22 The Family *Rhodospirillaceae*

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Abstract

Rhodospirillaceae Pfennig and Trüper 1971, 17[AL]

Rhodospirillaceae are a family within the order *Rhodospirillales* in the subclass of *Alphaproteobacteria*. The family *Rhodospirillaceae*, the so-called purple non-sulfur bacteria, have the type genus *Rhodospirillum* and embrace a total of 34 genera: *Azospirillum*, *Caenispirillum*, *Constrictibacter*, *Defluviicoccus*, *Desertibacter*, *Dongia*, *Elstera*, *Ferrovibrio*, *Fodinicurvata*, *Inquilinus*, *Insolitispirillum*, *Limimonas*, *Magnetospira*, *Magnetospirillum*, *Magnetovibrio*, *Marispirillum*, *Nisaea*, *Novispirillum*, *Oceanibaculum*, *Pelagibius*, *Phaeospirillum*, *Phaeovibrio*, *Rhodocista*, *Rhodospira*, *Rhodospirillum*, *Pararhodospirillum*, *Rhodovibrio*, *Roseospira*, *Skermanella*, *Telmatospirillum*, *Thalassobaculum*, *Thalassospira*, *Tistlia*, and *Tistrella*. According to 16S rRNA gene sequence similarities, the genera within the *Rhodospirillaceae* can be grouped into three big clusters: *Azospirillum–Skermanella–Desertibacter–Rhodocista–Dongia–Elstera–Inquilinus, Magnetospirillum–Nisaea–Thalassobaculum–Oceanibaculum–Fodinicurvata–Pelagibius –Tistlia–Phaeospirillum–Telmatospirillum–Defluviicoccus–Tistrella–Constrictibacter–Rhodovibrio–Limimonas*, and *Rhodospirillum–Pararhodospirillum–Roseospira–Rhodospira–Phaeovibrio–Novispirillum–Marispirillum–Insolitispirillum–Caenispirillum–Thalassospira–Magnetospira–Magnetovibrio–Ferrovibrio*. Some genera in the family *Rhodospirillaceae* grow photoheterotrophically under anoxic conditions in the light and chemoheterotrophically in the dark, while others grow heterotrophically under aerobic/microaerobic conditions. The members of the *Rhodospirillaceae* stain Gram negative and form rod shaped to spirillum-formed cells. The chemoheterotrophs include the facultative anaerobic genera *Skermanella*, *Telmatospirillum*, *Caenispirillum*, *Thalassobaculum*, and *Nisaea* and the strictly aerobic and microoxic genera *Azospirillum*, *Conglomeromonas*, *Magnetospirillum*, *Thalassospira*, *Tistrella*, and *Inquilinus*. The genus *Azospirillum* contains several diazotrophic, plant-associated bacteria having plant growth-promoting potential with agricultural application. Other genera include strains with interesting biotechnological potentials. Some genera also harbor opportunistic pathogenic bacteria, whose risk potential is not yet clear.

Taxonomy, Historical and Current

Short Description of the Family

Rhodospirillaceae (*Rho.do.spi.ril.la´ce.ae*: M.L. neut. n. *Rhodospirillum*, type genus of the family; L. suff. -aceae, ending

to denote a family; N.L. fem. pl. n. *Rhodospirillaceae*, the *Rhodospirillum* family; Pfennig and Trüper 1971a).

Phylogenetically, the family of *Rhodospirillaceae* is a member of the order *Rhodospirillales*, subclass *Alphaproteobacteria* in the phylum Proteobacteria (Stackebrandt et al. 1988). According to the 16S rRNA gene sequence comparison of the type species, the next related family within the the order *Rhodospirillales* is the family *Acetobacteraceae* (Gillis and De Ley 1980). The family *Rhodospirillaceae* contain the genera *Azospirillum*, *Caenispirillum*, *Constrictibacter*, *Defluviicoccus*, *Desertibacter*, *Dongia*, *Elstera*, *Ferrovibrio*, *Fodinicurvata*, *Inquilinus*, *Insolitispirillum*, *Limimonas*, *Magnetospira*, *Magnetospirillum*, *Magnetovibrio*, *Marispirillum*, *Nisaea*, *Novispirillum*, *Oceanibaculum*, *Pelagibius*, *Phaeospirillum*, *Phaeovibrio*, *Rhodocista*, *Rhodospira*, *Rhodospirillum*, *Rhodovibrio*, *Roseospira*, *Skermanella*, *Telmatospirillum*, *Thalassobaculum*, *Thalassospira*, *Tistlia*, and *Tistrella*. Some genera of the family *Rhodospirillaceae* grow photoheterotrophically under anoxic conditions in the light and chemotrophically in the dark (Pfennig and Trüper 1971a), while others grow chemoheterotrophically under aerobic conditions. They stain Gram negative and form rod shaped to spirillum-formed cells. Members of *Rhodospirillaceae* have varying metabolic and nutritional properties, which include photoheterotrophs, photoautotrophs, and chemoheterotrophs. The major respiratory lipochinones are ubiquinones 9, 10, and 11 and/or menaquinone 10 (MK-10). Unsaturated straight chain fatty acids are the predominant acyl groups of the family; among these are summed feature 8 ($C_{18:1}$ ω7c and/or $C_{18:1}$ ω6*c*), summed feature 3 ($C_{16:1}$ ω7c and/or $C_{168:1}\omega$6c), and summed feature 2 (consisting of $C_{14:0}$ 3 OH and or iso-$C_{16:0}$ 3-OH). The polar lipids consist mainly of phosphatidylglycerol, phosphatidylcholine, and other lipids which differ from species to species level. The type genus of the family is *Rhodospirillum*.

Azospirillum–Skermanella–Desertibacter–Rhodocista–Dongia–Elstera–Inquilinus

The genus *Azospirillum* (Tarand et al. 1979) forms a subcluster within the family *Rhodospirillaceae* together with the genera *Skermanella, Rhodocista, Desertibacter, Dongia*, *Elstera*, and *Inquilinus* (*Fig. 22.1*). These bacteria belong to the large group of "hydrobacteria," a clade of prokaryotes that originated in marine environments (Battistuzzi and Hedges 2009). Nearly all known representatives of the family *Rhodospirillaceae* are found in aquatic habitats, suggesting that *Azospirillum* represents a lineage which might have transitioned to terrestrial environments much later than the Precambrian split of "hydrobacteria" and "terrabacteria" (Wisniewski-Dyé et al. 2011). *Azospirillum* spp. are members of the α-subclass of *Proteobacteria*, and this genus was initially described by Krieg and Döbereiner (1984) to include a species previously named as *Spirillum lipoferum* (Beijerinck 1925). The growth of a spirillum-like bacterium in nitrogen-deficient malate- or lactate-based media, which had been inoculated heavily with garden soil, was first observed by Beijerinck in 1925. When this new bacterium was cultivated in malate medium, the nitrogen content increased, which led to the original species name *Azotobacter spirillum*. Three years later, it was renamed into *Spirillum* genus. In 1978 a group of isolates was utilized in a detailed taxonomic study by Tarrand et al. (1978). Based on the DNA homology group II bacteria *Azospirillum lipoferum* genus and species were described. This group of isolates seemed to correspond in several ways to Beijerinck's original description of *Spirillum lipoferum*, particularly with regard to growth with glucose or mannitol and to the formation of spirillum-shaped cells under certain conditions (Krieg and Döbereiner 1984).

These bacteria are spiral or slightly curved rod-shaped non-spore-forming cells with polyhydroxybutyrate (PHB) granules, which can form cysts. The Gram staining is negative and the cells are very motile with a single polar flagellum and several lateral flagella, shorter in length. Cells are polymorphic and positive for oxidase as well as catalase reaction (Tarrand et al. 1978). *Azospirillum* has a large amount of $C_{18:1}$ ω7*c* lipids (55·3 %) and contains also 16:1ω7*c*, 16:0 as a major component; the major hydroxy fatty acids are 3-OH $C_{14:0}$ and 3-OH $C_{16:0}$. When grown aerobically, species of this genus exhibit a quinone system with ubiquinone 10 (Q-10). The polar lipids consist mainly of phosphatidylglycerol, phosphatidylcholine, and one unidentified phospholipid. The DNA G+C content varies between 64 and 71 mol%.

The occurrence of *Azospirillum* spp. is widespread in the environment and has significant agricultural importance specifically as aerobic nitrogen-fixing species with considerable plant growth-promoting abilities. *A. brasilense* and *A. lipoferum* are known to associate with, and stimulate the growth of, numerous grasses and cereals. Most of the species were described from plant roots and soil samples. These organisms have a plant root tissue origin, especially in soils of tropical and subtropical regions, but also in temperate regions (Lavrinenko et al. 2010). *Azospirillum lipoferum* and *A. brasilense* are the two species which were described at first. Later, Magalhães et al. (1983) described the third species, *A. amazonense*. Four years later, another species was described, *A. halopraeferens* (Reinhold et al. 1987) from Kallar grass (*Leptochloa fusca*) in Pakistan. In 1989, Khammas isolated the 5th species of this genus, *A. irakense*, using root samples of rice. The taxonomy of the species *Conglomeromonas largimobilis* subsp. *largomobilis hodobium* was questioned as its similarity of the species *A. lipoferum*. The strains of this species were renamed to a new *Azospirillum* species called *A. largomobilis* and then corrected to *A. largimobile* (Sly and Stackebrandt 1999). In 2001, a new species was described and received the name of the famous scientist Johanna Döbereiner, calling *A. doebereinerae* (Eckert et al. 2001). In 2005, another species was described in China, also from rice samples, *A. oryzae* (Xie and Yokota 2005). Again a new species was described using plant tissue collected in China, *A. melinis* (Peng et al. 2006), using roots and stem of a plant called *Melinis*

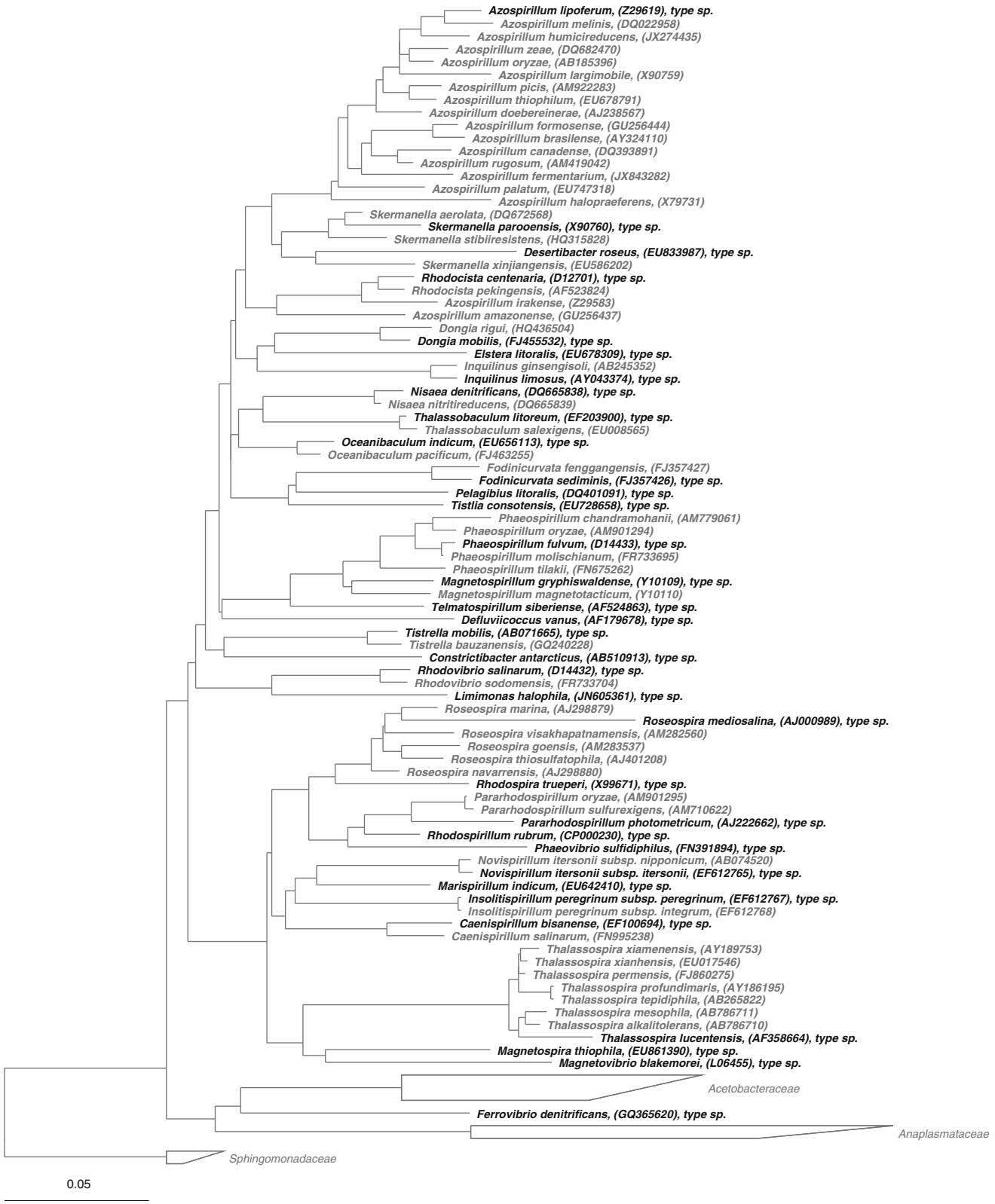

Fig. 22.1

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

minutiflora. In this case, a modification of the culture medium to a high pH contributed to discover not only this species but also two new ones: *A. canadense* (Mehnaz et al. 2007a) and *A. zeae* (Mehnaz et al. 2007b). In 2008, a new species was described using contaminated soils collected in Taiwan. This species was named *A. rugosum* as its colony morphology was different from the closely related ones (Young et al. 2008). This species was isolated from discarded road tar soil. In 2009, two new species were described: *A. palatum* (Zhou et al. 2009) and *A. picis* (Lin et al. 2009). *A. picis* was isolated from oil-contaminated soil samples in Taiwan, and it fixes nitrogen and possesses nitrate reduction activity, differing from *A. palatum*. Both species do not have indole production. *A. thiophilum* was isolated from sulfide spring collected in Russia (Lavrinenko et al. 2010). *A. formosense* was isolated from agricultural soil collected in Taiwan (Lin et al. 2012), and the last species, until to date (February 2014), is *A. humicireducens* that was isolated from microbial fuel cell and also fixes nitrogen. *A. fermentarium* was isolated from a fermentative tank in Taiwan.

More recently, the relatedness of *Roseomonas fauriae* and R. genomospecies 6 (originally members of the *Roseomonas* genus in the *Acetobacteraceae*) and *Azospirillum* spp. became apparent in comparative studies using phenotypic methods and molecular techniques. Conventional biochemical tests could not differentiate between the two taxa, and 16S rRNA and DNA–DNA hybridization experiments revealed rather high values for relatedness between *R. fauriae* with several type strains of *Azospirillum*. It was suggested that strains previously identified as *R. fauriae* and R. genomospecies 6 should be reclassified as *A. brasilense*, with the name *Roseomonas fauriae* as a later heterotypic synonym of *Azospirillum brasilense* (Helsel et al. 2006). Thus, *R. fauriae* and R. genomospecies 6, which possess a pink pigment like *A. brasilense*, were included into the genus *Azospirillum*; however, clinical isolates are also listed as *R. fauriae*.

Skermanella parooensis was first described as *Conglomeromonas largomobilis* subsp. *parooensis* together with *Conglomeromonas largomobilis* subsp. *largomobilis*. However, 16S rRNA comparison and nucleic acid hybridization (Falk et al. 1986; Ben Dekhil et al. 1997) showed that the latter was closely related to the genus *Azospirillum* and was therefore transferred as *Azospirillum largimobile* (Ben Dekhil et al. 1997) and *Conglomeromonas largomobilis* subsp. *parooensis* was elevated to *Conglomeromonas parooensis* (Ben Dekhil et al. 1997) since it was more distant from *Azospirillum* species. However, according to Rule 37a (1) of the International Code of Nomenclature of Bacteria, it should be classified in a different genus; thus, it was transferred to *Skermanella* gen. nov. as *Skermanella parooensis* gen. nov. (Sly and Stackebrandt 1999). New isolates from air (Weon et al. 2007) and soil from coal mine (Luo et al. 2012) made amendments to the genus description necessary as strictly aerobic (Weon et al. 2007), variable ability of ferment glucose and other phenotypic characteristics (Luo et al. 2012). The fourth species of genus was isolated from desert sand (An et al. 2009).

Recently, a bacterial isolate from sandy soil of the Taklimakan desert in Xinjiang, China, was described as a new genus, *Desertibacter roseus*. Based on 16S rRNA sequence comparison, it is more closely related to *Skermanella* than to *Azospirillum* (Liu et al. 2011).

Description of the genus *Rhodocista* was proposed by Kawasaki et al. (1992), to include a previously named *Rhodospirillum centenum* strain isolated from water of a hot spring (Favinger et al. 1989) as *Rhodocista centenaria* (Kawasaki et al. 1992). The presence of clearly distinct phenotypic, biochemical, and genetic properties from *Rhodospirillum rubrum* (type species of *Rhodospirillum* genus) supported the reclassification of this organism. The second species of this genus was described later by Zhang et al. (2003) that was named *Rhodocista pekingensis*, isolated from wastewater treatment plant. Rhodocista cells are vibrioid to spiral-shaped, anaerobic phototrophs or aerobic chemoorganotrophs; they are mesophilic and possess bacteriochlorophyll *a*. Colonies are pink pigmented, differentiating into dormant thermotolerant cysts when growing aerobically. *Rhodocista* type species form a close cluster with two species of the genus *Azospirillum* (*A. irakense* and *A. amazonense*, sharing 94.1 % and 91 % 16S rRNA gene sequence similarity). It encompasses 89.9 % 16S rRNA gene sequence similarity with the *Rhodospirillum rubrum* type species.

The bacterial genera *Dongia*, *Elstera*, and *Inquilinus* form a subcluster of strictly aerobic chemoheterotrophic bacteria. *Dongia mobillis* was isolated from freshwater wetland in Korea (Liu et al. 2010), while *Elstera litoralis* from biofilms on stones in the littoral zone of Lake Constance in Germany (Rahalkar et al. 2012). *Inquilinus* isolates are derived from clinical samples of, e.g., a cystic fibrosis patient (Coenye et al. 2001).

Magnetospirillum–Phaeospirillum–Nisaea–Thalassobaculum–Oceanibaculum–Fodinicurvata–Pelagibius–Tistlia–Telmatospirillum–Defluviicoccus–Tistrella–Constrictibacter–Rhodovibrio–Limimonas

According to the 16S rRNA gene sequence analysis (neighbor joining and maximum likelihood), a second branch of bacterial genera around *Magnetospirillum* and *Phaeospirillum* is evident (◉ *Fig. 22.1*).

Magnetotactic bacteria (MTB) is a general name used to group microorganisms capable of showing magnetotaxis and synthesizing intracellular organelles filled with crystals of a variety of mineral sources, named magnetosome. They are phylogenetically distributed into Alpha-, Gamma-, and Deltaproteobacteria classes of the Proteobacteria phylum, the Nitrospirae phylum, and the candidate division OP3, part of the Planctomycetes–Verrucomicrobia–Chlamydiae (PVC) bacterial superphylum (Bazylinski and Frankel 2004; Kolinko et al. 2012; Lefèvre et al. 2012a). Among the *Alphaproteobacteria* families, the *Rhodospirillaceae* comprises 3 genera representative of MTB: *Magnetospirillum*, *Magnetospira*, and *Magnetovibrio*. Characterization of the second isolated MTB that could be axenically cultivated, named MSR-1, and other

proteobacteria showed that it was closely related to *Aquaspirillum magnetotacticum* (94.1 %) than to *A. serpens* or to other reference organisms of the alpha subclass of *Proteobacteria* (84.1–88.9 %). Based on these results, Scheifer et al. (1991) proposed a new genus description, *Magnetospirillum*, in which both *A. magnetotacticum* and strain MSR-1 could be placed.

The genus *Phaeospirillum* was originally described as part of the study of Imhoff et al. (1998) which reclassified the *Rhodospirillum* species known so far into different genera mainly based on 16S rRNA gene sequence analysis. At this work *Rhodospirillum fulvum* (van Niel 1944) and *Rhodospirillum molischianum* (Giesberger 1947) were transferred to *Phaeospirillum fulvum* and *Phaeospirillum molischianum*, respectively. Reclassification of *Phaeospirillum* species was followed by the description of *P. chandramohanii*, *P. oryzae*, and *P. tilakii* (Kumar et al. 2009; Lakshmi et al. 2011a; Raj et al. 2012). These organisms were found in freshwater-rich environments such as mud and rhizosphere soil; are spiral shaped, mesophilic, and photoheterotrophic with photosynthetically grown cell suspension showing brown to brown-orange/brown-red color; present bacteriochlorophyll *a*, and major quinones are Q-9 and MK-9. *Phaeospirillum* type species share 94.4 % 16S rRNA gene sequence similarity with *Magnetospirillum* type species and 89.1 % 16S rRNA gene sequence similarity with *Rhodospirillum* type species.

A strain named P24 was shown to form a deep branch within the family *Rhodospirillaceae* based on comparative 16S rRNA gene sequence analysis (Lai et al. 2009a). The strain clustered closely to *Thalassobaculum litoreum* CL-GR58T (92.7 %), but the highest 16S rRNA gene sequence similarity was shared with strain SL3.14 (99 %), a bacterium isolated from the Silver Lake throughflow playa (Navarro et al. 2009; Lai et al. 2009a). Based on many peculiar phenotypic, biochemical, and molecular traits, a new genus, *Oceanibaculum* (Lai et al. 2009b emend. Dong et al. 2010) was proposed within the family *Rhodospirillaceae*. The type species is *Oceanibaculum indicum* P24^T (Lai et al. 2009b).

The genus *Pelagibius* was described by Choi et al. (2009) to include a marine bacterial strain isolated from seawater of the east coast of Korea. *Pelagibius* is a monospecific genus affiliated with the *Rhodospirillaceae* family according to 16S rRNA sequence analysis. The type species *Pelagibius litoralis* forms slightly curved or straight rods, is a mesophilic non-fermentative heterotroph, and is strictly aerobic forming circular, convex, and creamy colonies when grown on marine agar. The type species of *Pelagibius* share 92.9 % 16S rRNA gene sequence similarity with *Fodinicurvata* type species.

Tistlia consotensis is the only species decribed for this genus. This aerobic, slightly halophilic bacterium was recently isolated from a saline spring in Colombia (Diaz-Cárdenas et al. 2010).

Telmatospirillum siberiense was the first and only species described in the genus. Three isolates of this species were recovered from acidic wetland in Northern Russia. Based on 16S rRNA comparison, these isolates were allocated to *Telmatospirillum* nov. gen. as *Telmatospirillum siberiense*.

The genus *Rhodovibrio* comprises two species named *Rhodovibrio salinarum*, which is the type species, and *Rhodovibrio sodomensis* (Imhoff et al. 1998), formerly classified as *Rhodospirillum salinarum* and *Rhodospirillum sodomense*, respectively.

Rhodospirillum–Pararhodospirillum–Roseospira–Rhodospira–Phaeovibrio–Novispirillum–Marispirillum–Insolitispirillum–Caenispirillum–Thalassospira–Magnetospira–Magnetovibrio–Ferrovibrio

The genus *Rhodospirillum* is the type genus, and at present it consists of only one species, *Rhodospirillum rubrum* (Skerman et al. 1980; Molisch 1907; Imhoff et al. 1998), as the type species. The genus has ever contained other 11 species, including *Rhodospirillum photometricum*, *Rhodospirillum sulfurexigens*, *Rhodospirillum oryzae* (now *Pararhodospirillum photometricum*, *Pararhodospirillum sulfurexigens*, and *Pararhodospirillum oryzae*, respectively (Lakshmi et al. 2014)), *Rhodospirillum tenue* (now *Rhodocyclus tenuis* after (Imhoff et al. 1984)), *Rhodospirillum centenum* (presently *Rhodocista centenaria* after (Kawasaki et al. 1992)), and *Rhodospirillum fulvum*, *Rhodospirillum molischianum*, *Rhodospirillum salinarum*, *Rhodospirillum sodomense*, *Rhodospirillum salexigens*, and *Rhodospirillum mediosalinum* (these have been transferred respectively to *Phaeospirillum fulvum*, *Phaeospirillum molischianum*, *Rhodovibrio salinarum*, *Rhodovibrio sodomensis*, *Rhodothalassium salexigens*, and *Roseospira mediosalina* (Imhoff et al. 1998). The genus *Pararhodospirillum* consists of three species named *Pararhodospirillum photometricum*, *Pararhodospirillum sulfurexigens*, and *Pararhodospirillum oryzae* (Lakshmi et al. 2014), which have been classified previously as *Rhodospirillum photometricum*, *Rhodospirillum sulfurexigens*, and *Rhodospirillum oryzae*, respectively. *Pararhodospirillum photometricum* is the type species.

Roseospira mediosalina was first described as *Rhodospirillum mediosalinum* by Kompantseva and Gorlenko (1984). In 1998 Imhoff et al. based on comparison of the 16S rRNA sequence proposed to transfer it to *Roseospira* gen. nov. as *Roseospira mediosalina* comb. nov. Later Guyoneaud et al. (2002) classified 3 new isolates as new species of the genus (*R. marina* sp. nov., *R. navarrensis* sp. nov., and *R. thiosultatophila* sp. nov.) based on 16S rRNA gene sequence, DNA–DNA hybridization, and phenotypic characteristics. These authors also emended the genus description to take into account the characteristics of the new species. Chakravarthy et al. (2007) isolated from water of the fishing harbor at Visakhapatnam (India) strain JA131, later classified as *Roseospira visakhapatnamensis* sp. nov., and strain JA135 from sediment of Kurka saltern, Goa (India), which was classified as *Roseospira goensis* sp. nov.

Reallocation of *Aquaspirillum itersonii* and *Aquaspirillum peregrinum* (Hylemon et al. 1973) to the family *Rhodospirillaceae* leads to their reclassification into 2 new genera, *Novispirillum* and *Insolitispirillum* (Ding and Yokota 2002; Yoon et al. 2007b).

The species *Phaeovibrio sulfidiphilus*, the only species of this genus, was isolated from brackish water (Lakshmi et al. 2011b). Cells are vibrioid, mesophilic, strictly anaerobic, photoheterotrophic, and able to grow in a limited number of carbon substrates (acetate, pyruvate, and succinate). Chimeric internal membranes of lamellar stacks and vesicles are present in a single cell, and photosynthetically grown cultures are light brown. *Phaeovibrio* type species share 91.5 % 16S rRNA gene sequence similarity with *Rhodospirillum* type species and 80–91 % sequence similarity to *Rhodocista*, *Phaeospirillum*, *Rhodovibrio*, *Rhodospira*, and *Roseospira* type species.

The genus *Rhodospira* comprehends only one species named *Rhodospira trueperi* (Pfennig et al. 1997). This genus was assigned to describe a marine photosynthetic non-sulfur bacteria strain isolated from salt marsh that forms vibrioid- to spirilloid-shaped cells, and mesophilic, peach-colored photoheterotrophic cultures were observed under anoxic conditions. *R. trueperi* presents bacteriochlorophyll *b* and forms elemental sulfur globules outside the cells in the presence of sulfide, with Q-7 and MK-7 as major quinones. *Rhodospira trueperi* shares 93.9 % 16S rRNA gene sequence similarity with *Roseospira mediosalina* (type species) and 92.8 % 16S rRNA gene sequence similarity with *Rhodospirillum* type species.

Phylogenetic Structure of the Family and Its Genera

According to the phylogenetic relationship based on 16S rRNA gene sequence analyses within the order *Burkholderiales*, the family *Rhodospirillaceae* (Pfennig and Trüper 1971a) is moderately affiliated to the family *Acetobacteraceae* (Gillis and De Ley 1980). Extensive 16S rRNA gene sequence analyses of type species and strains constitute the phylogenetic structure within the family *Rhodospirillaceae* (Fig. 22.1).

Azospirillum–Skermanella–Desertibacter–Rhodocista–Dongia–Elstera–Inquilinus

The 16S rRNA gene sequence analysis within the genus *Azospirillum* reveals that *A. lipoferum, A. largimobile, A. brasilense, and A. halopraeferens* have 96.6 %, 96.6 %, 95.9 %, and 93.6 % similarity, respecticvely, with *A. doebereinerae* (Eckert et al. 2001). *A. formosense* is closely related to *A. brasilense* (98 % 16S rRNA similarity). *A. canadense* and *A. rugosum* are 96 % similar, while *A. thiophilum* and *A. picis* present a lower level of similarity (72 %). *A. halopraeferens* formed another cluster with 86 % of similarity to the above species described. *Skermanella aerolata* and *Skermanella parooensis* are included into the 16S rRNA tree as the closely related genus of *Azospirillum*. *A. amazonense* is the species with lower level of similarity together with *A. irakense* and forms a branch with *Rhodocista centenaria* and *Rhodocista pekingensis* (Lin et al. 2012). *A. fermentarium* strain CC-LY743^T revealed a high similarity level to *A. picis* DSM 19922^T (96.1 %), *A. oryzae* JCM 21588^T (96.0 %), and *A. rugosum* DSM 19657^T (96.0 %), while these values were lower (<96.0 %) for other species (Lin et al. 2013). The recently described *A. humicireducens* is closely related to *A. lipoferum* forming a subclade with 98 % similarity and also presents high levels to *A. thiophilum* (97.6 %) and *A. oryzae* (97.1 %) (Zhou et al. 2013).

Skermanella parooensis was originally classified as *Conglomeromonas largomobilis* subsp. *paraooensis*. The transfer of *Conglomeromonas largomobilis* subsp. *largomobilis* to the genus *Azospirillum* and the low 16S rRNA similarity (93 %) (Ben Dekhil et al. 1997) and DNA–DNA hybridization (5–12 %) between the two subspecies led to the elevation of the former to a new species *Skermanella parooensis* (Falk et al. 1986). Phylogenetic analyses of 16S rRNA gene sequences showed that species of the *Skermanella* genus form a cluster with *Desertibacter roseus* and are related phylogenetically to members of the genera *Azospirillum* and *Rhodocista* within the *Alphaproteobacteria*. Comparison of the 16S rRNA gene sequence of *Skermanella aerolata* 5416 T-32 showed highest level of similarity (96.2 %) with *S. parooensis* DSM 9527 but has lower levels of sequence similarity (<92 %) with respect to other species (Weon et al. 2007). *Skermanella xinjiangensis* strain 10-1-101 has the highest degree of similarity to *S. aerolata* 5416 T-32 (94.07 %) and *S. parooensis* DSM 9527 (92.74 %) (An et al. 2009). Partial 16S rRNA gene sequence (1,420 bp) of *Skermanella stibiiresistens* strain SB22T showed the highest degree of similarity to *S. aerolata* 5416 T-32 (97.3 % similarity), *S. parooensis* ACM 2042^T (95.8 %), and *S. xinjiangensis* 10-1 (92.9 %) (Luo et al. 2012). The phylogenetic analysis revealed that strain SB22T was closely related to the members of the genus *Skermanella* and grouped in the same cluster with *S. aerolata* 5416 T-32, *S. parooensis* ACM 2042^T, and *S. xinjiangensis* 10-1-101.

Phylogenetic analysis of the 16S rRNA gene sequence showed that the nearest phylogenetic neighbors of *Desertibacter roseus* 2622^T are species of the genus *Skermanella*. It shared 91.7 % and 90.1 % similarities to the type strains of *S. xinjiangensis* and *S. aerolata*, respectively. These values were lower (89.8–88.1 %) when compared to the type strains of the genus *Azospirillum* (Liu et al. 2011).

The 16S rRNA gene sequence analysis of the genus *Rhodocista* indicated that it forms a distinct phylogenetic branch within the *Rhodospirillaceae* family. In fact, the work of Kawasaki et al. (1992) clarified the phylogenetic positioning of spiral-shaped purple non-sulfur bacteria on the basis of 16S rRNA gene sequences, highlighting the heterogeneity of *Rhodospirillum* genus. The phylogenetic positioning of the *Rhodocista* species indicates a close cluster with *Azospirillum irakense* but quite distant from the other *Azospirillum* species. This very close relationship was also shown by Zhang et al. (2003), who described the second species *Rhodocista pekingensis*. *Rhodocista* species and *Azospirillum irakense* share about 96–97 % sequence similarity, although the latter is not phototrophic while the former did not grow using malate as carbon source.

Dongia mobilis LM22^T exhibited the highest 16S rRNA sequence similarity with *Inquilinus limosus* AU0476^T (90.4 %) and less than 90 % similarity with other members of the family *Rhodospirillaceae* such as *Skermanella*, *Azospirillum*, and *Rhodocista* (Liu et al. 2011). *Inquilinus ginsengisoli* Gsoil 080^T (Jung et al. 2011) was most closely related to *I. limosus* strains AU0476^T and AU1979 (Wayne et al. 1987) with 98.9 % 16S rRNA sequence similarity level. Recently, Baik et al. (2013) described a bacterium enrichment culture clone 04SU4-P as *Dongia rigui* 04SU4-P^T. A phylogenetic analysis based on 16S rRNA gene sequences showed that strain 04SU4-P^T forms an evolutionary lineage within the genus *Dongia* and its nearest neighbor is *Dongia mobilis* LM22^T (98.0 %). The 16S rRNA gene sequence analysis indicated that *Elstera litoralis* Dia-1^T was closely related to representatives of the genera *Azospirillum* (90–91 %), *Skermanella* (88–89 %), *Rhodocista* (87–88 %), and *Dongia* (88–89 %) (Rahalkar et al. 2012).

Magnetospirillum–Phaeospirillum–Telmatospirillum–Thalassobaculum–Nisaea–Oceanibaculum–Fodinicurvata–Pelagibius–Tistlia–Defluviicoccus–Tistrella–Constrictibacter–Rhodovibrio–Limimonas

Magnetotactic bacteria (MTB) is a general name used to group microorganisms capable of showing magnetotaxis and synthesizing intracellular organelles filled with crystals of a variety of mineral sources, named magnetosome. They are phylogenetically distributed into Alpha-, Gamma-, and Deltaproteobacteria classes of the Proteobacteria phylum, the Nitrospirae phylum, and the candidate division OP3, part of the Planctomycetes–Verrucomicrobia–Chlamydiae (PVC) bacterial superphylum (Bazylinski and Frankel 2004; Kolinko et al. 2012; Lefèvre et al. 2012a). Among the *Alphaproteobacteria* families, the *Rhodospirillaceae* comprises 3 genera representative of MTB: *Magnetospirillum, Magnetospira, and Magnetovibrio.* Based on 16S rRNA gene identity, it was shown that the closest organism of *Magnetospirillum bellicus* was *Magnetospirillum gryphiswaldense* MSR-1, with 96 % sequence similarity to strain VDY^T. The species *Dechlorospirillum anomalous* strain WD was shown to be closely related *to the* magnetotactic *Magnetospirillum species* (Michaelidou et al. 2000), but at the time, none of the *Magnetospirillum* species tested (*M. gryphiswaldense, M. magnetotacticum*, and *Magnetospirillum* strain AMB-1) could couple growth to the reduction of perchlorate or chlorate, but later on, the presence of a homolog of the cld gene in strains VDY, WD, and MS-1 was reported by Bender et al. (2004). Meanwhile, according to Lefèvre et al. (2013a), the magnetospirilla are a large group that appears to phylogenetically span a number of genera. Current evidences suggest that a detailed study considering the phylogenetic relationship between *Phaeospirillum* and *Magnetospirillum* would be necessary. The phylogenetic positioning of the *Phaeospirillum* type species is distributed in three sister clades. *P. fulvum* and *P. molischianum* form one clade with 99.1 % 16S rRNA gene sequence similarity. Another clade encompasses *P. chandramohanii* and *P. oryzae*, which shares 98.2 % similarity. The third clade harbors *P. tilakii* type strain sharing approximately 97 % similarity with *P. chandramohanii*/*P.oryzae* clade and around 96.7 % similarity with *P. fulvum*/*P. molischianum* clade. The clades *P. fulvum*/*P. molischianum* and *P. chandramohanii*/*P.oryzae* share approximately 97 % similarity. The 16S rRNA gene sequence similarities between *P. tilakii* JA492^T and the other *Phaeospirillum* type strains ranged from 96.5 % to 97.4 %. Besides the neighbors, strains *P. fulvum* DSM 113^T and *P. molischianum* ATCC 14031^T share the same branch with a 99.1 % 16S rRNA gene sequence similarity. Phylogenetic tree based on 16S rRNA gene sequences of strains of *Telmatospirillum siberiense* formed a separate cluster with purple non-sulfur bacteria of the genera *Phaeospirillum* and *Magnetospirillum*, within the family *Rhodospirillaceae.* Sequence similarity of *Telmatospirillum siberiense* strains between each other was 98.3–98.9 %, but only 90.9–92.5 % when compared to *Phaeospirillum* and *Magnetospirillum.* Comparison of partial amino acid sequence obtained from amplified *nifH* gene (148 residues) also showed that 2 strains (26-4b1 and K-1) of *Telmatospirillum siberiense* formed a separate branch with higher similarity to each other (97.0 % of amino acid sequence identity) than to their closest *Azospirillum* relatives (92.0–94.1 %). In addition, comparison of partial sequences of the "red-like" *cbbL* gene encoding large (catalytic) subunits of RuBisCO, corresponding to 231 amino acid residues, also showed close relationship of *Telmatospirillum siberiense* strains to each other (96.9 %), forming a separate branch in the phylogenetic tree. Their similarity with *Azospirillum lipoferum* (88.1–91.3 %) "red-like" *cbb*L gene was similar to other *Alphaproteobacteria* (77.9–94.2 %).

The 16S rRNA gene sequence of *Thalassobaculum litoreum* strain CL-GR58^T showed 90.9 % similarity to the type strain of *Azospirillum lipoferum*, 89.8 % to *Azospirillum oryzae*, 89.7 % to *Azospirillum canadense*, 89.5 % to *A. doebereinerae*, and 79.3–89.5 % to the other type species of the family *Rhodospirillaceae* (Zhang et al. 2008). The 16S rRNA sequence analysis of strain *Thalassobaculum salexigens* CZ41-10a^T showed that it was phylogenetically affiliated to the family *Rhodospirillaceae* (Urios et al. 2010) and presented variable similarity relatedness values to the relatives *Thalassobaculum litoreum* CL-GR58^T (99 %), *Nisaea nitritireducens* DSM 19540^T (94 %), and *Nisaea denitrificans* DSM 18348^T (93 %). *Oceanibaculum* is closely related to *Thalassobaculum litoreum* CLGR58 and *Nisaea*, but each of them forms a separate clade, as independent monophyletic cluster in the family *Rhodospirillaceae* (Urios et al. 2008; Lai et al. 2009a, b).

Phylogenetic analysis of almost-complete 16S rRNA gene sequences of *Fodinicurvata sediminis* strain YIM D82^T and *Fodinicurvata fenggangensis* YIM D812^T revealed that they formed a distinct lineage within the family *Rhodospirillaceae* (Wang et al. 2009). The similarity between the 16S rRNA gene sequences of the two strains was 98.2 %. The levels of 16S rRNA

gene sequence similarities between strain YIM D82^T and the type strains of *Rhodovibrio sodomensis* and *Rhodovibrio salinarum* were 90.6 % and 90.5 %, respectively, while the sequence similarity levels were 90.2 % and 90.1 %, respectively, against *Fodinicurvata fenggangensis* YIM D812^T. The single species of the *Pelagibius* genus formed a branch closely related to *Tistlia* type species (Díaz-Cárdenas et al. 2010) showing about 91 % of 16S rRNA gene sequence similarity (Choi et al. 2009). The 16S rRNA gene sequence analysis of strain *Tistlia consotensis* USBA 355^T indicated that it formed a distant phylogenetic line of descent with members of the genus *Thalassobaculumn* (90 % gene sequence similarity). This level was much lower when strain USBA 355T was compared to all other members of the family *Rhodospirillaceae* (Díaz-Cárdenas et al. 2010).

The species *Defluvicoccus vanus* showed 16S rRNA gene sequence similarity to the species *Rhodospirillum rubrum* (87.5 %), *P. fulvum* (88.5 %), *Magnetospirillum gryphiswaldense* (88.2 %), *Magnetospirillum magnetotacticum* (88.5 %), and *Rhodocista centenaria* (89 %). A comparison of the inferred 16S rRNA gene sequence nucleotide signature between members of *Alphaproteobacteria* supports the view that type strain Ben 114^T is not closely related to any of them (Maszenan et al. 2005).

The almost complete 16S rDNA sequence of *Tistrella mobilis* strain IAM 14872^T (Shi et al. 2002) showed sequence similarity values of 86.0 % to *Craurococcus roseus* JCM 9933 T and 90.1 % to *Phaeospirillum molischianum* ATCC 14031^T. Phylogenetic analysis using the neighbor-joining method showed that strain BZ78*T* (1,493 bp) formed a distinct cluster with *T. mobilis* IAM 14872 *T*, supported by a relatively high bootstrap value (98.3 % 16S rRNA gene sequence similarity) within the family *Rhodospirillaceae* (Zhang et al. 2011). A similar tree topology was also found in the tree generated using the maximum-likelihood method. Levels of 16S rRNA gene sequence similarities between strain BZ78*T* and the type strains of other species in the family *Rhodospirillaceae* were 90.1 %.

The 16S rRNA gene sequence of *Constrictibacter antarcticus* (strain 262-8^T) indicated high sequence similarities (99–90 %) with sequences of uncultured bacteria found in environmental samples worldwide. In comparative analysis with type strains, the most closely related neighbors of strain 262-8^T were *Stella vacuolata* DSM 5901 T (90.2 %), *Stella humosa* DSM5900T (90.2), and *Tistrella mobilis* IAM 14872 T (89.7 %). In agreement with Yamada et al. (2011), the low similarity values suggested that it would be difficult to analyze the phylogenetic position of strain 262-8^T by DNA–DNA hybridization.

The genus *Rhodovibrio* comprises two species named *Rhodovibrio salinarum*, which is the type species, and *Rhodovibrio sodomensis* (Imhoff et al. 1998), formerly classified as *Rhodospirillum salinarum* and *Rhodospirillum sodomense*, respectively. *Limimonas* 16S rRNA sequence showed similarity with *Rhodovibrio sodomensis* DSM 9895^T (91.6 %) and *Rhodovibrio salinarum* NCIMB 2243^T (91.2 %) forming an independent cluster with the halophilic members of the family *Rhodospirillaceae* although in a separate clade (Amoozegar et al. 2013).

Rhodospirillum–Pararhodospirillum–Phaeovibrio–Roseospira–Rhodospira–Novispirillum–Marispirillum–Insolitispirillum–Caenispirillum–Thalassospira–Magnetospira–Magnetovibrio–Ferrovibrio

Analysis of the 16S rRNA sequence of *Rhodospirillum rubrum* ATCC11170^T, *Pararhodospirillum photometricum* (formerly *Rhodospirillum photometricum*) strains DSM122^T and E11, *Rhodovibrio sodomensis* (formerly *Rhodospirillum sodomense*) strain ATCC51195^T, and *Rhodovibrio salinarum* (previously *Rhodospirillum salinarum*) strain ATCC35394^T resulted in the separation of these two later into a phylogenetic clade and the proposal of the new genus *Rhodovibrio* (Imhoff et al. 1998).

The 16S rRNA sequence analysis between *Pararhodospirillum sulfurexigens* (formerly *Rhodospirillum sulfurexigens*) strain JA143^T with *Rhodospirillum rubrum* ATCC11170^T and *Pararhodospirillum photometricum* (formerly *Rhodospirillum photometricum*) strain DSM122^T showed sequence similarity of 95.72 % and 95.58 %, respectively, which justified the description of the former as the type strain of the novel species (Kumar et al. 2008). In the same analysis, *Rhodovibrio sodomensis* (previously *Rhodospirillum sodomense*) strain DSI^T and *Rhodovibrio salinarum* (originally *Rhodospirillum salinarum*) strain ATCC35394^T were grouped into a clade apart. Later on, phylogenetic relationships based on the 16S rRNA gene sequence analysis of *Pararhodospirillum oryzae* (formerly *Rhodospirillum oryzae*) strain JA318^T with *Pararhodospirillum sulfurexigens* (formerly *Rhodospirillum sulfurexigens*) strain JA143^T, *Pararhodospirillum photometricum* (formerly *Rhodospirillum photometricum*) strain DSM122^T, and *Rhodospirillum rubrum* ATCC 11170^T indicated that the *P.oryzae* clustered with type strains of the genus *Rhodospirillum* (which was then included in the *Pararhodospirillum*) (Lakshmi et al. 2014). The highest sequence similarity for *Pararhodospirillum oryzae* strain JA318^T was found with the type strain of *Pararhodospirillum sulfurexigens* (99.9 %).

Comparative 16S rDNA sequence analyses of *Roseospira marina* CE2105, *Roseospira navarrensis* SE3104, *Roseospira thiosulfatophila* AT2115 (AJ401208), *Roseospira mediosalina*, *Roseospira visakhapatnamensis* JA131, and *Roseospira goensis* JA135 showed that these strains form a subgroup together with *Rhodospira trueperi* within the *Rhodospirillaceae* family of the *Alphaproteobacteria*, well separated from *Rhodospirillum* genus, their closest relatives. *Roseospira marina* CE2105, *Roseospira navarrensis* SE3104, and *Roseospira thiosulfatophila* AT2115 (AJ401208) have similar salt requirements that are phylogenetically closely related, with 16S rRNA similarity ranging from 97.6 % to 96.5 %, whereas *Roseospira mediosalina* requires a higher optimal salt concentration salinity and similarity ranging from 95.6 % to 94.7 % in comparison with the former three species (Guyoneaud et al. 2002). Comparison of 16S rRNA sequences of *Roseospira visakhapatnamensis* JA131 and *Roseospira goensis* JA135 showed that they are closest to *Roseospira navarrensis* (95.9 %), *Roseospira marina* (95.5 %), *Roseospira mediosalina* (94.2 %), and *Roseospira thiosulfatophila* (96.1 %).

Sequence similarity between *Roseospira visakhapatnamensis* JA131 and *Roseospira goensis* JA135T is 96.6 % (Chakravarthy et al. 2007).

Analysis of the phylogenetic positioning of the genus *Rhodospira* indicates its close relationship with the genus *Roseospira*, with 16S rRNA gene sequence similarity of about 93–94 %. However, the presence of bacteriochlorophyll *b* and tetrahydrospirilloxanthin as main pigments in *Rhodospira* differentiates this genus from *Roseospira* which contains Bchl *a* and carotenoids of the normal spirilloxanthin series as main pigments.

Phaeovibrio genus formed by the species *Phaeovibrio sulphidiphilus* branches separately from other *Rhodospirillaceae* genera. The highest similarities of the 16S rRNA gene sequences are observed with representatives from the *Rhodospirillum* genus, sharing approximately 91–92 % similarity.

Phylogenetic analysis based on 16S rRNA gene revealed that species previously named as *Aquaspirillum itersonii* and *Aquaspirillum peregrinum* (Hylemon et al. 1973) were more closely related to the *Alphaproteobacteria* than to the *Betaproteobacteria* group and formed distinct phylogenetic lineages leading to their reallocation to the family *Rhodospirillaceae* and creation of the genera *Novispirillum* and *Insolitispirillum* (Ding and Yokota 2002; Yoon et al. 2007b).

Representatives of the genera *Marispirillum*, *Insolitispirillum*, and *Novispirillum* cluster independently in the same branch of *Caenispirillum*, but apart from the *Rhodospirillum* and *Rhodospira*, representatives of the photosynthetic group of *Rhodospirillaceae*. Comparisons of 16S rRNA gene sequences showed that *Marispirillum indicum* type strain was most closely related to the type strains of two *Insolitispirillum peregrinum* subspecies (93.0–93.1 % sequence similarity), two *Novispirillum itersonii* subspecies (92.8–92.9 %), and *Caenispirillum bisanense* (91.7 %); sequence similarities with respect to other taxa were below 90.5 % (Lai et al. 2009a). The phylogenetic analysis based on 16S rRNA and the neighbor-joining algorithm showed that strains K92T and K93 of *Caenispirillum bisanense* joined a phylogenetic clade comprising *Novispirillum itersonii* (formerly *Aquaspirillum itersonii*) and *Insolitispirillum peregrinum* (formerly *Aquaspirillum peregrinum*) exhibiting the highest 16S rRNA gene sequence similarity values (91.3–91.5 %). Similar analysis indicated that *Caenispirillum salinarum* strain AK4^T was most closely related to *Caenispirillum bisanense* (96.6 %). In contrast, it shared less than 93.2 % sequence similarity with other members of the family.

Phylogenetic analysis based on the partial 16S rRNA gene sequencing of the type strain *Thalassospira lucentensis* QMT2^T indicated a high sequence identity (89 %) to the well-characterized species *Rhodospirillum rubrum*, *Novispirillum itersonii* (formerly *Aquaspirillum itersonii*), and *Terasakiella pusilla* (formerly *Oceanospirillum pusillum*) microorganisms, which are representatives of the α-subclass of the *Proteobacteria* (López-López et al. 2002). Analysis of 16S rRNA gene sequences of *Thalassospira xiamenensis* M-5^T and *Thalassospira profundimaris* WP0211^T indicated that both species were closely related to *Thalassospira lucentensis* (96.1 % and 96.2 % gene sequence similarities, respectively). The 16S rRNA gene sequence analysis of *Thalassospira tepidiphila* 1-1B^T showed a very high level of similarity to *Thalassospira profundimaris* WP0211^T (99.8 %), *Thalassospira xiamenensis* M-5^T (98.2 %), and *Thalassospira lucentensis* DSM 14000^T (98.1 %). However, the levels of DNA–DNA relatedness between strain 1-1B^T and these type strains were 50.7 ± 17.2, 35.7 ± 17.8, and 32.0 ± 21.1 %, respectively. Very high level of similarity was also observed between *Thalassospira alkalitolerans* MBE#61^T and *T. mesophila* MBE#74^T (98.9 % similarity), and these strains shared the highest levels of similarity with *T. lucentensis* QMT2T (99.0 % and 98.5 %, respectively). High levels of similarity were also detected when these strains were compared to *T. xianhensis* P-4^T (97.9 % and 97.7 %, respectively), *T. profundimaris* WP0211^T (97.7 % and 97.2 %, respectively), *T. xiamenensis* M-5^T (97.5 % and 97.2 %, respectively), and *T. tepidiphila* 1-1B^T (97.5 % and 96.9 %, respectively) (Tsubouchi et al. 2014). Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences reveals that *Magnetospira thiophila* and *Magnetovibrio blakemorei* are closely related to *Thalassospira* spp. while other MTB representatives cluster closely to *Phaeospirillum* spp. within the second main group of *Rhodospirillaceae*. Lefèvre et al. (2012a) observed the same distribution when comparing their phylogenetic relationship based on 16S rRNA or Mam proteins cluster analysis and concluded that the evolution of MTB and magnetosomes is congruent, indicating that they were acquired by a common ancestor of the *Magnetospirillum* clade, except in one case. The congruency of the evolutionary path of MTB and magnetotaxis is also confirmed when composition and morphological properties of magnetosome minerals are taken into account (Lefèvre et al. 2013a; Pósfai et al. 2013).

Phylogenetic analysis, based on 16S rRNA gene sequence, showed that *Ferrovibrio denitrificans* Sp-1^T formed a cluster with members from two different orders: *Sneathiellales* and *Rhodospirillales* within the class *Alphaproteobacteria* (Sorokina et al. 2012). 16S rRNA gene sequence of strain Sp-1^T showed similarities with *Sneathiellales chinensis* (89.4 %) followed by *Inquilinus limosus* (89.0 %) and 88.9 % with both *S. glossodoripedis* and *I. ginsengisoli*.

Molecular Analyses

DNA–DNA Hybridization Studies

Almost all descriptions of the *Azospirillum* species include results of DNA–DNA hybridization (DDH) studies within the genus possessing approximately 64–71 mol% (Ben Dekhil et al. 1997). Only for the species *A. rugosum* the data are not available. In 1987, Reinhold et al. detected the generic relationship of *A. halopraeferens* and also confirmed the generic status of *A. amazonense* using the DNAs of five representative strains of the genus. The authors hybridized labeled rRNA from *A. brasilense* ATCC 29145^T. The strains Au 4^T and Au 5 of *A. halopraeferens* as well as the strains YI^T, Y9, and Y13 of *A. amazonense* are located at the same level on the *Azospirillum* rRNA branch, which forms

a trinity together with the rRNA branches of *Rhodospirillum rubrum* and some *Aquaspirillum* species obtained previously by De Smedt et al. (1980). Each of these branches deserved at least a separate generic rank. With Tm(e) values ranging from 73.4 °C to 75.3 °C, both *A. halopraeferens* strains are quite distinct from the *A. brasilense–Azospirillum lipoferum* cluster. One year earlier Falk et al. (1986) described the results of DNA–DNA hybridization of *Conglomeromonas largomobilis* subsp. *largomobilis* that proved the similarity to the species *A. lipoferum* althought the 47 % of this similarity was considered lower to the criteria of 70 % recommended by Wayne et al. (1987). Ben Dekhil et al. (1997) compared the binary sequence similarity values; corrected dissimilarity values indicate that *C. largomobilis* subsp. *largomobilis* is most closely related to *A. lipoferum* and *A. brasilense* with 97.1 % and 95.2 % similarities, respectively. Based on this comparison and also other features, they transfer the species into *Azospirillum largomobilis* comb. nov., and subsequently the name was corrected to *Azospirillum largimobile* by Sly and Stackebrandt (1999). Other study involving *Azospirillum* species was reported by (Peng et al. 2006). The DNA–DNA hybridization among strains of *A. melinis* isolated from molasses grass varied from 81 % to 95 %, with a mean of 88.7 %, indicating that they represented the same genomic species. As expected, the DNA–DNA relatedness was 54–57 % for *Azospirillum lipoferum* DSM 1691^T and 30–34 % for *Azospirillum brasilense* Sp 7^T hybridized against the three strains of *A. melinis*. The DNA–DNA hybridization value between *Skermanella stibiiresistens* SB22^T and *S. aerolata* KACC 11604^T (= 5416 T-32^T) was 43.3 %. DNA–DNA hybridization studies of *Roseospira marina* CE2105, *Roseospira navarrensis* SE3104, and *Roseospira thiosulfatophila* AT2115 (AJ401208) showed low homologies between them and supported the separation into three species (Guyoneaud et al. 2002). No data are available for the other species or genera. DDH analysis showed that *Inquilinus ginsengisoli* Gsoil 080^T exhibited 12 ± 3.2 % DNA–DNA relatedness with *Inquilinus limosus* AU0476^T, whereas reciprocal hybridization resulted in a higher value of 15 ± 2.7 %. DDH studies for the other genera are missing, and in many cases, the 16S rRNA has been used to create new species.

DNA–DNA hybridization study showed that a dissimilatory perchlorate-reducing bacteria (DPRB) strain VDY^T, described as *Magnetospirillum bellicus*, has only 46.2 % similarity with *Dechlorospirillum anomalous*, although 99 % similarity was observed in 16S rRNA gene sequence analysis (Thrash et al. 2007). No data about DNA–DNA hybridization is available for *Magnetospirillum* (*M. gryphiswaldense* and *M. magnetotacticum*). Studies of DNA–DNA hybridization (DDH) on *Phaeospirillum* type strains are available for *P. oryzae* and *P. tilakii* between the other type strains of the genus. The neighbors strain *P. oryzae* JA317T and *P. chandramohanii* JA145T which share 98.2 % 16S rRNA gene sequence similarity present 55 % DDH similarity, which is lower than the level generally accepted to distinguish species. In addition, values of DDH for *P. oryzae* and the type strains *P. fulvum* DSM 113 T and *P. molischianum* ATCC 14031 T are 42 % and 38 % similarities, while 16S rRNA gene sequence similarities are 97.1 % and 97.4 %, respectively (Lakshmi et al. 2011b). Studies perfomed by Raj et al. (2012) presented very low DDH values for *P. tilakii* JA492T and the other *Phaeospirillum* type strains: 10–12 %, 10.2–14.6 %, 35.3–39.5 %, and 35.3–38 % DDH similarities between DSM 113 T, ATCC 14031 T, JA317T, and JA145T, respectively. DDH analysis indicated DNA–DNA relatedness value of 66 ± 1 % between *Thalassobaculum litoreum* DSM 18839^T and *Thalassobaculum salexigens* strain CZ41-10a^T (Urios et al. 2010). DNA–DNA hybridization assays indicated that *Thalassospira lucentensis* QMT2^T was the closest phylogenetic neighbor, and *T. xiamenensis* M-5^T and *T. tepidiphila* 1-1B^T were distantly related neighbors. The species *T. alkalitolerans* MBE#61^T and *T. mesophila* MBE#74^T showed relatively high levels of DNA–DNA relatedness (%); however, they exhibited low levels of hybridization value with *T. lucentensis* QMT2^T (12.5–16.0 % and 7.1–11.0 %, respectively), with *T. xiamenensis* M-5^T (24.1–25.0 % and 8.0–15.8 %, respectively), and with *T. tepidiphila* 1-1B^T (11.3–19.4 % and 9.0–10.4 %, respectively). The DNA–DNA relatedness between *T. alkalitolerans* MBE#61^T and *T. mesophila* MBE#74^T is 7.3–15.1 %. Studies involving species of genus *Tristella* showed that the level of DNA–DNA relatedness between strains *T. bauzanensis* BZ78^T and *T. mobilis* JCM 21370^T was 37.3 %, which was well below the threshold value of 70 % recommended for the delineation of bacterial species. In the case of the genus *Tistlia*, the DNA **GC%** of strain USBA 355^T was calculated to be 71 ± 1 mol% (Díaz-Cárdenas et al. 2010). This GC% value is closer to the genera *Inquilinus* and *Caenispirillum* and in the same range of the genera *Azospirillum* and *Magnetospirillum*. In contrast, it is quite distant from the genera *Thalassospira* and *Nisaea*. DNA–DNA hybridization (DDH) performed between species *Fodinicurvata sediminis* YIM D82T and *Fodinicurvata fenggangensis* YIM D812T showed a DNA–DNA relatedness of 27.5 %.

DDH experiments carried out between strains JA318^T of *Pararhodospirillum oryzae* and JA143^T of *Pararhodospirillum sulfurexigens* and strains JA318^T of *Pararhodospirillum oryzae* and DSM122^T of *Pararhodospirillum photometricum* resulted in reassociation values of 52 ± 2 % and 45.1 ± 1 % (n = 5, including reciprocal analyses), respectively, and these hybridization values supported the classification of strain JA318^T as a distinct species (Lakshmi et al. 2014). There are no reports of DNA–DNA hybridization studies involving neither *Rhodospirillum rubrum* nor *Rhodovibrio* sp. DNA–DNA relatedness of 93 % was reported, when *Caenispirillum bisanense* strain K92T was hybridized with strain K93, suggesting that the two strains represent the same genomic species (Yoon et al. 2007a).

Riboprinting and Ribotyping

The use of the riboprinting and ribotyping methods for clustering and characterization of members of the *Rhodospirillaceae* family is scarce. So far, the methods have been used for clustering several strains from few *Azospirillum* species and *Phaeospirillum*. The intraspecific diversity of *Azospirillum amazonense* isolates was studied by Azevedo et al. (2005) and

Reis Junior et al. (2006). Both authors used the intergenic space of 16–23S rDNA as target region, where the applied restriction enzymes allowed a highly resolving diversity analysis. Azevedo et al. (2005) observed a genetic diversity within the *A. amazonense* species and divided the isolates into four clusters with 78 % of similarity using *Hae*III, *Alu*I, *Rsa*I, *Cfo*I, *Msp*I, and *Eco*RI restriction enzymes. Reis Junior et al. (2006) obtained two groups defined at 56 % of similarity using *Alu*I, *Rsa*I, and *Cfo*I for other strains from the same species. Peng et al. (2006) used the IS-PCR fingerprinting to discover a new species of *Azospirillum*, *A. melinis*.

Oda et al. (2002) studied the bacterial community of aquatic sediments using BOX-PCR, RFLP, and 16S rRNA gene sequencing and reported two *Phaeospirillum fulvum* isolates.

MALDI-TOF

The use of the MALDI-TOF method for genotypic characterization of members of the *Rhodospirillaceae* family is scarce. Recently the method was used to differentiate few species from the genus *Azospirillum* (Stets et al. 2013). The authors compared the *A. brasilense*, *A. amazonense*, and *A. lipoferum* species commonly found associated to grasses to validate the discriminatory and identification efficiency of the method; MALDI-TOF MS was proposed to classify also other bacteria isolated from wheat roots. Six strains (Sp7, Sp245, FP2, HM210, SF0, and SF5) of *A. brasilense*, two strains (Y2 and Y6) of *A. amazonense*, and one strain DSM 1691 of *A. lipoferum* were grown in DYGS medium and analyzed in biological triplicates following the same procedures used for wheat isolates. The three *Azospirillum* species were grouped into separated clusters, and the four derivative strains of *A. brasilense* Sp7 and Sp245 also clustered according to their parent strains. It is noteworthy that the replicates always clustered together, but different strains formed distinct branches, distinguishing parent and derivative strains of *A. brasilense*. The technique is still under development; therefore, new data are not available yet.

Rudney et al. (2010) identified peptides assigned to *Phaeospirillum molischianum* when studying the metaproteome of the salivary microbiota by tandem mass spectrometry (MS/MS) followed by a cation exchange step-gradient chromatography linked to a microcapillary reverse-phase liquid chromatography.

Genome Comparison

Azospirillum–Skermanella–Rhodocista–Inquilinus

The most studied strains of the *Azospirillum* genus belong to the *A. brasilense*, *A. lipoferum*, and *A. amazonense* species that have complete or draft genome sequences available. The first data on genome structure of *Azospirillum* was described by Martin-Didonet et al. (2000), and at that time only six species were described. The authors used 10 strains of five *Azospirillum* species: *A. brasilense*, strains Sp7 (ATCC 29145), Cd (ATCC 29710), FP2, and Sp245; *A. lipoferum*, strains Sp59b (ATCC 29707) and JA25; *A. amazonense*, strains Y2 (ATCC 35120) and Y6 (ATCC 35121); *A. irakense*; and *A. halopraeferens*. The results showed the presence of several megareplicons with molecular sizes ranging from 0.2 to 2.7 Mbp as determined by pulsed-field gel electrophoresis (PFGE). The PFGE DNA patterns differed within the same species, which indicates that they are strain specific. In all strains tested, the presence of 16S rDNA was detected in more than one replicon, suggesting that *Azospirillum* contains multiple chromosomes. This assumption was confirmed later on with the genomes of three members of the *Azospirillum–R. centenaria* group available: *Azospirillum* sp. B510 (Kaneko et al. 2010), *A. brasilense* Sp245 (http://genome.ornl.gov/microbial/abra/19sep08/), and *R. centenaria* SW (Lu et al. 2010) detailed below. *Azospirillum* is usually compared to *Rhodocista centenaria* (formerly *Rhodospirillum centenum*), since the latter species possesses multiple chemotaxis operons and is used as a model organism to study chemotaxis (Xie et al. 2010).

Plasmids are present in *A. lipoferum* and *A. brasilense* strains tested over several years. Some of the strains contain as many as six plasmids ranging in size from 4 MDa to over 300 MDa (Elmerich 1983, 1986). A plasmid with a size of 90 Mda is present in all strains of *A. brasilense* and in some of *A. lipoferum* (p90) and shares conserved regions and carries several genes involved in the *A. brasilense*–plant root interaction (Croes et al. 1991; Alexandre and Bally 1999). Another plasmid – also described in detail – is pRhico found in *A. brasilense* Sp7, responsible for the interaction with roots. The *A. amazonense* strain Y2 presents four replicons with the following estimated sizes: 2.7 Mb, 2.2 Mb, 1.7 Mb, and 0.75 Mb (Martin-Didonet et al. 2000).

The *A. brasilense* Sp245 genome carries seven replicons of 3, 1.76, 0.912, 0.778, 0.690, 0.191, and 0.167 Mbp (Wisniewski-Dyé et al. 2011). These genomes encode genes related to nitrogen/carbon metabolism, energy production, phytohormone production, quorum sensing, antibiotic resistance, chemotaxis/motility, and bacteriophytochrome biosynthesis, as well as those involved in nitrogen and carbon fixation.

The genome of *Azospirillum* spp. strain B510, isolated from surface-sterilized stems of rice plants (*Oryza sativa* cv. Nipponbare) in Japan (Xie and Yokota 2005), consists of a single chromosome and six circular plasmids (pAB510a (1, 455, 109 bp), pAB510b (723, 779 bp), pAB510c (681, 723 bp), pAB510d (628, 837 bp), pAB510e (537, 299 bp), and pAB510f (261, 596 bp)) with the total size of 7,599,738 bp with no linear plasmids that are present in *A. brasilense* and *A. lipoferum*. Also *A. lipoferum* has the largest number of chromids (intermediates between chromosomes and plasmids) among all prokaryotes sequenced, indicating a potential for genome plasticity (Wisniewski-Dyé et al. 2011).

One of the most surprising features of the *A. amazonense* Y2 genome sequenced recently (Sant'Anna et al. 2011) is the presence of a gene cluster implicated in carbon fixation (the Calvin–Benson–Bassham cycle). The main genes of this cluster are the genes *cbbL* and *cbbS*, and they encode, respectively, the large and

small subunits of ribulose-1,5-bisphosphate carboxylase (RuBisCO). At least *R. centenaria* (formerly *R. centenum*) and *A. lipoferum* are known to be capable of growing autotrophically by means of RuBisCO in contrast to *Azospirillum* sp. B510 and *A. brasilense* Sp245, which do not contain Form I or II of RuBisCOs ("true" RuBisCOs) encoded in their genomes (Sant'Anna et al. 2011).

In *A. brasilense*, at least three pathways for IAA biosynthesis exist, two tryptophan-dependent pathways (indole-3-acetamide (IAM) pathway and indole-3-pyruvate (IPyA) pathway) and one tryptophan-independent pathway (Steenhoudt and Vanderleyden 2000; Spaepen et al. 2007). Similarly, the genome of *Azospirillum* sp. B510 contains genes responsible for the IAM pathway and three putative plant hormone-related genes encoding tryptophan 2-monooxytenase (*iaaM*) and indole-3-acetaldehyde hydrolase (*iaaH*), which are involved in IAA biosynthesis (Kaneko et al. 2010). However, the *iaaM*, *iaaH*, and *ipdC* genes, related to the IAM or IPyA pathways, were not located in the *A. amazonense* genome, and no *ipdC* homologue and *iaaC* was found in the B510 genome (Kaneko et al. 2010; Sant'Anna et al. 2011), suggesting that another pathway is present in these bacteria. Comparison among genome sequences of *A. brasilense* strains Sp 245 (origin: Brazil), Az39 (origin: Argentina), and CBG497 (origin: Mexico); *A. lipoferum* 4 B (origin: France); *Azospirillum* sp. B510 (origin: Japan); and *A. amazonense* Y2 (origin: Brazil), using BLAST for putative genes involved in IAA biosynthesis, showed that ipdC is present in *A. brasilense*, aromatic aminotransferase hisC1 is absent only in *A. amazonense*, aldehyde dehydrogenase is present only in Sp245 and Az39 genomes and nitrilase is present in Sp245, Az39, and Y2 genomes (Cassán et al. 2013). Further analysis of the genome sequence of *A. amazonense* revealed a gene encoding a protein with about 70 % similarity to nitrilases from plant species, like *Arabidopsis thaliana* and *Zea mays*, which catalyze the conversion of indole 3-acetonitrile to IAA (Kriechbaumer et al. 2007; Vorwerk et al. 2001). Future studies may indicate if this gene is envolved in IAA biosynthesis in *A. amazonense*.

Other features are also described to the *Azospirillum* genus such as chemotaxis/motility (Bible et al. 2008) including flagellum gene distributions (Chang et al. 2007; Sant'Anna et al. 2011), type IV secretion system (Kaneko et al. 2010), quorum sensing (Lerner et al. 2009), transport (TonB-dependent transport), antibiotic resistance (multidrug resistance (MDR) transporters), and several others (Sant'Anna et al. 2011). All three *Azospirillum* species possess three chemotaxis operons that are orthologous to those in *R. centenum*; however, they also have additional chemotaxis operons that are absent from their close aquatic relative (Wisniewski-Dyé et al. 2011).

Two species of the genus *Skermanella*, namely, *Skermanella aerolata* KACC 11604 and *Skermanella stibiiresistens* SB22, are being sequenced, but no sequence is available to date (http://www.ncbi.nlm.nih.gov/bioproject/).

The genome of *Rhodocista centenaria* ATCC 43720 T (formerly *Rhodospirillum centenum* strain SW = ATCC 51521) was sequenced (INSDC ID CP000613.2) (Lu et al. 2010). The genome presents 4,355,548 bp, 4,003 proteins, and 4,102 genes with a DNA G+C content of 70.5 %. The G+C content determined by thermal denaturation method on purified DNA is 69.9 mol% (Kawasaki et al. 1992). Extrachromosomal elements were absent. Genome assembly presents a single circular chromosome containing 35 pseudogenes, 4,003 protein coding genes, and 64 genes coding for RNA (11 rRNA genes, 52 tRNA genes, and 1 miscellaneous RNA gene). Nitrogenase reductase genes include *nif*, *mod*, and *fix* clusters for nitrogenase biosynthesis, molybdenium transport, and electron transport respectively. The absence of nitrogenase enzymes DRAT and DRAG, which mediated posttranslational regulation of nitrogenase Fe protein, suggests different environmental requirements for nitrogen fixation by *R. centenaria*. Two forms of RuBisCO (subtypes IAq and IC) and a gene encoding PEPC reveal superior ability for carbon fixation. Two genes encoding bacteriophytochrome are present (*ppr* and RC1_3803), and two genes coding for flavin-binding photoreceptors (RC1_2193 and RC1_0351) are suggested to play a role in controlling the bacterial metabolism in response of light. The sequences of the four 16S rRNA gene copies in the genome of *R. centenaria* SW are identical. A total of 72 flagella genes distributed in five gene clusters were identified to accomplish for the dual flagella system of *R. centenaria*: a constitutive polar flagellum and an inducible lateral flagellum.

The first draft assembly genome sequence of *Inquilinus limosus* (INSDC AUHM00000000) was reported by Pino et al. (2012). This genome was 7,413,714 bp long with a 69.87 % GC content and contained 7,081 predicted genes with 6,998 protein coding genes and 83 RNA genes. Besides genes with unknown function (610; 9.34 %), the highest number of genes are involved in amino acid transport and metabolism (857; 13.12 %), followed by genes coding for general function prediction only (848; 12.98 %), transcription (627, 9.60 %), carbohydrate transport and metabolism (705, 10.79 %), and inorganic ion transport and metabolism (408; 6.25 %). Other 199 genes (3.05 %) were found to code for secondary metabolite biosynthesis, transport, and catabolism, and 85 (1.30 %) were found related to defense mechanisms. According to Pino et al. (2012) 89 genes are likely involved in susceptibility or resistance to antibiotics and toxic compounds. Up to 19 could be coded for multidrug resistance efflux pumps while 21 for different classes of β-lactam recognizing proteins as penicillin-binding proteins. From them, four are homologous to β-lactamases deposited in databases. No transposable elements or pathogenicity islands could be detected.

Magnetospirillum–Phaeospirillum–Oceanibaculum–Thalassobaculum–Nisaea–Fodinicurvata–Tistlia–Tistrella–Rhodovibrio

Complete genome sequences of magnetotactic spirilla, *Magnetospira* sp. QH-2 and *Magnetospirillum* sp. (*Magnetospirillum magneticum*) AMB-1, from marine and freshwater environment, respectively, raised very important data

Table 22.1

Morphological, physiological and molecular differentiating characteristics among the genera of the family *Rhodospirillaceae*

	Azospirillum	*Desertibacter*	*Dongia*
Morphology	Straight or slightly curved rods	Rods	Slightly curved to straight rods
Gram-stain	Negative	Negative	Negative
Motility	+	+	+
Metabolism	Aero- and microaerobic	Strictly aerobic	Strictly aerobic and heterotrophic
Nitrogen fixation	+	Negative	Negative
Nitrate reduction	+	+	+
Range for growth			
Temperature (°C)	5–42 optimum 30	47–40	30–35
pH	5–9 optimum 7.0	7–10	7.0–7.5
Major fatty acids	18:1ω7c, 16:1ω7c, 16:0	C18:1 ω7c, C16:0, C18:0	C18:1v7c, C19:0 cyclo v8c, C16:0 and C16:0 2-OH
Ubiquinone type	Q-10	Q-10	Q-10
Genome size	6.8–7.6		
G+C content	64–69.6	71.4	65.6
Habitat	Mainly rhizosphere soil of several plants specially members of *Poaceae* family	Sand soil	Reactor
Additional characteristics	Denitrification variable		

	Constrictibacter	*Defluviicoccus*	*Fodinicurvata*	*Limimonas*	*Magnetospirillum*	*Nisaea*
Morphology	Ovoid to rods	Cocci/coccobacilli in tetrads and clusters	Vibrioid and rod-shape	Rod	Helical (clockwise) spirilla	Rod
Gram-stain	Negative	Negative	Negative	Negative	Negative	Negative
Motility	+	–	–	–	+	+
Metabolism	Aerobically or micro-aerobically	Chemoheterotrophic	Facultative anaerobic	Strictly aerobic	Microaerobic	Facultative anaerobic, microaerophilic
Nitrogen fixation	Negative		Negative	nd	+, variable	nd
Nitrate reduction	+	+	+	–	+	Variable
Range for growth						
Temperature (°C)	5.0–30.0	20–30	15–42	30–50	20–45	15–44
pH	6.0–8.0	5.0–8.5	6.5–8.5	6.0–8.0	6.5–6.9	5.0–9.0
Major fatty acids	C18:1, C16:0, C18:0	nd	C18:1v7c, C18:1 2-OH and C16:0	$C_{19:0}$ cyclo ω7*c* and $C_{18:0}$	$C_{18:1}\omega7$, $C_{16:0}$	$C_{18:1}\omega7$, $C_{16:1}\omega7$
Ubiquinone type	Q-10	nd	Q-10	Q-10	Q-10	Q-10
Genome size				nd	4.1–9.21	4.63
G+C content	69.8	66	61.5–62.3	67.0	62.0–71.0	60.0
Habitat	A gap of white rock	Sludge	Salt mine	Saline muds	Chemically stratified water and sediments of freshwater, brackish, marine, and hypersaline habitats	Marine
Other important characteristics			Accumulation of Fe(III) oxides on the cell surface	Extremely halophilic. Growth occurs at 2.5–5.2 M NaCl (optimum 3.4 M = 23.8 %)	Magnetotaxis, Magnetosome production variable	Tolerates salinity up to 6 % (optimum growth at 2 %)

Elstera	*Inquilinus*	*Rhodocista*	*Skermanella*
Rods	Rods	Vibrioid to spiral	Rod-shaped, with rounded or tapered end, and a straight or slightly curved axis
Negative	Negative	Negative	Negative
–	Variable	+	+
Chemotrophic	Strictly aerobic; chemo-organotrophic	Anaerobic/aerobic	Aerobic, chemo-organotroph
No	Negative	+	–
–	–		Variable
20–25	15–30	25–44	5–35
6.5–7.0		6.5–8	4–9
18:1ω7c, 18:1 2-OH and 16:0	18:1W7c, 18:1-2OH, 18:0-3OH		C18:1 ω7c and C16:0
Q-10	Q-10	Q-9	Q-10
		4.36	nd
58.5–62.5	70.3–70.9	68.8–69.9	65–68.8
Biofilm on stone	Respiratory Secretions	Water sample at the edge of a thermophilic hot spring/wastewater treatment plant	Fresh water, soil, air
Accumulation of Fe(III) oxides on the cell surface		Cells are converted to cysts under aerobic incubation. Anaerobically grown colonies are pink	NaCl concentration 0–5 %

Oceanibaculum	*Pelagibius*	*Phaeospirillum*	*Rhodovibrio*	*Telmatospirillum*	*Thalassobaculum*	*Tistlia*	*Tistrella*
Rod	Slightly curved or straight rods	Vibrioid to spiral-shaped	Vibrioid to spiral shaped cells	Vibrioid to spiral shaped	Slightly curved and rod	Slightly curved to straight rods	Rods
Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
+	+	+	+	+	+	+	+
Aerobic	Strictly aerobic	Anaerobic/ microaerobic	Photoheterotrophic and anaerobic, chemotrophic and microarobic to aerobic	Chemo-organotroph under anoxic or microoxic conditions/ chemolithotrophic (H_2 oxidation) under microoxic conditions	Aero- and facultative anaerobic	Aerobic	Aerobic
nd	–	+	–(?)	+	–	+	–
Variable	+		?	nd	Variable	+	+
10–42	15–33	25–35	20–47	4–30	15–37	20.0–40.0	20.0–40.0
6.0–11.0	6–11	5–9	7–8	4–7	5.0–10.0	5.0–8.0	5.0–9.0
$C_{16:1}$ω7c, $C_{16:0}$	C18:1ω7c, C18:0 3-OH, C19:0 cyclo ω8c	C18:1ω7c, C16:0, C16:1 ω6c/C16:1 ω7c	$C_{18:1}$, $C_{18:0}$, $C_{16:0}$	C18:1 ω7c, 17:0 cyclopropane and C16:0	C18:1ω7c; C16:0	C19:0ω8c; C18:1ω7c; C18:0	C18:1ω7c
nd	Q-10	Q-9, MK-9	Q-10, MK-10	nd	Q-10	Q-10	Q-10
3.95		3.79–3.81	nd	nd			
64.8–67.7	66.3	60.5–64.8	66–67	61.6–64	65.0–68.0	71.0	65.8–67.5
Marine, Sediments of hydrothermal field	Coastal seawater	Freshwater, mud and rhizosphere soil	Anoxic zones of saline or hypersaline environments such as sea water, brines and salt lakes that are exposed to the light	Marsh water	Seawater	Water	Wastewater and soil
Moderately halophilic. Tolerates salinity up to 9 % (optimum varies among species)	Non-fermentative heterotroph; Salt required for growth	Non-fermentative. Fully pigmented under photosynthetic conditions; photoautotrophic	Cells contain internal photosynthetic membranes as vesicles. Photosynthetic pigments are bacteriochlorophyll a (esterified with phytol) and carotenoids of the spirilloxanthin series with spirilloxanthin as the major component. Photoheterotrophs under anaerobiosis in the light, or chemotrophs under microoxic to oxic conditions in the dark; complex nutrients are required	NaCl concentration up to 0.58 %	Halophilic	Slightly halophilic	

Table 22.1 (continued)

	Caenispirillum	*Ferrovibrio*	*Insolitispirillum*	*Magnetospira*	*Magnetovibrio*	*Marispirillum*
Morphology	Helical	Short and thin vibrios	Helical	Variable, truncated or fully helical spirillum	Vibrioid to helical	Helical
Gram-stain	Negative	Negative	Negative	Negative	Negative	Negative
Motility	+	+	+	+	+	+
Metabolism	Chemoheterotrophic; aerobic	Facultative anaerobes	Aerobic	Obligately microaerophilic	Facultative anaerobic, microaerophilic	Facultative anaerobic
Nitrogen fixation	Negative		+	+	+	nd
Nitrate reduction	+	+	−	+	+	+
Range for growth						
Temperature for growth (°C)	15–47	5–45	12–39	5–37	4–31	10–41
pH	6.5–10	5.5–8.0	5.0–8.0	6.9–7.1	7.0–7.5	5.0–10.0
Major fatty acids	$C_{18:1}$ ω7c	18:1x7c, 19:0 cyc and 16:0	$C_{18:1}\omega7c$, $C_{16:0}/C_{18:1}$ 2-OH	$C_{18:1}\omega7$, $C_{16:1}\omega7$	$C_{18:1}\omega7$, $C_{16:1}\omega7$	$C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH
Ubiquinone type	Q-10	Q-10	Q-9	nd	nd	nd
Genome size			nd	nd	3.7	nd
G+C content	70	64.2	62.0–66.0	47.2	52.9–53.5	
Habitat	Sludge	Iron–sulfide mineral spring	Freshwater, Oxidation ponds	Marine	Sediments	Marine
Other important characteristics		Accumulation of Fe(III) oxides on the cell surface	Coccoid non-motile organisms and microcysts forms differentiate subspecies	Polar magnetotaxis	Axial and polar magnetotaxis	Moderately halophilic. Tolerates salinity up to 12 % (optimally at 2–8 %)

Novispirillum	*Phaeovibrio*	*Rhodospira*	*Rhodospirillum*	*Pararhodospirillum*	*Roseospira*	*Thalassospira*
Spirilla, helical and coccoid	Vibrioid	Vibrioid to spirilloid	Vibrioid to spiral shaped cells	Spiral shaped cells	Vibrioid to spiral shaped	Vibrioid to spiral
Negative	Negative	Negative	Negative	Negative	Negative	Negative
+	+	+	+	+	Variable	Variable
Aerobic	Anaerobic	Anaerobic/microaerobic	Photoheterotrophic and anaerobic, photoautotrophic, chemotrophic and microaerobic to aerobic, fermentative	Phototrophic anaerobes	Photo-organotroph in anoxic conditions	Aero- and anaerobic
+	−		+	+/−	+ or −	−
+			−	nd	−	+
15–43	20–40	20–35	30–35	25–40	30–35	4.0–40.0
5.0–9.0	7–8	7–7.8	6.8–7.0	6–8	5.3–8.5	4.5–11.0
$C_{18:1}\omega7c$, $C_{16:0}$/$C_{18:1}$ 2-OH	$C_{18:1}\omega7c$ and $C_{16:0}$	$C_{18:1}$, $C_{16:0}$ and $C_{14:0}$	$C_{18:1}\omega7c$/C18:1ω6c, C16:0, C16:1ω7c/ C16:1ω6c, and also three hydroxyl acids of C14 and C16	C16:0, $C_{18:1}\omega7c$/ C18:1ω6c, and three hydroxy acids of C14, C15 and C16	nd	C16:0
Q-10		Q-7, MK-7 and RQ-7	Q-10, RQ-10	Q-8, RQ-8	nd	Q-10
4.29			4.35	3.8	nd	
63.0–65.0	67.8–68.8 mol%	65.7	63.8–65.8	60–65.8	66.6–72.3	47.0–54.7
Freshwater	Brackish water	Salt marsh	Freshwaters	Freshwaters, exceptionally in rhizosphere	Anoxic marine sediments; microbial mats in coastal waters; marine environments	Saline soil, marine environment, plant
Sensitive to 3.0 % NaCl, produces water-soluble yellowish green fluorescent or brown pigment, the last in presence of tyrosine and tryptophan	Non-fermentative. Brown pigmented under photoautotrophic growth	Anaerobically grown colonies are beige to peach-colored	Cells contain internal photosynthetic membranes as vesicles. Photosynthetic pigments are bacteriochlorophyll a (esterified with phytol or geranylgeraniol) and carotenoids of the spirilloxanthin series. Photoheterotrophs under anaerobiosis in the light, or photoautotrophs with molecular hydrogen or sulfide as electron donors, or chemotrophs under microoxic to oxic conditions in the dark, or fermentatives and oxidant-dependent	Cells contain internal photosynthetic membranes as lamellar stacks forming a sharp angle to the cytoplasmic membrane. Photosynthetic pigments are bacteriochlorophyll a and carotenoids lycopene and rhodopin. Strict anaerobes and obligate phototrophs with preference for neutral pH	NaCl concentration −0.2–15 %	Halophilic; growth up to 10 % NaCl

about peculiarities associated with their gene structural organization, ecosystem origin, and adaptative evolution (Matsunaga et al. 2005; Richter et al. 2007; Ji et al. 2013). A 130 kb region representing a putative genomic "magnetosome island" (MAI) of *M. gryphiswaldense* was shown to undergo frequent transposition and subsequent deletion under physiological stress conditions (Ullrich et al. 2005). In this region a great abundance of multiple copies of transposase genes that belongs to different families of IS elements was observed, suggesting that these mobile genetic elements play a major role in driving the hypervariability shown by the organizational differences in spontaneous magnetosome mutants obtained upon subculture in the laboratory. Comparison of magnetosome gene organization among some MTB of *Rhodospirillaceae* revealed that distinct variations in gene order and sequence similarity, as well as copy numbers, are present in the MAI of the MTB. Based on these data and on a detailed comparison study including MTB of other classes, it has been considered that at least a set of *mam* genes (*mamH, I, E, K, M, O, P, A, Q, L, B, S, T, C, D, Z*, and *X*), *mms6* and *mmsF* are universally shared by the MAI on MTB of the *Rhodospirillaceae* family (Richter et al. 2007; Lefèvre et al. 2013b). Genes for nitrogen fixation and assimilatory nitrate respiration are well conserved among freshwater magnetospirilla, but absent from the *Magnetospira* sp. QH-2 genome. As observed in the QH-2 genome by gene cluster synteny and gene correlation analyses, the insertion of the magnetosome island probably occurred after divergence between freshwater and marine magnetospirilla. The presence of a sodium-quinone reductase, sodium transporters, and other functional genes is evidence of the adaptive evolution of *Magnetospira* sp. QH-2 to the marine ecosystem. In contrast, marine *Magnetospira* sp. QH-2 neither has TonB and TonB-dependent receptors nor does it grow on trace amounts of iron (Ji et al. 2013). Further draft genome comparison of other *Magnetospirillum* spp. revealed that the genome size varies from 4,2 Mbp to 4,9 Mbp and annotated ORFs from 3,878 to 4,925 (Dzyuba et al. 2012). The presence of plasmid is indicated to *M. gryphiswaldense* type strain, and a 3.7-kb cryptic plasmid designated pMGT was found in *M. magneticum* MGT-1 (Bertani et al. 2001; Okamura et al. 2003).

The genome of *Phaeospirillum molischianum* strain DSM 120^T was sequenced (INSDC ID CAHP00000000.1) (Duquesne et al. 2012). The genome presents 3,805,617 bp, 3,803 protein sequences, and 3,888 genes with a G+C content of 61.5 %. The G+C content determined as buoyant density and by thermal denaturation was in this range (60.5–64.8 mol%) (Imhoff et al. 1998). Extrachromosomal elements were absent. The genome assembly presents 61 contigs (sizes of 522–416,194 bp), 11 pseudogenes, 3,803 protein coding genes, and 62 genes coding for RNA (5 rRNA genes, 49 tRNA genes, and 8 other miscellaneous RNA genes). Proteins involved in signal transduction include 60 histidine kinase-type sensors and 65 response regulators (*LuxR, Fis, CheY*, and *OmpR* families), which are believed to play important roles in the adaptability of *P. molischianum* to environmental changes. Nitrogenase reductase genes include two types of nitrogenases, a Mo-Fe-dependent nitrogenase and an alternative Fe-Fe nitrogenase. In addition, 5.89 % of the overall genome corresponds to repetitive sequences, and a total of 81 transposases were predicted. The genome of the *Phaeospirillum fulvum* strain MGU-K5, isolated from a lake mud in Khabarovsk (Russia), was fully sequenced, and the automatic annotation is available (AQPH00000000.1 at NCBI). The genome presents 3,789,403 bp, 3,462 proteins, and 3,510 genes with a DNA G+C content of 63.9 %. Nitrogenase reductase genes, including molybdenium–iron and vanadium–iron nitrogenase subunits, are present in the genome. A high number of proteins involved in signal transduction (including ~49 histidine kinase-type sensors), response regulators (including approximately 2 *LuxR*, 1 *Fis*, and 21 *CheY* families), and cell detoxification (including approximately 12 multidrug efflux pumps, 4 heavy metal efflux pumps, and 8 RND-related efflux transporters) are present.

Oceanibaculum indicum P24^T draft genome contains 3,952,792 bp corresponding to a total of 3,755 protein-coding and 45 tRNA genes. According to Lai and Shao (2012a), the proteins associated with amino acid transport and metabolism (COG initial, E) were the most abundant COG group (415 open reading frames (ORFs), 14.4 %), followed by the ones associated with inorganic ion transport and metabolism (P; 244 ORFs, 8.5 %) and transcription (K;216 ORFs, 7.4 %).

One ongoing draft genome sequencing project on *Thalassobaculum salexigens* strain DSM 19539 is available at the public GenBank (NCBI) with a total length of 5.08 Mb and a GC content of 67.4 %. No more information about the proteins and ribosomal genes is available.

The *Nisaea denitrificans* DR41_21^T genome sequenced (GenBank accession number AUFM00000000) by DOE Joint Genome Institute is represented by 20 scaffolds covering 4626718 bases; of this total 91.53 % are coding DNA (98.62 % of protein coding, 1.38 % of RNA, and 0.67 % of pseudogenes coding, that is not additive under total gene count since it could be counted as protein coding or RNA genes). Another draft genome related to this genus is that of *Nisaea* sp. BAL199 (GenBank accession number ABHC00000000), by J. Craig Venter Institute, that consists of a total of 69 DNA scaffolds covering 6102701 bases that represent 90.10 % of total DNA coding bases, and a total of 6182 genes (99.13 % protein coding and 0.87 % RNA coding). Up to date, although publically available at NCBI, no further details about these genomes have been published elsewhere.

Fodinicurvata sediminis DSM 21159 (YIM D82T) genome (INSDC ATVH00000000.1), analyzed in the course of the Genomic Encyclopedia of Bacteria and Archaea, showed 3,690,548 bp long with 60.63 % GC content. This value is similar to those determined by T_m and nuclease method performed on purified DNA (61 mol%, ❯ *Table 22.1*). Besides 28 pseudogenes, 3,551 genes have been predicted, of which 3,490 were protein-coding genes and 61 were RNA genes. The distribution of genes into clusters of orthologous groups (COGs) functional categories indicates that the highest number of genes is involved in amino acid transport and metabolism (435; 13.42 %), followed by genes coding for general function prediction only (403; 12.43 %), transcription (215, 6.63 %), energy production and conversion (176; 5.43 %),

and carbohydrate transport and metabolism (175, 5.4 %). A total of 141 genes (4.35 %) were found to code for secondary metabolite biosynthesis, transport, and catabolism, and 35 (1.08 %) were found related to defense mechanisms.

The genome of *Tistlia consotensis* was sequenced using the Roche GS FLX + System allowing for long sequence reads (Rubiano-Labrador et al. 2014). After assembling the 171,055 reads, 2,377 contigs were obtained comprising a total of 5,701,113 bp sequence. The high sequence coverage obtained (10.4×) indicates that the genome size should be slightly above 5.7 Mbp. Based on the nucleotide sequences, the G+C mole percentage was estimated at 70.4 %. Remarkably, this value is among the highest G+C ratio known for *Alphaproteobacteria*, but the GC content between 60 and 70 mol% is consistent with that reported to halophilic and halotolerant microorganisms in *Rhodospirillaceae* family (Rubiano-Labrador et al. 2014). A high GC content (above 60 %), as found in *T. consotensis*, could be a common feature in halophilic microorganisms, since it is correlated with the abundance of acidic residues, especially Asp, in halophilic proteins. The acidic nature of the proteins contributes to adaptation at high salt concentrations.

The complete genome sequence of *Tistrella mobilis* strain KA081020-065 totaling 6,513,401 bp was established by 454 pyrosequencing to reveal five replicons comprising a 3,919,492-bp circular chromosome and four circular plasmids ranging in size from 83,885 bp (pTM4) to 1,126,962 bp (pTM3); it has also a high average G+C content of 68 % (Xu et al. 2012). Its genome organization with three megaplasmids greater than 600 kb each is reminiscent of several other α-proteobacteria members such as the rice plant endophyte *Azospirillum* sp. B510 that harbors six plasmids, five of which are in excess of 500 kb.

One ongoing draft genome sequencing project on *Rhodovibrio salinarum* strain DSM 9154 is available at the public GenBank (NCBI) with a total length of 4.18 Mb and a GC content of 64 %. No more information about the proteins and ribosomal genes is available.

Rhodospirillum–Pararhodospirillum–Novispirillum–Caenispirillum–Thalassospira

The *Rhodospirillum rubrum* genome consists of a 4,352,825-bp-long chromosome with 65 % G+C content and a 53,732-bp plasmid with 60 % G+C content (Munk et al. 2011). A total of 3,850 protein-coding genes and 83 RNA genes were predicted, including four copies of rrn operon. The *Pararhodospirillum photometricum* genome consists of a 3,876,289-bp chromosome, which encodes 3,281 proteins and 3 rRNAs (Duquesne and Sturgis 2012).

Novispirillum itersonii subsp. *itersonii* ATCC 12639 draft genome (GenBank accession number ARMX00000000) corresponds to 34 scaffolds, including Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR), covering a total of 4290651 number of bases; of this total only 88.58 % was assigned as base coding for proteins (98.13 %) and RNA (1.87 %) genes.

A draft genome sequence of *Caenispirillum salinarum* AK4T (INSDC ANHY00000000) is available and consisted of 61 contigs of 4,952,365 bp. A total of 4,574 coding regions were found in the genome, where 3,092 (67.8 %) were functionally annotated. The number of genes transcribed from positive strands was 2,276 and from negative strands were 2,327. Besides genes with unknown function (401; 10.11 %), the distribution of genes into clusters of orthologous groups (COGs) functional categories indicates that the highest number of genes is involved only in general function prediction (458; 11.55 %), followed by genes coding for amino acid transport and metabolism (353; 8.9 %) and energy production and conversion and signal transduction mechanisms (both 295; 7.44 %). A total of 124 genes (3.13 %) were found to code for secondary metabolite biosynthesis, transport, and catabolism, and 47 (1.19 %) were found related to defense mechanisms. In agreement with Khatri et al. (2013), the functional comparison of genome sequences available on the RAST server revealed the closest neighbors of *Caenispirillum salinarum* to be *Rhodospirillum rubrum* ATCC 11170 (score 533) followed by *Magnetospirillum magneticum* AMB-1 (score 520), *Rhodospirillum rubrum* (score 490), *Magnetospirillum gryphiswaldense* MSR-1 (score 472), and *Alphaproteobacterium* BAL199 (score 318).

The genome of *Thalassospira profundimaris* WP0211^T was sequenced and contains 4,040 candidate protein-encoding genes (with an average size of 958 bp), giving a coding intensity of 88.4 % (Lai and Shao 2012b). A total of 3,157 proteins could be assigned to clusters of orthologous groups (COG) families. Forty-three tRNA genes for 18 amino acids (lacking Asn and Lys) were identified. The proteins associated with amino acid transport and metabolism (COG initial, E) were the most abundant group among the COGs (412 open reading frames (ORFs), 13.1 %), followed by the proteins associated with transcription (K; 360 ORFs, 11.4 %) and inorganic ion transport and metabolism (P; 263 ORFs, 8.3 %).

Phenotypic Analyses

The main features of the members of *Rhodospirillaceae* are listed in ❯ *Table 22.1*.

Azospirillum Tarrand et al. 1979 (1980), Emend. Falk et al. 1985

A.zo.spi.ril'lum. French noun azote, nitrogen; Greek noun spira, a spiral; spillum, a small spiral; *Azospirillum*, a small nitrogen spiral.

Bacteria belonging to the genus *Azospirillum* are Gram negative, aerobic, curved, or slightly curved rods, spiral with diameter varying from 0.6 to 1.5 μm, and lengths vary with the species and culture medium from 1.0 to 7.0 μm. Motile by a single polar flagellum and lateral several smaller ones, shorter in length. Generally contains granules of poly-β-hydroxybutyrate (PHB). Oxidase, catalase, and urease activity is present in some species. Also nitrate–nitrite and denitrification is present in some species but not all three pathways together and a single species,

Table 22.2

Morphological, physiological and molecular characteristics differentiating species within genus *Azospirillum*

	Azospirillum lipoferum Sp. 59b^T = ATCC 29707^T	*Azospirillum brasilense* Sp7^T = ATCC 29145^T	*Azospirillum amazonense* Am 14 (=Y1^T) = ATCC 35119	*Azospirillum halopraeferens* Au 4 = ATCC 43709^T	*Azospirillum irakense* KBC1^T = ATCC 51182^T	*Azospirillum largimobile* ACM 2041^T	*Azospirillum doebereinerae* GSF71^T = DSM 13131^T	*Azospirillum oryzae* COC8^T = CCTCC AB204051^T
Morphology	Rod, sometimes spiral	Rod, sometimes spiral	Rod, sometimes spiral	Vibroid, S-shape	Rod, sometimes spiral	Rod, curved axis	Rod or S-shape	Spiral or vibroid
Gram-stain	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Cell size (μm)	1.0 × 1.5	1.0 × 1.2	0.8 × 1.0	0.7–1.4 × 2.4–5.0	0.6–0.9	0.7–1.5 × 1.5–4.5	1.0–1.5 × 2.0–3.0	1.0–1.0 × 5.0–5.0
Pleomorphic cells	+	–	+	+	+	+	+	nd
Flagellum	1, polar	1, polar	1, polar	1, polar	1, polar	nd	1, polar	1, polar
Lateral flagella	+	+	–	–	+	–	–	–
Motility	+	+	+	+	+	+	+	+
Metobolism	Aerobic	Aerobic	Aerobic	Aerobic	Aerobic	Aerobic	Aerobic	Aerobic
N_2 fixation	+	+	+	+	+	+	+	+
Detection *nifD* and/or *nifH*	+	+	+	+	+	+	+	+
Nitrate reductase	+	+	v	+	v	+	+	+
Temperature for growth (°C):								
Range	nd	nd	nd	nd	30–33	10–37	nd	nd
Optimum	37	37	35	41	33	28	30	30
pH for growth:								
Range	5.7–6.8	6.0–8.0	nd	6.0–8.0	5.5–8.5	nd	6.0–7.0	6.0–7.0
Optimum	nd	nd	nd	7.2	nd	nd	nd	nd
PHB accumulation	+	+	+	+	+	+	+	+
Oxidase	–	nd	nd	+	+	+	+	+
Catalase	–	nd	nd	+	nd	+	+	nd
Urease	nd	nd	nd	nd	+	+	+	nd
Nitrite reduction	nd	nd	nd	nd	nd	nd	nd	nd
Growth with 3% NaCl	–	v	–	+	+	–	–	–
Denitrification	+	+	–	+	v	–	+	–
Biotin requirement	+	–	–	+	–	–	–	+
N-acetylglucosamine	+	+	v	nd	+	+	–	nd
D-galactose	+	v	+	–	+	+	v	+
myo-inositol	v (+)	–	+	nd	–	–	nd	–
L-rhamnose	–	–	v	nd	+	–	nd	+
L-arabinose	+	v	+	v	+	+	+	+
Malate	+	+	+	+	+	+	+	+
Sucrose	–	–	+	–	+	nd	–	–
D-glucose	+	v	+	–	–	+	v	+
D-fructose	+	+	+	+	v	+	+	+
D-mannitol	+	–	–	–	–	+	+	–
Glicerol	+	+	–	+	nd	+	+	nd
D-sorbitol	+	–	–	–	–	+	+	–
Indol production	+	+	+	+	+	+	+	nd

Azospirillum melinis TMCY 0552^T	*Azospirillum canadense* DS2^T = LMG 23617^T	*Azospirillum zeae* N7^T = LMG 23989^T	*Azospirillum rugosum* IMMIB AFH-6^T = CCUG 53966^T	*Azospirillum picis* IMMIB TAR-3^T = CCUG 55431^T	*Azospirillum palatum* ww 10^T = LMG 24444^T	*Azospirillum thiophilum* BV-S^T = DSM 21654	*Azospirillum formosense* CC-Nfb-7^T = BCRC 80273^T	*Azospirillum humicireducens* SgZ-5T = CCTCC AB 2012021^T	*Azospirillum fermentarium* CC-LY723^T = BCRC 80505^T
Rod, sometimes spiral	Short rods	Rod, sometimes spiral	Rods, slighty curved	Rods, slighty curved	Rod	Spiral, curved rods	Rods, slighty curved	Spiral, curved rods	Spiral, curved rods
Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
0.7–0.8 × 1.0–1.5	0.9–1.8 × 2.5	0.9–1.5 × 1.9–6.8	nd	nd	0.6–1.0 × 2.0–2.6	1.1–2.0 × 3.6–7.0	0.8 × 2.5	1.2–1.8 × 2.5–5.5	1.0–1.2 × 2.4–2.6
–	nd	nd		nd	nd	nd	nd	nd	nd
–	1, polar	1, polar	1, polar	1, polar	Peritrichous	nd	1, polar	1, polar	1, polar
–	–		–	–	+	nd	nd	nd	nd
–	+	+	+	+	+	+	+	+	+
Facultative anaerobic	Aerobic	Aerobic	Aerobic	Aerobic	Aerobic	Mixotrophic–anaerobic growth with nitrate	Aerobic	Facultative anaerobic	Aerobic
+	+	+	+	+	–	+	+	+	+
+	*nif H*	+		*nif H*	–	nd	*nif H*	*nif H*	
+	+	+	+	+	–	+	+	+	+
5–37	20–37	20–41	22–37	22–37	15–42	15–40	20–37	25–37	20–37
20–33	25–30	30	30	37	30–37	37	30	30	30
4.0–8.0	nd	5.0–7.0	nd	6.5–9.0	5.0–9.0	6.5–8.5	5.0–9.0	5.5–8.5	6.0–8.0
nd	5.0–7.0	5.3–8.0	nd	nd	6.0–8.0	7.5	7.0	7.2	7.0
+	+	+	+	+	+	+	+	+	+
nd	+	nd	+	+	w	+	–	+	+
nd	+	+	+	+	+	+		+	+
nd	nd	+	nd	+	+	–		nd	nd
nd	nd	–	nd		–	nd	nd	–	+
+	–	–	–	–	–	+	–	+	+
nd	nd	+	nd	nd	nd	–	nd	–	nd
–	–	nd	nd		nd	nd	–	–	–
+	–	v	–	+	v	–	–	+	+
+	–	+	–	+	–	+	+	–	+
+	–	–	–	nd	–	–	–	+	+
+	–	–	–	nd	–	–	–	+	+
+	–	+	–	+	v	+	+	+	–
+	+	+	+	+	+	+	+	+	+
–	–	–	–	–	+	+	–	+	+
+	–	v	+	+	+	+	+	+	–
+	–	+	–	nd	+	+	+	+	–
+	–	+	–	+	+	+	–	+	–
+	–	+	+	nd	–	+	+	+	–
+	–	+	–	+	–	+	+	+	+
nd	–	+	–	–	–	–			+

Table 22.2 (continued)

	Azospirillum lipoferum Sp. 59b^T = ATCC 29707^T	*Azospirillum brasilense* Sp7^T = ATCC 29145^T	*Azospirillum amazonense* Am 14 (=Y1^T) = ATCC 35119	*Azospirillum halopraeferens* Au 4 = ATCC 43709^T	*Azospirillum irakense* KBC1^T = ATCC 51182^T	*Azospirillum largimobile* ACM 2041^T	*Azospirillum doebereinerae* GSF71^T = DSM 13131^T	*Azospirillum oryzae* COC8^T = CCTCC AB204051^T
Predominant cellular fatty acids – Genus *Azospirillum* has 18:1ω7c, 16:1ω7c, 16:0 as a major component.	C18:1 ω7c or C18:1 ω6c (60.71%), C16:1 ω7c or C16:1 ω6c (4.61%), C14:0 3–OH and/or iso-16:1 (3.62%), C 16:0 3-OH (2.07%) C18:1 2-OH (0.30%)	C18:1 ω7c or C18:1 ω6c (60.2%), C16:1 ω7c or C16:1 ω6c (13.1%), C14:0 3-OH and/or iso-16:1 (4.8%), C 16:0 (4.1%). C 16:0 3-OH (3.7%) C18:1 2-OH (5.9%)	nd	nd	nd	nd	nd	C18:1 ω7c or C18:1 ω6c (64.04%), C16:1 ω7c or C16:1 ω6c (8.54%), C14:0 3-OH and/or iso-16:1 (4.44%), C 16:0 (7.32%), C 16:0 3-OH (2.64%) C18:1 2-OH (5.35%)
Predominant ubiquinone	Q–10	Q–10	Q–10	Q–10	Q–10	Q–10	Q–10	Q–10
Genome size	Genome sequenced of strain 4B (isolated from rice–France)– Genome size 6.8 Mbp and seven replicons	Genome sequenced of strain Sp 245 (isolated from wheat–Brazil). Genome size of 7.5 Mbp and seven replicons. Strain CBG497 genome size of 6.5 Mbp and six replicons	Draft genome of Y2T 0f 7.0 Mbp and four replicons	nd	nd	nd	nd	* strain close to type strain COC8– Genome size of 7.6 Mbp and six replicons
DNA G+C (mol%)	69–70	69–71	66–68	70	64–67	70	69	66.8
Habitat	Roots, stem and leaves-several plants	Roots, stem and leaves-several plants	Roots, stem and leaves-several plants	Saline soils and rhizosphereof Kallar grass	Rhizosphere	Water	Roots, stem and leaves-several plants	Rice (*Oryza sativa*) roots

Azospirillum melinis TMCY 0552^T	*Azospirillum canadense* DS2^T = LMG 23617^T	*Azospirillum zeae* N7^T = LMG 23989^T	*Azospirillum rugosum* IMMIB AFH-6^T = CCUG 53966^T	*Azospirillum picis* IMMIB TAR-3^T = CCUG 55431^T	*Azospirillum palatum* ww 10^T = LMG 24444^T	*Azospirillum thiophilum* BV-S^T = DSM 21654	*Azospirillum formosense* CC-Nfb-7^T = BCRC 80273^T	*Azospirillum humicireducens* SgZ-5T = CCTCC AB 2012021^T	*Azospirillum fermentarium* CC-LY723^T = BCRC 80505^T
nd	18:1ω7c (54.9%), 16:0 (12.3%), Summed feature 3 (12%).	C18:1 ω7c (54.4%), C 16:0 (5.6%), 16:0 3-OH (4.3%), Summed feature 3 (14.4%) and summed feature 2 (5.6%)	C18:1ω7c (39.61%), C16:0 (12.40%), C14:0 3-OH (5.6%), C16:0 3-OH (3.2%)	C18:1ω7c (57.86 %), C16:1ω7c (6.94 %), C16:0 (19.48 %), C14:0 3-OH (4.21 %), C16 3-OH (47.42 %) and C18:0 3-OH (14.07 %), C14:0 3-OH (4.21 %), C16 3-OH (47.42 %) and C18:0 3-OH (14.07 %)	C18:1ω7c (35.9%), C16:0 (13.0%), cyclo-C19:0 ω8c (32.1%)	C18:1 ω7c or C18:1 ω6c (60.65%), C16:1 ω7c or C16:1 ω6c (14.79%), C 16:0 (7.61%), C14:0 3-OH and/or iso-16:1 (3.76), C 16:0 3-OH (1.71%) C 18:0 (1.33%), C18:1 2-OH (3.66%)	C18:1 ω7c or C18:1 ω6c (56.4%), C16:1 ω7c or C16:1 ω6c (15.1%), C14:0 3-OH and/or iso-16:1 (5.8%), 16:0 (4.47%), C 16:0 3-OH (4.2%) C18:1 2-OH (6.9%)	C18:1 ω7c or C18:1 ω6c (62.37%), C16:1 ω7c or C16:1 ω6c (7.75%), C14:0 3-OH and/or iso-16:1 (4.61%), 16:0 (9.47%), C 16:0 3-OH (2.23%) C18:1 2-OH (4.84%)	C18:1 ω7c or C18:1 ω6c (46.7%), C16:1 ω7c or C16:1 ω6c (12.5%), C14:0 3-OH and/or iso-16:1 (5.5%), 16:0 (13.6%), C 16:0 3-OH (4.2%) C18:1 2-OH (1.4%)
Q–10	Q–10	Q–10	Q–10	Q–10	Q–10	Q–10	Q–10	Q–10	Q–10
nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
68.7	67.6	64–65	nd	68.7	67.3	67	65.5	67.7	69.6
Molasses grass	Rhizosphere soil of corn (*Zea mays*)	Rhizosphere soil of corn (*Zea mays*)	Oil contaminated soil	Dicarded road tar	Forest soil	Mat of the sulfide spring	Soil	Microbial fuel cell	Microbial fuel cell

A. palatum, described as a non-nitrogen-fixing bacterium. Most bacteria of the genus *Azospirillum* grow aerobically, but three species were classified as facultative anaerobic such as *A. melinis, A. thiophilum*, and *A. humicireducens*. Sugars are oxidized but not fermented. It is considered a mesophilic genus with growth at 20–37 °C, optimum temperature at 30 °C. Some species can grow at 5 ° C and a maximum temperature of 41 °C was found for *A. halopraeferens*. The pH range can vary from 4.0 to 8.0, but neutral pH is the optimum for most of the species. Growth in 3 % NaCl is also variable, but it is a special feature for *A. halopraeferens*. Bacteria of this genus also utilize several carbon sources such as organic acids, sugars, amino acids, and sugar alcohols; the pattern of carbon utilization has been used for discriminatory purpose between the species of the genus. The use of the nitrogen-free NFb semisolid medium allows the enrichment and isolation of several species although new media can also be used. Several new isolates obtained from soil and other environments confirm that this genus is widespread in nature and in different regions of the world. *Azospirillum* lipids have a large amount of $C_{18:1}\omega7c$ (55.3 %) and also contain $C_{16:1}\omega7c$ and $C_{16:0}$ as major components; the major hydroxy fatty acids are $C_{14:0}$ 3-OH and $C_{16:0}$ 3-OH. When grown aerobically, the species of this genus exhibit a quinone system with ubiquinone 10 (Q-10). The polar lipids consist mainly of phosphatidylglycerol, phosphatidylcholine, and one unidentified phospholipid. The DNA G+C content varies between 64 and 71 mol%. This genus is closely related to *Rhodocista* and *Skermanella* based on the 16S rRNA gene sequence comparison. The type species of the genus is *A. lipoferum* and the type strain is 59b (= ATCC 29707 = CIP 106280 = DSM 1691 = JCM 1247 = LMG 13128 = NBRC 102290 = NCAIM B.01801 = NRRL B-14654 = VKM B-1519). Besides the type species, the genus embraces 16 other species, isolated either from the rhizosphere or from endophytic plant tissues, agricultural or contaminated soil samples, water, and fermented tank, as follows: *A.brasilense* (Tarrand et al. 1978), *A. amazonense* (Magalhães et al. 1983), *A. halopraeferens* (Reinhold et al. 1987), *A. irakense* (Khammas et al. 1989), *A. largimobile* (Ben Dekhil et al. 1997), *A. doebereinerae* (Eckert et al. 2001), *A. oryzae* (Xie and Yokota 2005), *A. melinis* (Peng et al. 2006), *A. canadense* (Mehnaz et al. 2007a), *A. zeae* (Mehnaz et al. 2007b), *A. rugosum* (Young et al. 2008), *A. picis* (Lin et al. 2009), *A. palatum* (Zhou et al. 2009), *A. thiophilum* (Lavrinenko et al. 2010), *A. formosense* (Lin et al. 2012), *A. humicireducens* (Zhou et al. 2013), and *A. fermentarium* (Lin et al. 2013). The type strains and additional characteristics for these species are listed in *Table 22.2*.

Conglomeromonas Skerman et al. 1983

Con. glom. e. ro. monas, L. pp. conglomeratus to form in a [rounded] mass; Gr. fem. n. monas a unit, monad; M. L. fem. n. *Conglomeromonas* monad forming in a [rounded] mass.

The genus *Conglomeromonas* has one species, *Conglomeromonas largomobilis*, and two subspecies, *Conglomeromonas largomobilis* subsp. *largomobilis* and *Conglomeromonas largomobilis* subsp. *parooensis*, as was proposed by Skerman et al. (1983). This genus was proposed to incorporate the strains described as follows: Gram-negative, non-spore-forming organisms which exhibit unicellular and multicellular phases of growth. Unicellular phase cells are rod shaped, with rounded or tapered ends and a straight or slightly curved axis; cells are arranged singly or in pairs. Motile cells have mixed flagellation, with a single polar flagellum and one or more distinctive lateral flagella of different thickness and length. The DNA G+C content was 67.0 mol%. Although these organisms were isolated from freshwater sources rather than from soil or plant roots and are not able to fix nitrogen under aerobic conditions, other characteristics suggested that they may be related to the genus *Azospirillum* (Skerman et al. 1983). Ben Dekhil et al. (1997) transferred the type species of the genus *Conglomeromonas largomobilis* subsp. *largomobilis* to the genus *Azospirillum* as *A. largomobile* on the basis of phylogenetic evidence based on16S rRNA gene sequence comparisons and earlier nucleic acid hybridization studies (Falk et al. 1986). Furthermore, the genus *Conglomeromonas* became invalid, and consequently, a new genus was required to accommodate *C. largomobilis* subsp. *parooensis*. Thus, Sly and Stackebrandt (1999) created a new genus *Skermanella* and transferred the subspecies of *C. largomobilis* subsp. *parooensis* as *Skermanella parooensis*.

Skermanella Sly and Stackebrandt (1999), Emended Weon et al. (2007) and Luo et al. 2012

Sker.ma.nel'la. M.L. dim. ending -ella; M.L. fem. dim. n. *Skermanella* named after V. B. D. Skerman who first isolated this bacterium, and in honor of his contribution to bacterial systematics.

Bacteria of this genus are Gram negative and non-spore forming, which exhibits unicellular and multicellular phases of growth. Unicellular-phase cells are rod shaped, with rounded or tapered ends and a straight or slightly curved axis, arranged singularly or in pairs. Motile cells have mixed flagellation, with a single polar flagellum and one or more distinct lateral flagella of different lengths. Multicellular conglomerates arise from single cells, which lose motility, become optically refractile, and reproduce by multi-planar septation. Under suitable conditions conglomerates dissociate into single motile cells, which produce water-clear colonies in which the sparse number of cells move in a sluggish manner. No filamentous structures are formed and no buds are produced. The bacterium is an obligate chemoorganotroph and strictly aerobic. All members of the genus are positive for catalase, oxidase, alkaline phosphatase, acid phosphatase, esterase (C4), naphthol-AS-BI-phosphohydrolase, and leucine arylamidase, but negative for α-galactosidase, β-galactosidase, α-mannosidase, β-fucosidase, N-acetyl-β-glucosaminidase, α-chymotrypsin, and trypsin. Carbohydrate metabolism is fermentative and glucose fermentation is variable. Cells do not fix dinitrogen under microaerophilic

conditions. Strains of this genus have a high DNA G+C content (65.0–69.6 mol%), and the major respiratory quinone is Q-10. The major polar lipids are diphosphatidylglycerol, phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, and an unknown aminolipid. The nearest phylogenetic relative is *Azospirillum largimobile* (93 % 16S rRNA gene sequence similarity). The type species is *Skermanella parooensis* and the type strain is ACM 2042 (= CIP 106994 = DSM 9527 = UQM 2042). The other species of the genus are *S. xinjiangensis* (An et al. 2009), *S. stibiiresistens* (Luo et al. 2012), and *S. aerolata* (Weon et al. 2007). The *S. stibiiresistens* strain SB22^T is highly resistant to antimony, growing in the presence of 4 mM Sb(III) in R_2A broth. The type strains and additional characteristics for these species are listed in ● *Table 22.3*.

Desertibacter Liu et al. 2011

De.ser.ti.bac'ter. L. n. *desertum* desert; N.L. masc. n. *bacter* rod; N.L. masc. n. *Desertibacter* a desert bacterium.

The cells are Gram-negative rods, motile by means of a single polar flagellum and strictly aerobic. They are catalase and oxidase positive and are able to reduce nitrate to nitrite. *Desertibacter* is not able to fix nitrogen. Colonies of the type strain 2622^T are pink, circular, and convex with regular margins. Cells contain PHB. Growth occurs at 12–42 °C (optimum 37–40 °C), at pH 7–10 (optimum pH 8), and at NaCl concentrations of up to 1.5 % (optimum 0.5 % NaCl). Hydrolyse aesculin and gelatin but not casein, tyrosine, or starch. In API ZYM tests, positive for alkaline phosphatase, esterase (C4), esterase/lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, naphthol-AS-BI-phosphohydrolase, β-galactosidase (weakly), and α-glucosidase (weakly) and negative for lipase (C14), trypsin, α-chymotrypsin, acid phosphatase, α-galactosidase, β-glucuronidase, N-acetyl-b-glucosaminidase, β-glucosidase, α-mannosidase, and α-fucosidase. API 20NE tests show positive reactions for nitrate reduction, aesculin hydrolysis, gelatin hydrolysis, urease, and β-galactosidase and negative reactions for arginine dihydrolase, indole production, and glucose fermentation. It does not assimilate D-glucose, L-arabinose, maltose, D-mannose, D-mannitol, N-acetylglucosamine, adipic acid, capric acid, malic acid, potassium gluconate, trisodium citrate, or phenylacetic acid. The DNA G+C content of strain 2622^T is 71.4 mol%. The type species is *Desertibacter roseus* and type strain 2622^T (= CCTCC AB 20812 T = KCTC 22436 T), isolated from a gamma-irradiated sand sample from the Taklimakan desert in Xinjiang, China. The type strains and additional characteristics for these species are listed in ● *Table 22.4*.

Rhodocista Kawasaki et al. 1994

Rho.do.ci'sta. L. fem. n. *rhodos* the rose; L. fem.n. *cista* a basket; M.L. fem.n., *Rhodocista* red basket.

Cells have vibrioid to spiral cell form with a size of 0.6–2 µm; they are motile by means of a polar flagellum. Growth is mesophilic. Photosynthetic membranes are present as lamellae lying parallel to cytoplasmatic membrane when cells are grown phototrophically. Growth occurs phototrophically under anaerobic conditions in the light and chemoheterotrophically under aerobic conditions in the dark. Anaerobically grown colonies are pink. Bacteriochlorophyll *a* and carotenoids of the spirilloxanthin series are present. Cells are converted to cysts under aerobic incubation, becoming resistant to desiccation and heat. The major cellular ubiquinone is ubiquinone Q-9. The DNA G+C content is 68.8–69.9 mol%. The type species is *Rhodocista centenaria* ATCC 43720^T (= DSM 9894^T = IAM 14193^T = NRBC 16667^T = JCM 21060^T) isolated from a water sample at the edge of a thermophilic hot spring at Wyoming, USA (Kawasaki et al. 1994). This species is a homotypic synonym of *Rhodospirillum centenum* (Favinger et al. 1989). *Rhodocista pekingensis* is the second species of the genus, and the type strain 3-p^T (= AS 1.2194^T = JCM 11669^T) was isolated from a municipal wastewater treatment plant at Beijing, China (Zhang et al. 2003). The type strains and additional characteristics for these species are listed in ● *Table 22.5*.

Dongia Liu et al. 2010

Don'gi.a. N.L. fem. n. *Dongia* after Professor Xiu-Zhu Dong, a bacteriologist and bacterial taxonomist in China.

The cells are Gram negative, 0.3–0.5 µm wide and 0.6–1.0 µm long, non-spore forming, motile, and slightly curved to straight rods. They have a strictly aerobic metabolism and are heterotrophic – never phototrophic. Internal membrane systems and bacteriochlorophyll a are absent. Cells reduce nitrate to nitrite and oxidase is variable. Dongia cells are negative for ß-galactosidase, urease, catalase, and production of indole and H_2S. They hydrolyze Tweens 20 and 80 weakly, but starch, L-tyrosine, casein, arginine, gelatin, and aesculin are not hydrolyzed. Using the standard mineral base according to Dong and Cai (2001), weak growth on L-arabinose, cellobiose, glucose, lactose, maltose, raffinose, sucrose, D-xylose,erythritol, glycerol, and D-glucitol was observed; no growth occurred with sodium acetate, casein, citrate, citric acid, inositol, malic acid, methanol, L-rhamnose, sorbitol, and succinic acid. API ZYM tests are positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, phosphatase acid, and naphthol-AS-BI-phosphohydrolase and weakly positive for lipase (C14), valine arylamidase, and cystine arylamidase. The type species of the genus is *Dongia mobilis*. Type strain is LM22^T (5CGMCC 1.7660 T 5JCM15798T), isolated from a sequencing batch reactor for the treatment of malachite green effluent. A second species, *Dongia rigui*, type strain 04SU4-P^T (KCTC 23341 = JCM 17521), was isolated from freshwater collected from the Woopo wetland, Republic of Korea (Baik et al. 2013). Both species have as majors fatty acids (>10 % of the total) $C_{19:0}$ω8c cyclo, $C_{16:0}$, and $C_{18:0}$ω7c. The major ubiquinone is Q-10. The DNA G+C content varies from 65.6 to 71.5 mol%. The type strains and additional characteristics for these species are listed in ● *Table 22.6*.

Table 22.3

Morphological, physiological and molecular characteristics differentiating species within genus *Skermanella*

	Skermanella aerolata 5416T-32^T (kit API 20Ne; API ID 32 GN API 50 CH)	*Skermanella parooensis* ACM 2042^T (kit API)	*Skermanella stibiiresistens* SB22^T (kit API 20Ne; API ID 32 GN)	*Skermanella xinjiangensis* 10–1-101^T (kit API 20Ne; API ID 32 GN API 50 CH)
Morphology	Rod-shaped	Rod-shaped	Rod-shaped	Rod-shaped
Cell size (mm)	0.6–1.2 × 1.5–3.5	1.0–1.5 × 1.5–3	nd	0.8–0.9 × 1.1–1.6
Flagellation	Single polar or subpolar flagellum	Single polar and one or more lateral flagella	nd	Single polar or sub-polar flagellum
Motility	+	+	+	+
N_2 fixation	–	–	–	–
PHB accumulation or other characteristics	+	+	nd	+
Detection *nifD* and/or *nifH*	nd	nd	nd	nd
Main carotenoids	nd	nd	nd	nd
Photolithoautotrophic growth (electron donor)	nd	nd	nd	nd
Temperature for growth (°C):				
Range	5–35	10–37	4–37	17–37
Optimum	25–35	28	28	28–37
pH for growth:				
Range	4.0–9.0	6.0–9.0	5–9	6–9
Optimum	6.0–7.0		7	7–8
Oxidase	+	+	+	+
Catalase	+	+	+	+
Color of cell suspension	nd	nd	nd	nd
Urea hydrolise	+	+	–	+
Nitrate reduction	+	+	–	–
Nitrite reduction	nd	nd	nd	nd
NaCl concentration for growth (%)	0–5%	0–2%	0–4%	0–4%
Utilization of:				
Acetate	–	+	–	+
Aspartate	nd	nd	nd	nd
Benzoate	nd	nd	nd	nd
Butyrate	nd	nd	nd	nd
Citrate	–	+	–	–
Crotonate	nd	nd	nd	nd
N-acetyl-D-glucosamine	–	–	–	–
meso-inositol	–	–	+	–
L-rhamnose	–	+	+	+
meso-erythritol	–	–	nd	–
Adonitol	–	–	nd	–
L-arabinose	+	+	–	+
D-arabinose	+	nd	–	+
Malate	+	+	+	+
Sucrose	–	–	–	–
D-glucose	+	+	+	+
D-fructose	–	+	–	–

Table 22.3 (continued)

	Skermanella aerolata 5416T-32^T (kit API 20Ne; API ID 32 GN API 50 CH)	*Skermanella parooensis* ACM 2042^T (kit API)	*Skermanella stibiiresistens* SB22^T (kit API 20Ne; API ID 32 GN)	*Skermanella xinjiangensis* 10–1-101^T (kit API 20Ne; API ID 32 GN API 50 CH)
Mannitol	+	+	–	+
Glycerol	–	–	nd	–
Adipate/adipic acid	–	–	nd	–
Azelaic acid	nd	nd	nd	nd
Sebacic acid	nd	nd	nd	nd
Phenol	nd	nd	nd	nd
4-Chlorophenol	nd	nd	nd	nd
Predominant ubiquinone	Q-10 and Q-8	Q-10	Q-10	Q-10
Predominant cellular fatty acids	C18:1 ω7c and C16:0	C18:1 ω7c and C16:0	C18:1 ω7c (63.5%), summed feature 2 (C14:0 3-OH and/or iso-C16:1 I (10.8%)) and C16:0 (9.9%)	C18:1 ω7c and C16:0
Indol production	–	–	–	–
DNA G+C (mol%)	65.0	67 (66.4–68.0)	69.6	68.8
Genome size	nd	nd	nd	nd
Habitat	Air	Fresh water	Soil of a coal mine	Soil

nd, not determined

Elstera Rahalkar et al. 2012

Els'te.ra. N.L. fem. n. *Elstera* named after Hans-Joachim Elster, a German limnologist working on Lake Constance who was one of the first to establish the importance of the littoral zone for the lake ecosystem.

Cells are Gram-negative rods; they are catalase and oxidase positive. They grow chemoheterotrophically and use sugars, some organic acids, and alcohols as preferred substrates. Ubiquinone Q-10 is the dominant quinone and putrescine is the dominant polyamine. Cells are slightly curved rods, 1.0 × 2.0–5.0 μm in size, and nonmotile, with a minimum doubling time of approximately 40 h. Based on API 20NE and Biolog PM1 tests, cells are negative for the reduction of nitrate, sulfate, and iron (III); nitrogen fixation; and indole production from tryptophan. The cells grow in the presence of many carbon sources such as D-glucose, L-rhamnose, D-fructose, D-galactose, L-arabinose, D-xylose, D-mannitol, D-sorbitol, D-glucuronic acid, glycerol, ethanol, and L-malate. Weak or no growth was observed in the presence of trehalose, D-maltose, dulcitol, adonitol, D-saccharic acid, N-acetyl-β-*o*-mannosamine, glucuronamide, D-glucosaminic acid, formate, acetate, propionate, pyruvate, L-lactate, D-malate, fumarate, succinate, glycolate, glyoxylate, citric acid, m-tartrate, 2-oxoglutarate, and Tweens 20, 40, and 80. The DNA G+C content of strain Dia-1 is 61.0 ± 1.5 mol%. The type species is *Elstera litoralis* and type strain is Dia-1^T (= DSM 19532 T = LMG 24234 T). It was isolated from biofilms on stones in the littoral zone of Lake Constance, Germany. The type strains and additional characteristics for this species are listed in *Table 22.7*.

Inquilinus Coenye et al. 2002

In.qui'li.nus. L. masc. n., *Inquilinus* an inhabitant of a place that is not its own.

Cells are Gram negative, strictly aerobic, chemoorganotrophic, nonmotile, nonsporulating, and rod shaped; *I. ginsengisoli* has a cell size of 0.6–0.8 × 2.5–4.0 μm when grown for 2 days at 30 °C on R_2A agar. Catalase activity is present. Growth is observed at 25–42 °C and in 1 % NaCl. *I. ginsengisoli* cannot grow with 3 % (w/v) NaCl and at 37 °C. Growth is observed on BCSA at 32 °C. There are no denitrification and indole production. Based on RapID NF Plus (Remel) and API 20E (bioMerieux, Hazelwood, MO), lysine decarboxylase, ornithine decarboxylase, or arginine dihydrolase activities are negative. There are no pigment production, lipase, phosphatase, N-acetylglucosaminidase, β-glucosidase, proline aminopeptidase, pyrrolidonyl aminopeptidase, and tryptophan aminopeptidase; N-benzyl-arginine aminopeptidase activity may be present. Utilization of carbon sources (API 20NE and API ID 32 GN kits) is variable between species (Jung et al. 2011). The predominant ubiquinone is Q-10, and the majority of cellular fatty acids are $C_{18:0}$ω7c, $C_{18:1}$2-OH, and $C_{18:0}$3-OH. The G+C content of the genome is between 69.9 and 70.9 mol%. The genus *Inquilinus* was originally described by Coenye et al. (2002).

Table 22.4

Morphological, physiological and molecular characteristics differentiating species within genus *Desertibacter*

	Desertibacter roseus CCTCC AB 208152^T
Morphology	Rod
Cell size (μm)	nd
Flagellation	Single polar flagellum
Motility	+
N_2 fixation	–
PHB accumulation or other characteristics	+ / Gamma radiation-resistant bacteria
Detection *nifD* and/or *nifH*	nd
Temperature for growth (°C):	
Range	12.0–42.0
Optimum	37.0–40.0
pH for growth:	
Range	7.0–10.0
Optimum	8.0
Oxidase	+
Catalase	+
Urease hydrolise	+
Nitrate reduction	+
Nitrite reduction	nd
NaCl concentration for growth (%)	0–1.5
Utilization of (less than 20):	
4-Chlorophenol	nd
Adipate adipic acid	–
Adonitol	nd
Azelaic acid	nd
citrate	nd
D-arabinose	–
D-fructose	nd
D-glucose	–
Glycerol	nd
L-rhamnose	–
Malate	–
Mannitol	–
meso-erythritol	nd
meso-inositol	nd
N-acetyl-D-glucosamine	–
Phenol	nd
Sebacic acid	nd
Sucrose	–
Predominant ubiquinone	Q-10
Predominant cellular fatty acids	C18:1v7c
Indol production	–
DNA G+C (mol%)	71.4
Genome size	nd
Habitat	Sand
Biochemical characteristics kit	API ZYM, API 20E, API 20NE

+, positive; –, negative; nd, not determined; v, variable; na, not available

Table 22.5

Morphological, physiological and molecular characteristics differentiating species within genus *Rhodocista*

	Rhodocista centenaria ATCC 43720^T	*Rhodocista pekingensis* 3-p^T
Morphology	Vibrioid to spiral	Vibrioid to spiral
Cell size (μm)	1–2 × 3	0.6–0.8 × 0.8–1.5
Flagellation	1, polar	1, polar
Motility	+	+
N_2 fixation	+	nd
PHB accumulation or other characteristics	+	nd
Detection *nifD* and/or *nifH*	+	nd
Temperature for growth (°C):		
Range	39–47	25–44
Optimum	39–45	31–42
pH for growth:		
Range	5.7–7	6.5–8
Optimum	6.8	7
Oxidase	nd	nd
Catalase	nd	nd
Urease hydrolise	nd	nd
Nitrate reduction	nd	nd
Nitrite reduction	nd	nd
NaCl concentration for growth (%)	0	0
Utilization of:		
N-acetyl-D-glucosamine	nd	nd
meso-inositol	nd	nd
L-rhamnose	nd	nd
meso-erythritol	nd	nd
Adonitol	nd	nd
D-arabinose	nd	nd
Malate	–	–
Sucrose	nd	–
D-glucose	–	–
D-fructose	–	nd
Mannitol	nd	–
Glicerol	nd	–
Adipate adipic acid	nd	nd
Azelaic acid	nd	nd
Sebacic acid	nd	nd
Phenol	nd	nd
4-Chlorophenol	nd	nd
D-galactose	nd	nd
D-xylose	nd	nd
Trehalose	nd	nd
Predominant ubiquinone	Q-9	Q-9
Predominant cellular fatty acids	nd	nd

Table 22.5 (continued)

	Rhodocista centenaria ATCC 43720^T	*Rhodocista pekingensis* 3-p^T
Indol production	nd	nd
carotenoids	Spirilloxanthin	Spirilloxanthin
DNA G+C (mol%)	68.8	69.9
Genome size	4.36	na
Habitat	Water sample at the edge of a themophilic hot spring	Wastewater treatment plant

nd, not determined

Table 22.6
Morphological, physiological and molecular characteristics differentiating species within genus *Dongia*

	Dongia mobilis CGMCC 17660	*Dongia rigui* 04SU4-P^T
Morphology	Slightly curved to straight rods	Curved to twisted rod-shaped
Cell size (μm)	0.3–0.5 × 0.6–1.0	0.3 × 0.7–1.7
Flagellation	Single polar flagellum	Single polar flagellum
Motility	+	+
N_2 fixation	–	–
PHB accumulation or other characteristics	nd	nd
Detection *nifD* and/or *nifH*	nd	nd
Temperature for growth (°C):		
Range	20.0–40.0	15–37
Optimum	30.0–35.0	25–30
pH for growth:		
Range	6.0–10.0	5.0–11.0
Optimum	7.0–7.5	7
Oxidase	–	+
Catalase	–	–
Urease hydrolise	–	+
Nitrate reduction	+	–
Nitrite reduction	nd	nd
NaCl concentration for growth (%)	0.5	0
Utilization of (less than 20):		
4-Chlorophenol	nd	nd
Adipate adipic acid	nd	nd
Adonitol	nd	nd
Azelaic acid	nd	nd
Citrate	–	nd
D-arabinose	+	nd

Table 22.6 (continued)

	Dongia mobilis CGMCC 17660	*Dongia rigui* 04SU4-P^T
D-fructose	nd	nd
D-glucose	+	nd
Glycerol	nd	nd
L-rhamnose	–	nd
Malate	–	nd
Mannitol	nd	nd
meso-erythritol	nd	nd
meso-inositol	–	nd
N-acetyl-D-glucosamine	nd	nd
Phenol	nd	nd
Sebacic acid	nd	nd
Sucrose	+	nd
Predominant ubiquinone	Q-10	Q-10
Predominant cellular fatty acids	C18:1v7c, C19:0 cyclo v8c, C16:0	C19:0 ω8c cyclo, C16:0 and C18:1 ω7c
Indol production	–	–
DNA G+C (mol%)	65.6	71.5
Genome size	nd	nd
Habitat	Reactor	Freshwater
Biochemical characteristics kit	API ZYM and API 20NE	API ZYM

+, positive; –, negative; nd, not determined; v, variable; na, not available

It comprises the species *Inquilinus limosus*, isolated from respiratory secretions of a cystic fibrosis patient in the USA (Coenye et al. 2002), and *Inquilinus ginsengisoli*, isolated from ginseng field soil (Jung et al. 2011). The *Inquilinus limosus* AU0476^T (= LMG 20952 T = CCUG45653T) is the type species. The type strains and additional characteristics for these species are listed in *Table 22.8*.

Magnetospirillum Scheifer et al. 1992

Mag.ne'to.spir.il'lum, Gr.n.magnes, magnet, comb. form magneto-, Gr.n.*spira* a spiral; M.L.dim.neut.n.*spirillum* a small spiral; *Magnetospirillum* a small magnetic spiral.

Bacteria are characterized by a helical (clockwise) spirillum cell shape; cells are 0.2–0.7 μm wide and 1.0–20.0 μm long. The cells have a Gram-negative cell wall and are motile by means of a single flagellum at each pole. Each magnetotactic cell contains membrane enveloped crystals, named magnetosomes, which are arranged in a chain within the cytoplasm. Mobility and magnetic behavior can be diminished or lost after several subcultivations. The cells are microaerophilic and chemoorganotrophic. Catalase and oxidase can be present or absent. Growth occurs on various organic acids; carbohydrates

Table 22.7
Morphological, physiological and molecular characteristics differentiating species within genus *Elstera*

	Elstera litoralis DSM 19532^T
Morphology	Slightly curved rods
Cell size (μm)	1.06 × 2.0–5.0
Flagellation	nd
Motility	–
N_2 fixation	–
PHB accumulation or other characteristics	nd
Detection *nifD* and/or *nifH*	nd
Temperature for growth (°C):	
Range	10.0–25.0
Optimum	20.0–25.0
pH for growth:	
Range	5.5–8.0
Optimum	6.5–7.0
Oxidase	+
Catalase	+
Urease hydrolise	nd
Nitrate reduction	–
Nitrite reduction	nd
NaCl concentration for growth (%)	nd
Utilization of (less than 20):	
4-Chlorophenol	nd
Adipate adipic acid	nd
Adonitol	–
Azelaic acid	nd
Citrate	–
D-arabinose	+
D-fructose	+
D-glucose	+
Glycerol	+
L-rhamnose	+
Malate	+
Mannitol	+
meso-erythritol	nd
meso-inositol	nd
N-acetyl-D-glucosamine	+
Phenol	nd
Sebacic acid	nd
Sucrose	–
Predominant ubiquinone	Q-10
Predominant cellular fatty acids	18:1v7c, 18:1 2-OH and 16:0.
Indol production	–
DNA G+C (mol%)	61.0± 1.5
Genome size	nd
Habitat	Biofilms on stones
Biochemical characteristics kit	API 20NE and Biolog (PM1)

+, positive; –, negative; *nd*, not determined; *v*, variable; *na*, not available

are utilized only occasionally. *Magnetospirillum* is nitrogen fixation positive as indicated by acetylene reduction assay, *nif*HDK hybridization, and growth in N-free media (Bazylinski et al. 2000). The G+C content of DNA is 64–71 mol%. The type species is *Magnetospirillum gryphiswaldense* and the type strain is MSR-1 (= DSM 6361 = IFO – now NBRC 15271 = JCM 21280). The other species of the genus is *Magnetospirillum magnetotacticum*, type strain MS-1 (= ATCC 31632 = DSM 3856 = IFO – now NBRC 15272 = JCM 21281 = LMG 10894). This species is the basonym of *Aquaspirillum magnetotacticum* (Maratea and Blakemore 1981 emended by Scheifer et al. 1992). The type strains and additional characteristics for these species are listed in ➤ *Table 22.9*.

Phaeospirillum Imhoff et al. 1998

Phae.o.spi.ril´lum. Gr. adj. *phaeos*, brown; M.L. neut. n. *Spirillum*, a bacterial genus; M.L. neut. n., *Phaeospirillum*, brown *Spirillum*.

Phaeospirillum forms vibrioid- to spiral-shaped cells, 0.5–1.2 μm in size. The cells are motile by means of polar flagella. Carotenoid glycosides and bacteriochlorophyll *a* are present on intracytoplasmatic photosynthetic membranes as lamellar stacks. Growth is mesophilic. The major fatty acids are $C_{18:1}\omega7c$, $C_{16:0}$, and $C_{16:1}\omega6c$ and/or $C_{16:1}\omega7c$. Growth is preferably photo-organotrophically under anaerobic conditions in the light or in the dark under microaerobic conditions. Cell suspensions are dark brown to brown-orange/brown-red colored. Cells harbors ubiquinone Q-9 and menaquinone MK-9 as major components. The DNA G+C content is 60.5–64.8 mol%. This genus name was created by Imhoff et al. (1998) after reclassification of brown-colored spiral-shaped phototrophic purple non-sulfur bacteria formerly classified originally as *Rhodospirillum*, based on genetic and phenotypic characteristics. The species of this genus have been isolated from freshwater, mud, and rhizosphere soil and show no salt requirement for growth. The type species is *Phaeospirillum fulvum* and the type strain ATCC 15798^T (= ATCC 53113^T = DSM 113^T) was isolated from sewage pond (van Niel 1944; Imhoff et al. 1998). *P. molischianum* was described as the second species, and the type strain ATCC 14031^T (= DSM 120^T = LMG 4354^T) was isolated from mud from a ditch (Giesberger 1947; Imhoff et al. 1998). The third species is *P. chandramohanii*, type strain JA145^T (= JCM 14933^T = KCTC5703^T = NBRC 104961^T), isolated

Table 22.8
Morphological, physiological and molecular characteristics differentiating species within genus *Inquilinus*

	Inquilinus limosus LMG 20952^T	*Inquilinus ginsengisoli* Gsoil 080^T
Morphology	Rods	Rod-shaped
Cell size (μm)	nd	0.6–0.8×2.5–4.0
Flagellation	nd	nd
Motility	–	–
N_2 fixation	nd	nd
PHB accumulation or other characteristics	nd	nd
Detection *nifD* and/or *nifH*	nd	nd
Temperature for growth (°C):		
Range	25–42	15–30
Optimum	32	nd
pH for growth:	nd	nd
Range	nd	nd
Optimum	nd	nd
Oxidase	+	+
Catalase	+	+
Urease hydrolise	–	–
Nitrate reduction	–	–
Nitrite reduction	nd	nd
NaCl concentration for growth (%)	1.0	2
Utilization of (less than 20):		
4-Chlorophenol	nd	nd
Adipate adipic acid	Weakly positive	–
Adonitol	nd	nd
Azelaic acid	nd	nd
Citrate	–	+
D-arabinose	+	+
D-fructose	nd	nd
D-glucose	+	+
Glycerol	nd	nd
L-rhamnose	–	nd
Malate	+	–
Mannitol	+	+
meso-erythritol	nd	nd
meso-inositol	+	+
N-acetyl-D-glucosamine	+	+
Phenol	nd	nd
Sebacic acid	nd	nd
Sucrose	+	+
Predominant ubiquinone	Q-10	Q-10
Predominant cellular fatty acids	18:1W7c, 18:1–2OH, 18:0–3OH	C18:1v9c/v12t/v7c and C19:0 cyclo v8c
Indol production	nd	–
DNA G+C (mol%)	70.3	69.9
Genome size	nd	nd
Habitat	Respiratory secretions	Soil
Biochemical characteristics kit	RapIDNF Plus (Remel) and API20E bioMerieux, Hazelwood, Mo.)	API 20 NE and API ID32GN kits

+, positive; –, negative; nd, not determined, v, variable; na, not available

from freshwater reservoir at Mudasarlova, India (Kumar et al. 2009). The fourth species described is *P. oryzae*, and the type strain JA317^T (= KCTC 5704^T = NBRC 104938^T) was isolated from rhizosphere soil of a paddy at Nadergul, India (Lakshmi et al. 2011); the fifth species of the genus is *P. tilakii*, and the type strain JA492^T (= KCTC 15012^T = NBRC 107650^T) was isolated from a water/mud from Nelapattu Bird Sanctuary, India (Raj et al. 2012). The type strains and additional characteristics for these species are listed in *Table 22.10*.

Nisaea Urios et al. 2008

Nis´ae.a. L. fem. n., *Nisaea* nymph of the sea (1 of the 50 daughters of Nereus and Doris), referring to the marine origin.

Cells are motile, Gram-negative rods growing optimally at 30 °C, pH 6.0, and 20 g l^{-1} NaCl. The major fatty acids are $C_{18:1}\omega7c$ (69.1 %), $C_{16:1}\omega7c$ (13.9 %), and $C_{16:0}$ (11.3 %). The cells harbor Q-10 and the polar lipid phosphatidylglycerol. On Biolog GN2 plates positive reactions are obtained for fructose, glucose, raffinose, acetate, γ-hydroxybutyrate, and propionate. Positive reactions with API ZYM are obtained for alkaline phosphatase, acid phosphatase, and leucine arylamidase. Oxidase and catalase are positive. The genus *Nisaea* was created after characterization of strains isolated from one of the major sites of water-column denitrification among the world's oceans (Urios et al. 2008). It comprises two species, the type species *Nisaea denitrificans* (type strain DR41_21 = OOB 129 = CIP 109265 = DSM 18348) and *N. nitritireducens* (type strain DR41_18 = OOB 128 = CIP 109601 = DSM 19540). The DNA G+C content is around 60.1–60.2 mol%. The type strains and additional characteristics for these species are listed in *Table 22.11*.

Table 22.9

Morphological, physiological and molecular characteristics differentiating species within genus *Magnetospirillum*

	Magnetospirillum gryphiswaldense MSR-1 = DSM 6361	*Magnetospirillum magnetotacticum* MS-1= ATCC 31632= DSM 3856	*Magnetospirillum magneticum* AMB-1 = ATCC 700264	*Magnetospirillum bellicus* VDY= DSM 21662 = ATCC BAA-1730	*Magnetospirillum aberrantis* SpK = VKPM B-11049
Morphology	Helical (clockwise) spirilla	Helical (clockwise) spirilla	Helical	Spirillum-shaped	Spirilla
Cell size (μm)	0.7 × 1–20. Younger cells are usually shorter (3 to 4 mm)	0.2–0.4 × 4.0–6.0. Tendency to form long chains and coccoid bodies in older cultures	0.4–0.6 × > 3.0	0.5 × 3	0.3–0.4 × 1.5–5.0
Magnetosome number and structure	Variable, enveloped particles arranged in a chain	Variable, enveloped particles arranged in a chain	Arranged in a chain of over 15 magnetosomes	–	Sparse magnetic particles (30–40 nm), single or in small cluster
Flagellation	Single flagellum at each pole	Single flagellum at each pole	Single flagellum at each pole	–	Polar flagella
Motility	+	+	+	+	+
N_2 fixation	+	+	+	nd	Growth on semisolid forming subsuperficial pelicle
PHB accumulation or other characteristics	+	+	nd	nd	+
Detection *nifD* and/or *nifH*	+	+	+	nd	nd
Temperature for growth (°C):					
Range	nd	15–37	nd	<10–42	20–45
Optimum	28–34	30	28–34	42	31
pH for growth:					
Range	nd	nd	5.8–8.2	6.0–7.5	nd
Optimum	7.0–7.5	7.0–7.5	7.0–7.5	6.8	6.5–6.9
Oxidase	+	– or faintly positive with toluene treated cells	nd	–	+
Catalase	+	–	–	–	–
Urease hydrolise	nd	–	nd	nd	nd
Nitrate reduction	+	+	+	+	+
Nitrite reduction	+	+	+	+	+
NaCl concentration for growth (%)	nd	< 1	nd	1.5	nd
Utilization of:					
Lactate	+	+	+	+	+
Acetate	+	+	+	+	+
Fumarate	nd	+	+	+	+
Malate	+	nd	+	–	nd
Peptone	+	nd	nd	nd	nd
Pyruvate	+	+	+	+	+
Succinate	+	+	+	+	+
Sucrose	–	nd	nd	–	–
Aspartic acid	–	nd	nd	nd	nd

Table 22.9 (continued)

	Magnetospirillum gryphiswaldense MSR-1 = DSM 6361	*Magnetospirillum magnetotacticum* MS-1= ATCC 31632= DSM 3856	*Magnetospirillum magneticum* AMB-1 = ATCC 700264	*Magnetospirillum bellicus* VDY= DSM 21662 = ATCC BAA-1730	*Magnetospirillum aberrantis* SpK = VKPM B-11049
Galactose	–	nd	nd	nd	nd
α-ketoglutarate	–	nd	+	nd	nd
Lactose	–	nd	nd	nd	nd
Maltose	–	–	nd	+	nd
D-sorbose	–	nd	nd	nd	nd
Tartrate	–	nd	nd	nd	+
N-butyrate	nd	–	+	+	+
β-hydroxybutyrate	nd	nd	+	nd	nd
Propionate	nd	–	+	+	+
Predominant ubiquinone	na	na	na	na	Q-10
Predominant cellular fatty acids	$C_{14:0}$ 3OH, $C_{18:1}$, $C_{16:1}$	$C_{14:0}$ 3OH, $C_{18:1}$, $C_{16:0}$ 3OH	na	C18:1ω7, C16:0	C18:1ω7, C16:0
Pigment production	nd	–	nd	nd	nd
Indol production	nd	nd	nd	nd	nd
DNA G+C (mol%)	71.0	64.5–66.4	65.1	64.8	62.6
Genome size (Mb)	4.26	4.50	4.97	nd	4.15
Habitat	Fresh water sediments	Microaerobic zones from freshwater sediments	Freshwater	Bioelectrical reactor (BER) inoculated from creek water	Freshwater sediments

+, positive; –, negative; *nd*, not determined; *v*, variable; *na*, not available

Thalassobaculum Zhang et al. 2008, Emend. Urios et al. 2010

Tha.las´so.ba.cu.lum. Gr. n. *Thalassa*, the sea; L. neut. n. *baculum*, stick; N.L. neut. n. *Thalassobaculum* rod-shaped bacterium from the sea.

Cells are Gram negative, slightly curved, and straight rod shaped; they are motile by means of a polar flagellum. Growth is heterotrophic and some of the strains are facultative anaerobes. Cells are positive for oxidase and catalase. Bacteriochlorophyll a is not present. Cells do not fix atmospheric N_2 under anoxic conditions. Optimal growth occurs at 30 °C, at pH 8.0, and at high salinity ranging from 34 to 40 g l^{-1}. Cells are tested positive for leucine arylamidase and valine arylamidase activities with the API ZYM kit. Carbon source utilization is variable according to the kit applied. The major fatty acids are $C_{18:1}\omega7c$, $C_{16:0}$, $C_{17:0}$, and summed feature 3 ($C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH). The isoprenoid quinone is Q-10. The G+C content of the DNA is 65–68 mol%. The type species of the genus is *Thalassobaculum litoreum* and type strain CL-GR58^T (= KCCM 42674^T = DSM 18839^T) that was isolated from coastal seawater, Korea (Zhang et al. 2008). The second species is *T. salexigens*, type strain CZ41-10a^T (= DSM 19539^T = CIP 109064^T = MOLA 84^T), isolated from the water column in the bay of Banyuls-sur-Mer, France (Urios et al. 2010). The type strains and additional characteristics for these species are listed in Table 22.12.

Oceanibaculum Lai et al. 2009, Emend. Dong et al. 2010

O.ce.a'ni.ba'cu.lum. Gr. n. *oceanus*, ocean; L. neut. n. *baculum*, stick; N.L. neut. n. *Oceanibaculum*, rod-shaped bacterium from the ocean.

The genus is characterized by rod-shaped cells, motile by means of a single polar flagellum. The cells stain Gram negative and are oxidase positive. Catalase activity and nitrate reduction are variable. The type species share similar characteristics of growth on 216 L agar plates, forming smooth, gray colonies with regular edges, 1–2 mm in diameter after 72 h incubation at 28 °C, and are nonpigmented and slightly raised in the center. The strains are unable to ferment glucose; they are moderately halophilic, but optimum NaCl concentration varies among them. The dominant fatty acids are $C_{16:1}\omega7c$, $C_{16:0}$, $C_{18:0}$, $C_{18:1}\omega7c$, $C_{18:1}$2-OH, and $C_{19:0}\omega8c$ cyclo. Bacteriochlorophyll *a* is not present. The DNA G+C content is 64.8–67.7 mol%. The type species is *Oceanibaculum indicum* and the type strain is P24 (= CCTCC AB 208226 = LMG 24626 = MCCC 1A02083).

Table 22.10

Morphological, physiological and molecular characteristics differentiating species within genus *Phaeospirillum*

	Phaeospirillum fulvum ATCC 15798^T	*Phaeospirillum molischianum* ATCC 14031^T	*Phaeospirillum chandramohanii* JA145^T	*Phaeospirillum oryzae* JA317^T	*Phaeospirillum tilakii* JA492^T
Morphology	Vibrioid to spiral-shaped	Vibrioid to spiral-shaped	Spiral-shaped	Spiral-shaped	Spiral-shaped
Cell size (μm)	0.5–0.7 × 3.5	0.7–1.0 × 4–6	0.8–1 × 4–8	0.8–1.2 × 2–6	0.5–0.8 × 2–6
Flagellation	1, polar	1, polar	1, polar	1, polar	1, polar
Motility	+	+	+	+	+
N_2 fixation	+	+	+	+	nd
PHB accumulation or other characteristics	nd	nd	nd	nd	nd
Detection *nifD* and/or *nifH*	nd	+	nd	nd	nd
Temperature for growth (°C):					
Range	25–30	25–30	25–35	25–35	25–35
Optimum	25	25	30	30	30
pH for growth:					
Range	6–8.5	6–8.5	6.5–8	6–8	5–9
Optimum	7.3	7.3	7	7	6–6.5
Oxidase	nd	nd	nd	nd	nd
Catalase	nd	nd	nd	nd	nd
Urease hydrolise	nd	nd	–	+	nd
Nitrate reduction	nd	nd	–	nd	nd
Nitrite reduction	nd	nd	–	nd	nd
NaCl concentration for growth (%)	0	0	0	0	0
Utilization of:					
N-acetyl-D-glucosamine	nd	nd	nd	nd	nd
meso-inositol	nd	nd	nd	nd	nd
L-rhamnose	nd	nd	nd	nd	nd
meso-erythritol	nd	nd	nd	nd	nd
Adonitol	nd	nd	nd	nd	nd
D-arabinose	nd	nd	nd	nd	nd
Malate	+	+	+	–	(+)/–
Sucrose	nd	nd	nd	–	nd
D-glucose	+	–	–	–	–
D-fructose	–	–	–	–	–
Mannitol	–	–	(+)	–	–
Glicerol	–	–	–	–	–
Adipate adipic acid	nd	nd	nd	nd	nd
Azelaic acid	nd	nd	nd	nd	nd
Sebacic acid	nd	nd	nd	nd	nd
Phenol	nd	nd	nd	nd	nd
4-Chlorophenol	nd	nd	nd	nd	nd
D-galactose	nd	nd	nd	nd	nd
D-xylose	nd	nd	nd	nd	nd
trehalose	nd	nd	nd	nd	nd
Predominant ubiquinone	Q-9:MK-9 (8:2)	Q-9:MK-9 (8:2)	Q-9:MK-9 (9:1)	Q-9:MK-9 (8:2)	Q-9:MK-9 (7:3)

Table 22.10 (continued)

	Phaeospirillum fulvum ATCC 15798^T	*Phaeospirillum molischianum* ATCC 14031^T	*Phaeospirillum chandramohanii* JA145^T	*Phaeospirillum oryzae* JA317^T	*Phaeospirillum tilakii* JA492^T
Predominant cellular fatty acids	$C_{18:1}\omega7c$, $C_{16:0}$, and $C_{16:1}\omega6c$/$C_{16:1}\omega7c$	$C_{18:1}\omega7c$, $C_{16:1}\omega6c$/$C_{16:1}\omega7c$ and $C_{16:0}$	$C_{16:1}\omega7c$/$C_{16:1}\omega6c$, $C_{16:0}$ and $C_{18:1}\omega7c$	$C_{18:1}\omega7c$, $C_{16:0}$ and $C_{16:1}\omega6c$/$C_{16:1}\omega7c$	$C_{18:1}\omega7c$, $C_{16:0}$ and $C_{16:1}\omega6c$/$C_{16:1}\omega7c$
Indol production	nd	nd	nd	nd	nd
Carotenoids[a]	LY, RP, HLG	LY, RP, HLG, DHLYDG	LY, RP, HLG, DHLYDG	LY, RP, HLG	LY, RP, HLG, DHLYDG
DNA G+C (mol%)	64.8	60.5–64.8	60.5	63.3	62.7
Genome size	3.79 Mb	3.81 Mb	nd	nd	nd
Habitat	Sewage pond	Mud	Freshwater	Rhizosphere soil	Salt marsh

nd, not determined

[a]DHLYDG, Dihydroxylycopene diglucoside; HLG, hydroxylycopene glucoside; LY, lycopene; RP, rhodopin; SP, spirilloxanthin

The second species of the genus is *Oceanibaculum pacificum*, type strain LMC2up-L3 (= MC2UP-L3 = CCTCC AB 209059 = LMG 24859 = MCCC 1A02656), and was isolated from a hydrothermal field sediment of the southwest Pacific Ocean (Dong et al. 2010). The type strains and additional characteristics for these species are listed in *Table 22.13*.

Fodinicurvata Wang et al. 2009

Fo.di.ni.cur.va'ta. L. fem. n. *fodina*, mine; L. adj. curvatus -a -um curved; N.L. fem. n. *Fodinicurvata*, curved-shaped bacterium isolated from a mine.

Cells have a Gram-negative cell wall structure and a size of 0.2–0.5 × 0.5–1.5 μm. They are facultatively anaerobic, vibrioid, and rod shaped. Neither flagella nor endospores are present. Catalase and oxidase are positive. Colonies are cream–white, circular, convex, and opaque with irregular margins after growth on NA supplemented with 5 % at 28 °C for 5 days. Growth occurs under anaerobic conditions. The temperature range for growth is 15–42 °C (optimum, 28 °C), and the pH range for growth is 6.5–8.5 (optimum, 7.5). The carbon sources L-arabinose, D-mannitol, and sucrose are used by *F. sediminis*, while *F. fenggangensis* utilizes myoinositol. Growth occurs at NaCl concentrations of 1.5–20 % (w/v) (optimum, 5 %). Bacteriochlorophyll *a* is not present. Cells accumulate PHB granules and are able to reduce nitrate. The two species strains do not produce H_2S or L-phenylalanine deaminase. Biochemical tests for nitrate reduction, arginine dihydrolase, and urease are positive. Hydrolysis of aesculin and gelatin, indole production, glucose acidification, and phenylalanine deaminase and b-galactosidase are negative. The predominant polar lipids consist of diphosphatidylglycerol, phosphatidylmethylethanolamine, and phosphatidylcholine. Phosphatidylinositol is variable. The DNA G+C content varies from 61.5 to 62.3 mol%. The type species of the genus is *F. sediminis* and the type strains is YIM D82^T (= DSM 21159 T = KCTC22351T). The second species is *F. fenggangensis* strain YIM D812^T (= CCTCC AA 208037 T = DSM 21160 T). Both species were isolated from a salt mine of Fenggang in Yunnan, southwest China. The type strains and additional characteristics for these species are listed in *Table 22.14*.

Pelagibius Choi et al. 2009

Pe.la.gi.bi'us. L. n. *pelagus*, the sea; N.L. masc. n. *bius* from Gr. N. *bios* life; N.L. masc. N., *Pelagibius*, sea life.

Pelagibius forms slightly curved or straight rods, motile by means of a polar flagellum. Cells are strictly aerobic, non-fermentative heterotrophs; they require salt for growth. Cells are oxidase and catalase positive. Growth is mesophilic. Poly-β-hydroxybutyrate granules are formed. Dominant fatty acids are $C_{18:1}\omega7c$, $C_{18:0}$ 3-OH, and $C_{19:0}$ cyclo $\omega8c$. Ubiquinone 10 (Q-10) is the major isoprenoid quinone. Chemotactic and phenotypic characteristics differentiate *Pelagibius* from other related genera in the family *Rhodospirillaceae*, such as temperature range for growth (15–33 °C), pH 6–11, salt tolerance range (2–6 %), and absence of bacteriochlorophyll *a*. Amylase and gelatinase are not produced. Cells grow on L-tyrosine, but casein, hypoxanthine, Tween 80, and xanthine are not hydrolyzed. Cells reduce nitrate to nitrite. According to API ZYM substrate panel, the type strain produces alkaline and acid phosphatases, esterase (C4), leucine arylamidase, naphthol-AS-BI-phosphohydrolase, and β-galactosidase. Aesculin is hydrolyzed (API 20NE). Cells utilize L-arabinose, D-galactose, D-glucose, inositol, inulin, D-mannitol, D-mannose, pyruvic acid, succinate, tartrate, and D-xylose as sole carbon sources. The DNA G+C content of the type species is 66.3 mol%. The type species is *Pelagibius litoralis*, and the type strain CL-UU02^T (= KCCM 42323^T = JCM 15426^T) was isolated from seawater of the east coast of Korea (Choi et al. 2009). The type strains and additional characteristics for this species are listed in *Table 22.15*.

Table 22.11
Morphological, physiological and molecular characteristics differentiating species within genus *Nisaea*

	Nisaea denitrificans DR41_21 =DSM 18348=CIP 109265=OOB 129	*Nisaea nitritireducens* DR41_18 = DSM 19540=CIP 109601=OOB 128
Morphology	Rod	Rod
Cell size (μm)	0.9±0.2×2.5±0.6	0.9±0.2×2.5±0.6
Flagellation	Single polar flagellum	Single polar flagellum
Motility	+	+
N_2 fixation	nd	nd
PHB accumulation or other characteristics	nd	nd
Temperature for growth (°C):		
Range	15–44	15–44
Optimum	30	30
pH for growth:		
Range	5.0–9.0	5.0–9.0
Optimum	6.0	6.0
Oxidase	+	+
Catalase	+	+
Urease	nd	nd
Nitrate reduction	+	–
Nitrite reduction	+	+
NaCl concentration for growth (%)	0–6.0	0–6.0
Utilization of:		
Citrate	–	–
Glycogen	–	–
D-fructose	+	+
Glycerol	–	–
Malate	nd	nd
Aesculin	nd	nd
Pyruvate	nd	nd
Succinate	–	–
Sucrose	+	+
Glucose	+	+
Aspartic acid	–	–
Galactose	–	–
L-rhamnose	–	–
α-ketoglutarate	–	–
Lactose	–	–
Maltose	–	–
D-sorbose	nd	nd
Tartrate	nd	nd
N-butyrate	nd	nd
γ-hydroxybutyrate	+	+
Propionate	+	+
Predominant ubiquinone	Q-10	Q-10
Predominant cellular fatty acids	C18:1w7c, C16:1w7c/iso-15 2-OH	C18:1w7c, C16:1w7c/iso-15 2-OH, C19:0w8c cyclo
Indol production	nd	nd
DNA G+C (mol%)	60.1–60.5	60.1–60.5
Genome size	4.63	nd
Habitat	water column, Marine	water column, Marine

+, positive; – negative; nd, not determined; v, variable; na, not available

Tistlia Díaz-Cárdenas et al. 2010

Tistlia Tist'li.a. N.L. fem. n. *Tistlia* named after Tistl, honoring Michael Tistl, a geologist, for his rediscovery of the Salado de Consotá saline spring.

Cells of *Tistlia* are strictly aerobic, slightly curved to straight rods which do not possess pili or form spores. Gram reaction is negative. In *Tistlia consotensis* (Díaz-Cárdenas et al. 2010) cells reveal a Gram-positive cell-wall ultrastructure. Cell sizes are 0.6–0.7 × 3.0–3.5 μm. Cells multiply by binary fission and show tumbling motility. Growth is mesophilic and slightly halophilic with optimum growth occurring at 30 °C, pH 6.5–6.7, and a salinity of 0.5 % (w/v). Growth is chemoheterotrophic; cells grow on glucose or peptone as a sole carbon source. Yeast extract is not required for growth but increases the biomass yields. Growth occurs with pyruvate, butyrate, succinate, glucose, mannose, xylose, galactose, arabinose, trehalose, cellobiose, lactose, sucrose, rhamnose, fructose, maltose, peptone, casamino acids, tryptone, pepticase, gelatin, arginine, alanine, leucine, isoleucine, valine, glutamate, glycerol, inositol, and starch, but formate, acetate, methanol, lactate, citrate, α-ketoglutarate, ribose, raffinose, methionine, threonine, lysine, glycine, histidine, Tween 80, ethyl oleate, olive oil, benzoate, and cinnamate cannot be used as substrates. Cells are able to fix dinitrogen, showing very high acetylene reduction activity, and were found to possess the *nifH* gene. Urea, nitrate, and glutamate can serve as sole nitrogen sources. Q-10 is the predominant ubiquinone and $C_{19:0}\omega8c$ cyclo, $C_{18:1}\omega7c$, and $C_{18:0}$ are the dominant fatty acids. The DNA G+C content is 71 ± 1 mol%. The type species is *T. consotensis*, strain USBA 355 T (= JCM 15529 T = KCTC 22406 T), isolated from the Salado de Consotá saline spring, Colombia (Díaz-Cárdenas et al. 2010). The type strains and additional characteristics for this species are listed in *Table 22.16*.

Table 22.12
Morphological, physiological and molecular characteristics differentiating species within genus *Thalassobaculum*

	Thalassobaculum litoreum DSM 18839^T	*Thalassobaculum salexigens* DSM 19539^T
Morphology	Curve-straight rods	Rods
Cell size (μm)	1.3–1.5	1.6+-0.3
Flagellation	Polar	Single, polar
Motility	+	+
N_2 fixation	–	–
PHB accumulation or other characteristics	+	nd
Detection *nifD* and/or *nifH*	–	–
Temperature for growth (°C):		
Range	10.0–35.0	15–37
Optimum	30–35	30
pH for growth:		
Range	7.0–9.0	5.0–10.0
Optimum	8	8
Oxidase	+	+
Catalase	+	+
Urease hydrolise	nd	nd
Nitrate reduction	+	nd
Nitrite reduction	nd	nd
NaCl concentration for growth (%)	2.0–4.0	3.4–4.0
Utilization of:		
N-acetyl-D-glucosamine	–	–
meso-inositol	–	nd
L-rhamnose	–	nd
meso-erythritol	nd	+
Adonitol	nd	nd
D-arabinose	+	–
Malate	nd	nd
Sucrose	+	–
D-glucose	–	–
D-fructose	–	+
Mannitol	–	+
Glicerol	nd	–
Adipate adipic acid	nd	nd
Azelaic acid	nd	nd
Sebacic acid	nd	nd
Phenol	nd	nd
4-Chlorophenol	nd	nd
Predominant ubiquinone	Q-10	Q-10
Predominant cellular fatty acids	C18:1w7c; C16:0; C17:0	C18:1w7c; C17:0; C16:0; C16:1w7c
Indol production	nd	nd
DNA G+C (mol%)	68.0	65.0
Genome size	nd	nd
Habitat	Water	Water

+, positive; –, negative; nd, not determined

Telmatospirillum Sizova et al. 2007

Tel.ma.to.spi.ril'lum Gr.n. *telma -atos*, marsh, swamp, fen; N.L. dim neut. n. *Spirillum*, a bacterial genus; N.L. neut. n. *Telmatospirillum*, a fen *Spirillum*.

Cells are Gram negative, vibrioid to spiral shaped, and motile by means of polar or subpolar flagella. Major cellular fatty acids are $C_{18:1}\omega7c$, $C_{17:0}$ cyclopropane, and $C_{16:0}$. Cells grow chemoorganotrophically under anoxic conditions or at low oxygen pressures in the dark as well autotrophically on $H_2 + CO_2$ at low oxygen pressure, being tolerant up to 5 kPa of oxygen. Cells are catalase and oxidase negative. The growth temperature range between 4 °C and 30 °C and the pH range is 4–7. Growth is supported by several organic acids and glucose. Cells can fix atmospheric N_2. Liquid medium is superior to solid agar medium. The G+C content of the DNA is 61.6–64 mol%. The type species is *Telmatospirillum siberiense*, and the type strain 26-4b1 (= ATCC BAA-1305 = KACC 11899) was isolated from northern acidic wetlands under *Sphagnum*. The type strains and additional characteristics for this species are listed in Table 22.17.

Defluvicoccus Maszenan et al. 2005

De.flu.vi.coc'cus. L. neut. n. *defluvium*, sewage; N.L. (Gr. derived) masc. n. *coccus*, berry (spherical microbe); N.L. masc. n. *Defluvicoccus*, a coccus from sewage.

Cells are Gram-negative, non-spore-forming, and nonmotile cocci; they grow chemoheterotrophically under aerobic conditions with a mean cell size of 1.5–4.0 μm. Cells are usually arranged in clusters or tetrads, stain very faintly, and appear empty after staining. Oxidase is negative and catalase is positive. It grew optimally at 25–30 °C and at a pH of 7.5–8.0. Urease and gelatin liquefaction are weakly positive. Many carbon sources (Biolog GN and GP systems), including adonitol, malate, and D-arabinose, are utilized by strain Ben 114^T as presented in description of species and summarized in Table 22.1. Cells are positive for the following enzyme activities as detected with the API ZYM system: alkaline phosphatase, esterase, esterase lipase, leucine arylamidase, acid

Table 22.13
Morphological, physiological and molecular characteristics differentiating species within genus *Oceanibaculum*

	Oceanibaculum indicum P24=CCTCC AB 208226=LMG 24626=MCCC 1A02083	*Oceanibaculum pacificum* LMC2up-L3=CCTCC AB 209059=LMG 24859 =MCCC 1A02656
Morphology	Rod	Rod
Cell size (μm)	0.6–1.5 × 2.3–2.5	0.5–0.7 × 1.7–2.1
Flagellation	Single polar flagellum	Single polar flagellum
Motility	+	+
N_2 fixation	nd	nd
PHB accumulation or other characteristics	nd	nd
Detection *nifD* and/or *nifH*	nd	nd
Temperature for growth (°C):		
Range	10–42	10–45
Optimum	25–37	28–37
pH for growth:		
Range	6.0–11.0	6.0–11.0
Optimum	7.0–9.0	7.0–9.0
Oxidase	+	+
Catalase	+	+
Urease	–	+
Nitrate reduction	+	–
Nitrite reduction	–	–
NaCl concentration for growth (%)	0–9.0	0–9.0
Utilization of:		
Citrate	–	–
Glycogen	–	–
D-fructose	–	+
Glycerol	–	–
Malate	nd	nd
Aesculin	nd	nd
Pyruvate	nd	nd
Succinate	–	–
Sucrose	–	–
Glucose	–	–
Aspartic acid	–	–
Galactose	+	–
L-rhamnose	+	–
α-ketoglutarate	v	–
Lactose	–	–
Maltose	–	–
D-sorbose	nd	nd
Tartrate	nd	nd
N-butyrate	nd	nd
γ-hydroxybutyrate	–	–
Propionate	–	–
Predominant ubiquinone	nd	nd
Predominant cellular fatty acids	$C_{16:0}$, $C_{18:0}$, $C_{18:1}$w7c, $C_{18:1}$ 2-OH and $C_{19:0}$w8c cyclo/$C_{18:0}$ 3–OH	$C_{16:1}$, $C_{16:0}$, $C_{18:1}$w7c
Pigment production	–	–
Indol production	–	–
DNA G+C (mol%)	64.8–65.5	67.7
Genome size	3.95	nd
Habitat	Deep water, Marine	Sediments of hydrothermal field

+, positive; –, negative; *nd*, not determined; *v*, variable; *na*, not available
Utilization of carbon source was tested using API 20NE and Biolog GN2

phosphatase, and naphthol-AS-BI-phosphohydrolase. The DNA G+C content is 66 mol%. The type species is *Defluvicoccus vanus* and the type strain is Ben 114 T (= NCIMB 13612 T = CIP107350T), isolated from a sample of biomass from an enhanced biological phosphorus removal (EBPR) activated sludge plant in the Czech Republic (Maszenan et al. 2005). The type strains and additional characteristics for this species are listed in *Table 22.18*.

Tistrella Shi et al. 2002

Tistr.el'la. M. L. dim. fem. ending -ella; N. L. fem. n. *Tistrella*, arbitrary name formed from the acronym of Thailand Institute of Scientific and Technological Research, TISTR, where the isolation of strain IAM 14872^T was performed.

Cells are Gram negative and rod shaped, with variable sizes (0.7–1.2 × 1.5–1.2 μm). They often occur in chains with a length of approximately 12 mm and are highly motile by means of a single polar flagellum. The cells show binary fission. Bacteria are strictly aerobic and chemoorganotrophic. They are non-photosynthetic and the cells lack intracytoplasmic membrane systems and bacteriochlorophyll a. Cells accumulate polyhydroxyalkanoates. Optimal growth temperatures and pH depend on the species and are between 25 °C and 30 °C and pH 7–7.4, respectively. The salt requirement is variable. Malic acid is readily used as carbon source. The use of other carbon sources such as

Table 22.14
Morphological, physiological and molecular characteristics differentiating species within genus *Fodinicurvata*

	Fodinicurvata sediminis DSM 21159^T	*Fodinicurvata fenggangensis YIM D812^T*
Morphology	Rod and vibroid	Rod and vibroid
Cell size (μm)	0.3–0.5 × 0.7–1.5	0.2–0.5 × 0.5–1.3
Flagellation	Absent	Absent
Motility	–	–
N_2 fixation	–	–
PHB accumulation or other characteristics	+	+
Detection *nifD* and/or *nifH*	–	–
Temperature for growth (°C):		
Range	15.0–42.0	15.0–42.0
Optimum	28.0	28.0
pH for growth:		
Range	6.5–8.5	6.5–8.5
Optimum	7.5	7.5
Oxidase	+	+
Catalase	+	+
Urease hydrolise	+	+
Nitrate reduction	+	+
Nitrite reduction	nd	nd
NaCl concentration for growth (%)	1.5–20	1.5–20
Utilization of (less than 20):		
4-Chlorophenol	nd	nd
Adipate adipic acid	nd	nd
Adonitol	–	–
Azelaic acid	nd	nd
Citrate	+	+
D-arabinose	+	–
D-fructose	–	–
D-glucose	+	+
Glycerol	+	+
L-rhamnose	–	–
Malate	nd	nd
Mannitol	+	+
meso-erythritol	nd	nd
meso-inositol	–	+
N-acetyl-D-glucosamine	nd	nd
Phenol	nd	nd
Sebacic acid	nd	nd
Sucrose	+	–
Predominant ubiquinone	Q-10	Q-10
Predominant cellular fatty acids	C18:1v7c, C18:1 2-OH and C16:0.	C18:1v7c, C18:1 2-OH and C16:0.
Indol production	nd	nd
DNA G+C (mol%)	61.5	62.3

Table 22.14 (continued)

	Fodinicurvata sediminis DSM 21159^T	*Fodinicurvata fenggangensis YIM D812^T*
Genome size	nd	nd
Habitat	Salt mine	Salt mine
Biochemical characteristics kit	Carbon utilization was tested using artificial seawater medium	Carbon utilization was tested using artificial seawater medium

+, positive; –, negative; *nd*, not determined, v, variable; na, not available

L-arabinose, D-mannitol, N-acetylglucosamine, and adipic acid is variable. The cells produce indole, reduce nitrate to nitrite, but do not fix dinitrogen. Aesculin, gelatin, and arginine are hydrolyzed. Cells are positive for catalase and oxidase. The major ubiquinone is Q-10. The major cellular fatty acid is $C_{18:1}\omega7c$. Both 2-hydroxy and 3-hydroxy fatty acids are present, and the major hydroxy fatty acids are $C_{18:0}$ 2-OH and $C_{14:0}$ 3-OH. The $C_{19:0}$ ω8c cyclo may be present. The G–C content of DNA is 65.8–67.5 mol%. The type species is *T. mobilis* and the type strain is IAM 14872 T (= TISTR 1108 T), isolated from wastewater in Thailand (Shi et al. 2002). A second species, named *T. bauzanensis*, type strain BZ78T (= DSM 22817 T = CGMCC 1.10188 T = LMG 26047 T), was isolated from hydrocarbon-contaminated soil in Bozen, South Tyrol, Italy, and was described by Zhang et al. (2011). The type strains and additional characteristics for these species are listed in ❯ *Table 22.19*.

Constrictibacter Yamada et al. 2011

Cons.tric.ti.bac'ter. L. adj. *constrictus*, compressed, contracted; N.L. masc. n. *bacter*, a rod; N.L. masc. n. *Constrictibacter*, rod with compressed parts.

Cells of *Constrictibacter antarcticus* are ovoid to rod shaped and often occur in pairs or chains. Cells are motile and do not form spores and grow aerobically or micro-aerobically; they have a diameter of 0.8–1.0 μm and a length of 1.5–2.0 μm. Colonies are white and circular, 0.2 mm in diameter on 0.256LB/MA agar. Biochemical characteristics, analyzed using the API 20NE and API ZYM, indicate that catalase is produced, but oxidase is not produced. Produces acid phosphatase, alkaline phosphatase, cystine arylamidase, esterase (C4), esterase lipase (C8),b-glucosidase, leucine arylamidase, lipase (C14), naphthol-AS-BI-phosphohydrolase, trypsin, and valine arylamidase. *Constrictibacter* cells reduce nitrate and complex nutrients (tryptone or yeast extract) are essential for growth. The respiratory quinones are Q-10 and Q-8. The major cellular fatty acids are $C_{18:1}$, $C_{16:0}$, and $C_{18:0}$. The DNA G+C content of the type strain/species is 69.8 mol%. The type species is *Constrictibacter antarcticus* 262-8^T (= JCM, ATCC16422T, BAA1906T) that was isolated from a cavity within white rock collected in the Skallen region of Antarctica. The type strains and additional characteristics for this species are listed in ❯ *Table 22.20*.

Table 22.15
Morphological, physiological and molecular characteristicas differentiating species within genus *Pelagibius*

	Pelagibius litoralis KCCM 42323^T
Morphology	Slightly curved or straight rods
Cell size (um)	0.5–1.0 × 1.2–2.5
Flagellation	1, polar
Motility	+
N_2 fixation	nd
PHB accumulation or other characteristics	+
Detection *nifD* and/or *nifH*	nd
Temperature for growth (°C):	
Range	15–33
Optimum	28–30
pH for growth:	
Range	6–11
Optimum	7–8
Oxidase	+
Catalase	+
Urease hydrolise	–
Nitrate reduction	+
Nitrite reduction	nd
NaCl concentration for growth (%)	2–6%
Utilization of:	According to the API 20NE system
N-acetyl-D-glucosamine	–
meso-inositol	+
L-rhamnose	–
meso-erythritol	nd
Adonitol	nd
L-arabinose	+
Malate	nd
Sucrose	–
D-glucose	+
D-fructose	–
Mannitol	+
Glicerol	–
Adipate adipic acid	nd
Azelaic acid	nd
Sebacic acid	nd
Phenol	nd
4-Chlorophenol	nd
D-galactose	+
D-xylose	+
Trehalose	–
Predominant ubiquinone	Q-10
Predominant cellular fatty acids	C18:1ω7c, C18:0 3-OH, C19:0 cyclo ω8c

Table 22.15 (continued)

	Pelagibius litoralis KCCM 42323^T
Indol production	–
Carotenoids[a]	nd
DNA G+C (mol%)	66
Genome size	nd
Habitat	Coastal seawater

[a]*DHLYDG* Dihydroxylycopene diglucoside, *HLG* hydroxylycopene glucoside, *LY* lycopene, *RP* rhodopin, *SP* spirilloxanthin

Rhodovibrio Imhoff et al. 1998

Rho.do.vi'bri.o. Gr. n. *rhodon*, the rose; M.L. masc. n. *Vibrio*, a bacterial genus; M.L. masc. n., *Rhodovibrio*, the rose *Vibrio*.

Cells of the genus *Rhodovibrio* are vibrioid to spiral shaped, 0.6–0.9 µm in size. They are motile by means of polar flagella and multiply by binary fission (Imhoff et al. 1998; Imhoff 2005a). Cell staining is Gram negative. Internal photosynthetic membranes are present as vesicles. They contain bacteriochlorophyll a as well as carotenoids of the spirilloxanthin series. Cells harbor ubiquinones and menaquinones with 10 isoprene units (Q-10 and MK-10). Major cellular fatty acids are $C_{18:1}$ and $C_{18:0}$. The polyamines putrescine and spermidine may be present (Haitiana et al. 2001). Bacteria of this genus grow preferably photoheterotrophically under anoxic conditions in the light, but it is also possible to grow the cells chemotrophically under microoxic to oxic conditions in the dark (Imhoff et al. 1998; Imhoff 2005a). Complex nutrients are required, as no growth is observed in the complete absence of yeast extract or peptone. Under low concentration of yeast extract, lactate or casamino acids increase growth markedly in case of *R. salinarum*, while acetate, malate, succinate, or pyruvate has a similar effect in *R. sodomensis* (Nissen and Dundas 1984; Mack et al. 1993). These species are halophiles, require NaCl or sea salt for growth, and have salt optima above seawater salinity. Cell growth is mesophilic with the preference for neutral pH. Both species show best growth in the presence of 0.1 M Mg^{2+} (Mack et al. 1993). Their DNAs have G+C contents between 65 and 69 mol%. *R. salinarum* is the genus type species and the type strain is ATCC 35394^T (= DSM 9154). The type strain of the species *R. sodomensis* is DSI (= ATCC 51195 = DSM 9895). The type strains and additional characteristics for these species are listed in *Table 22.21*.

Limimonas Amoozegar et al. 2013

Li.mi.mo'nas. L. n. *limus*, mud; L. fem. n. *monas*, a unit, monad; N.L. fem. n. *Limimonas*, a unit (bacterium) isolated from mud.

Cells are Gram negative, strictly aerobic, nonmotile, and rod shaped. They are catalase and oxidase positive and extremely halophilic. Optimal growth occurs with 3.4 M NaCl, at pH 7.0 and 40 °C. The polar lipid pattern consists of phosphatidylglycerol,

Table 22.16
Morphological, physiological and molecular characteristics differentiating species within genus *Tistlia*

	Tistlia consotensis JCM 15529^T
Morphology	Curved-straight rods
Cell size (μm)	3.0–3.5
Flagellation	−
Motility	−
N_2 fixation	+
PHB accumulation or other characteristics	nd
Detection *nifD* and/or *nifH*	+
Temperature for growth (°C):	
Range	20–40
Optimum	30
pH for growth:	
Range	5.0–8.0
Optimum	6.5–6.7
Oxidase	nd
Catalase	+
Urease hydrolise	+
Nitrate reduction	+
Nitrite reduction	+
NaCl concentration for growth (%)	0.5
Utilization of:	
N-acetyl-D-glucosamine	nd
meso-inositol	+
L-rhamnose	+
meso-erythritol	nd
Adonitol	nd
D-arabinose	+
Malate	nd
Sucrose	+
D-glucose	+
D-fructose	+
Mannitol	nd
Glicerol	+
Adipate adipic acid	nd
Azelaic acid	nd
Sebacic acid	nd
Phenol	nd
4-Chlorophenol	nd
Predominant ubiquinone	Q-10
Predominant cellular fatty acids	C19:0 cyclo w8c; C18:1w7c; C18:0
Indol production	nd
DNA G+C (mol %)	71.0
Genome size	nd
Habitat	Water

+, positive; −, negative; nd, not determined

Table 22.17
Morphological, physiological and molecular characteristics differentiating species within genus *Telmatospirillum*

	Telmatospirillum siberiense 26–4b1^T (BAA-1305^T)
Morphology	vibriod to spiral shaped
Cell size (mm)	0.2–0.6 (mm) diameter
Flagellation	1–2 polar or subpolar flagella
Motility	+
N_2 fixation	+
PHB accumulation or other characteristics	nd
Detection *nifD* and/or *nifH*	+
Main carotenoids	nd
Photolithoautotrophic growth (electron donor)	nd
Temperature for growth (°C):	
Range	4–30
Optimum	25–28
pH for growth:	
Range	4–7
Optimum	5.7–6.5
Oxidase	–
Catalase	–
Color of cell suspension	nd
Urease hydrolise	nd
Nitrate reduction	nd
Nitrite reduction	nd
NaCl concentration for growth (%)	0–0.58%
Utilization of:	
Acetate	+ (microaerophilic)
Aspartate	nd
Benzoate	nd
Butyrate	nd
Citrate	nd
Crotonate	nd
N-acetyl-D-glucosamine	nd
meso-inositol	nd
L-rhamnose	nd
meso-erythritol	nd
Adonitol	nd
L-arabinose	nd
D-arabinose	nd
Malate	nd
Sucrose	nd
D-glucose	nd
D-fructose	nd
Mannitol	nd
Glicerol	nd
Adipate adipic acid	nd

Table 22.17 (continued)

	Telmatospirillum siberiense 26–4b1^T (BAA-1305^T)
Azelaic acid	nd
Sebacic acid	nd
Phenol	nd
4-Chlorophenol	nd
Predominant ubiquinone	nd
Predominant cellular fatty acids	C18:1 ω7c, 17:0 cyclopropane and C16:0
Indol production	
DNA G+C (mol%)	64
Genome size	nd
Habitat	Acidic wetlands under Sphagnum (bogs, fens)

nd, not determined

Table 22.18
Morphological, physiological and molecular characteristics differentiating species within genus *Defluvicoccus*

	Defluvicoccus vanus NCIMB 13612^T
Morphology	Cocci/coccobacill
Cell size (μm)	1.5–4.0
Flagellation	Absent
Motility	−
N_2 fixation	nd
PHB accumulation or other characteristics	+
Detection *nifD* and/or *nifH*	nd
Temperature for growth (°C):	
Range	20.0–30.0
Optimum	25.0–30.0
pH for growth:	
Range	5.0–8.0
Optimum	7.5–8.0
Oxidase	−
Catalase	+
Urease hydrolise	+
Nitrate reduction	+
Nitrite reduction	nd
NaCl concentration for growth (%)	nd
Utilization of (less than 20):	
4-Chlorophenol	nd
Adipate adipic acid	nd
Adonitol	+
Azelaic acid	nd
Citrate	nd
D-arabinose	+
D-fructose	nd

Table 22.18 (continued)

	Defluvicoccus vanus NCIMB 13612^T
D-glucose	nd
Glycerol	nd
L-rhamnose	nd
Malate	+
Mannitol	nd
meso-erythritol	nd
meso-inositol	nd
N-acetyl-D-glucosamine	+
Phenol	nd
Sebacic acid	nd
Sucrose	nd
Predominant ubiquinone	nd
Predominant cellular fatty acids	nd
Indol production	−
DNA G+C (mol%)	66
Genome size	nd
Habitat	Activated sludge biomass
Biochemical characteristics kit	Biolog GN and GP systems; Microbact 24E system tests (Oxoid)

+, positive; − negative; nd, not determined, v, variable; na, not available

diphosphatidylglycerol, four unidentified phospholipids, three unidentified amino lipids, and two other unidentified lipids. Ubiquinone Q-10 is the major isoprenoid quinone. The predominant fatty acids are $C_{19:0}$ω7c cyclo and $C_{18:0}$. The DNA G+C content of the type strain is 67.0 mol%. The type species is *Limimonas halophila* and the type strain is IA16^T (= IBRC-M 10018^T = DSM 25584^T). The type strains and additional characteristics for this species are listed in *Table 22.22*.

Rhodospirillum Molisch 1907, Emend. Lakshmi et al. 2013

Rho.do.spi.ril'lum. Gr. n. *rhodon*, the rose; M.L. neut. n. *Spirillum*, a bacterial genus; M.L. neut. n., *Rhodospirillum*, the rose Spirillum.

Cells of the genus *Rhodospirillum* are vibrioid to spiral shaped and motile by means of bipolar flagella and multiply by binary fission. They are Gram negative and mesophilic and prefer neutral pH. Cells contain internal photosynthetic membranes as vesicles. Photosynthetic pigments are bacteriochlorophyll *a* (esterified with phytol or geranylgeraniol) and carotenoids of the spirilloxanthin series, such as spirilloxanthin itself and rhodovibrin. Ubiquinones and rhodoquinones with 10 isoprene units are present. Main cellular fatty acids include $C_{18:1}$ω7c/$C_{18:1}$ω6c, $C_{16:1}$ω7c/$C_{16:1}$ω6c, $C_{16:0}$, $C_{14:0}$3-OH, and $C_{16:0}$3-OH. They grow generally well using fatty acids as carbon sources, except formate and propionate. No appreciable development occurs with tartrate, gluconate, or citrate (Van Niel 1944).

Table 22.19
Morphological, physiological and molecular characteristics differentiating species within genus *Tistrella*

	Tistrella mobilis TISTR 1108^T	*Tistrella bauzanensis* DSM 22817^T
Morphology	Rods	Rods
Cell size (μm)	0.7–1.0	1.0–1.2
Flagellation	Single, polar	Single, polar
Motility	+	+
N_2 fixation	nd	nd
PHB accumulation or other characteristics	+	+
Detection *nifD* and/or *nifH*	nd	nd
Temperature for growth (°C):		
Range	20–40	1.0–25.0
Optimum	30	20
pH for growth:		
Range	5.0–9.0	nd
Optimum	7.4	7.0
Oxidase	+	+
Catalase	+	+
Urease hydrolise	–	–
Nitrate reduction	+	+
Nitrite reduction	–	nd
NaCl concentration for growth (%)	nd	nd
Utilization of:		
N-acetyl-D-glucosamine	+	–
meso-inositol	nd	–
L-rhamnose	nd	–
meso-erythritol	nd	nd
Adonitol	nd	nd
D-arabinose	+	+
Malate	+	+
Sucrose	nd	–
D-glucose	nd	–
D-fructose	nd	–
Mannitol	+	–
Glicerol	nd	–
Adipate adipic acid	+	nd
Azelaic acid	nd	nd
Sebacic acid	nd	nd
Phenol	nd	nd
4-Chlorophenol	nd	nd
Predominant ubiquinone	Q-10	Q-10
Predominant cellular fatty acids	C18:1w7c	C18:1w7c; C19:0w8c cyclo
Indol production	+	+
DNA G+C (mol%)	67.5	65.8
Genome size	nd	nd
Habitat	Water	Soil

+, positive; –, negative; nd, not determined

Ethanol is a good substrate, whereas carbohydrates and their corresponding polyalcohols are not utilized. Alanine, asparagine, and aspartic and glutamic acids result in satisfactory growth; glycine and leucine give rise, at best, to slight development. Bacteria of this genus grow preferably photoheterotrophically under anaerobic conditions in the light, but they can also grow photoautotrophically with molecular hydrogen and sulfide, but not with thiosulfate as photosynthetic electron donor. They also can grow chemotrophically under microoxic to oxic conditions in the dark. Fermentation and oxidant-dependent growth may occur. Their DNA has a G+C content between 63 and 66 mol%. *Rhodospirillum rubrum* is the type species of the genus and the type strain is S1^T (= ATCC 11 170^T = NCIB 8355^T) (Skerman et al. 1980, Pfennig and Trüper 1971b; Lakshmi et al. 2014). The type strains and additional characteristics for this species are listed in ❯ *Table 22.23*.

Pararhodospirillum Lakshmi et al. 2014

Pa.ra.rho.do.spi.ril'lum. Gr. prep. *para*, beside, alongside of, near, like; N.L. neut. n. *Rhodospirillum*, a bacterial generic name; N.L. neut. n. *Pararhodospirillum*, resembling *Rhodospirillum*.

Cells of the genus *Pararhodospirillum* are spiral shaped and motile by means of bipolar flagella and multiply by binary fission. They are Gram negative and grow under mesophilic conditions with preference for neutral pH. Cells contain internal photosynthetic membranes as stacks of lamellae that form a sharp angle to the cytoplasmic membrane. Photosynthetic pigments are bacteriochlorophyll *a* (esterified with phytol or geranylgeraniol) and carotenoids of the spirilloxanthin series, which include lycopene and rhodopin, although spirilloxanthin itself may be absent in some *P. photometricum* strains. Ubiquinones and rhodoquinones with 8 isoprene units are present. Main cellular fatty acids include $C_{18:1}\omega7c/C_{18:1}\omega6c$, $C_{16:0}$, $C_{14:0}$3-OH, $C_{15:0}$3-OH, and $C_{16:0}$3-OH. Bacteria of this genus are strictly anaerobes and obligate phototrophs. Growth factors are required for growth. Their DNA has a G+C content between 60.0 and 65.8 mol%. *Pararhodospirillum photometricum* is the type species of the genus (DSM 122^T = ATCC 49918^T). Other species are *Pararhodospirillum sulfurexigens* (JA143^T = DSM 19785^T = JCM 14885^T = NBRC 104433^T) and *Pararhodospirillum oryzae* (JA318^T = KCTC 5960^T = NBRC 107573^T). The type strains and additional characteristics for these species are listed in ❯ *Table 22.24*.

Table 22.20

Morphological, physiological and molecular characteristics differentiating species within genus *Constrictibacter*

	Constrictibacter antarcticus JCM 16422^T
Morphology	Ovoid to rods
Cell size (μm)	0.8–1.0 × 1.5–2.0
Flagellation	Few polar flagellum
Motility	+
N_2 fixation	–
PHB accumulation or other characteristics	nd
Detection *nifD* and/or *nifH*	nd
Temperature for growth (°C):	
Range	5.0–30.0
Optimum	25
pH for growth:	
Range	6.0–8.1
Optimum	7.0
Oxidase	−
Catalase	+
Urease hydrolise	nd
Nitrate reduction	+
Nitrite reduction	nd
NaCl concentration for growth (%)	0.5
Utilization of:	nd
4-Chlorophenol	−
Adipate adipic acid	−
Adonitol	nd
Azelaic acid	nd
citrate	nd
D-arabinose	−
D-fructose	+
D-glucose	+
Glycerol	+
L-rhamnose	nd
Malate	−
Mannitol	−
meso-erythritol	−
meso-inositol	−
N-acetyl-D-glucosamine	nd
Phenol	nd
Sebacic acid	nd
Sucrose	+
Predominant ubiquinone	Q-10
Predominant cellular fatty acids	C18:1, C16:0, C18:0
Indol production	nd
DNA G+C (mol%)	69.8
Genome size	nd
Habitat	White rock
Biochemical characteristics kit	API 20 NE and API ZYM

+, positive; −, negative; nd, not determined, v, variable; na, not available

Table 22.21

Morphological, physiological and molecular characteristics differentiating species within genus *Rhodovibrio*

	Rhodovibrio salinarum ATCC 35394^T	*Rhodovibrio sodomense* DSI^T
Morphology	Rod to spiral-shaped cells	Vibrio-shaped cells
Cell size (μm)	0.3 × 1–3	0.6–0.7 × 1.6–2.5
Flagellation	A pair at one end of the cell	Presumably polar flagella
Motility	+	Weakly motile
N_2 fixation	nd	−
PHB accumulation or other characteristics	−	+
Detection *nifD* and/or *nifH*	nd	−
Temperature for growth (°C):		
Range	20–45	25–47
Optimum	42	35–40
pH for growth:		
Range	nd	nd
Optimum	7.5–8.0	7.0
Oxidase	+	+
Catalase	nd	nd
Urease hydrolise	nd	nd
Nitrate reduction	nd	nd
Nitrite reduction	nd	nd
NaCl concentration for growth (%)	4 (3–24)	12 (6–20)
Utilization of:		
Malate	ǂ	†
Sucrose	ǂ	
Mannitol	ǂ	
Glicerol	ǂ	
Acetate	ǂ	†
Butyrate		ǂ
Citrate	ǂ	ǂ
Fructose	ǂ	
Fumarate	ǂ	
Glutamate	ǂ	
Lactate	†	
Propionate	ǂ	ǂ
Pyruvate	ǂ	†
Succinate	ǂ	†
Predominant ubiquinone	Q-10, MK-10	nd
Predominant cellular fatty acids	$C_{18:1}$, $C_{18:0}$, $C_{16:0}$	nd
Indol production	nd	nd

Table 22.21 (continued)

	Rhodovibrio salinarum ATCC 35394^T	*Rhodovibrio sodomense* DSI^T
DNA G+C (mol%)	67.4	66.2–66.6
Genome size	nd	nd
Habitat	Saltern	Salt lakes

+, positive; −, negative; nd, not determined
ǂ = Does not stimulate growth significantly in the presence of reduced complex nutrients (required)
† = Stimulates growth in the presence of reduced complex nutrients (required)

Table 22.22
Morphological, physiological and molecular characteristics differentiating species within genus *Limimonas*

	Limimonas halophila IA16 =IBRC-M 10018 =DSM 25584
Morphology	Rod
Cell size (μm)	0.1–0.2 × 1.5– 2.0
Flagellation	−
Motility	−
N_2 fixation	na
PHB accumulation or other characteristics	na
Detection *nifD* and/or *nifH*	na
Temperature for growth (°C):	
Range	30–50
Optimum	40
pH for growth:	
Range	6.0–8.0
Optimum	7.0
Oxidase	+
Catalase	+
Urease	−
Nitrate reduction	−
Nitrite reduction	nd
NaCl concentration for growth (%)	15–30
Utilization of:	
Citrate	nd
Glycogen	nd
D-fructose	nd
Glycerol	+
Malate	nd
Aesculin	nd
Pyruvate	nd
Succinate	−
Sucrose	+
Glucose	+

Table 22.22 (continued)

	Limimonas halophila IA16 =IBRC-M 10018 =DSM 25584
Aspartic acid	+
Galactose	+
L-rhamnose	nd
α-ketoglutarate	nd
Lactose	+
Maltose	nd
D-sorbose	nd
Tartrate	nd
N-butyrate	nd
γ-hydroxybutyrate	nd
Propionate	nd
Predominant ubiquinone	Q-10
Predominant cellular fatty acids	$C_{19:0}$ cyclo ω7c and $C_{18:0}$
Pigment production	−
Indol production	−
DNA G+C (mol%)	67.0
Genome size	nd
Habitat	Mud of hypersaline Lake

+, positive; −, negative; nd, not determined, v, variable; na, not available
Utilization of carbon source were (1%, w/v) was performed using salts of MGM broth, without peptone and containing 0, 1 g l^{-1} of yeast extract, as indicated by Amoozegar et al. (2013)

Roseospira Imhoff et al. 1998, Emend. Guyoneaud et al. 2003

Ro.se.o.spi'ra. L. adj. *roseus*, rosy; Gr. n. *spira*, the spiral; M.L. fem. n., *Roseospira*, the rosy spiral.

The cells of this genus are vibrioid or spiral shaped, 0.4–1.0 μm in size, and motile by means of polar or bipolar flagella and divide by binary fission. Cells are staining Gram negative. Intracytoplasmic photosynthetic membranes are present as vesicles and contain bacteriochlorophyll *a* as well as various carotenoids as photosynthetic pigments. *Roseospira* are slightly halophilic bacteria requiring NaCl or sea salt for growth. Optimum NaCl concentrations are between 0.5 % and 7 % (w/w). Growth occurs preferably photo-organotrophically under anoxic conditions in the light, but cells can also grow under microoxic conditions in the dark. Phototrophic grown cells contain intracytoplasmic membranes of the lamellar type together with bacteriochlorophyll *a* as well as carotenoids. Growth factors niacin, thiamine, *p*-aminobenzoic acid, and yeast extract are required. Their DNAs have G+C contents between 65.0 and 72.3 mol%. The type species of the genus is *Roseospira mediosalina* and type strain is strain BN 280. The genus embraces four other species: *R. marina*, *R. visakhapatnamensis*, *R. goensis*, and *R. navarrensis*. A new species, *R. thiosulfatophila*, has been proposed (Guyoneaud et al. 2002) but, to date,

Table 22.23
Morphological, physiological and molecular characteristics differentiating species within genus *Rhodospirillum*

	Rhodospirillum rubrum S1^T
Morphology	Vibrioid to spiral-shaped cells
Cell size (μm)	0.8–1.0 × 7–10
Flagellation	Bipolar
Motility	+
N_2 fixation	+
PHB accumulation or other characteristics	+
Detection *nifD* and/or *nifH*	+
Temperature for growth (°C):	
Range	nd
Optimum	30–35
pH for growth:	
Range	6.0–8.5
Optimum	6.8–7.0
Oxidase	+
Catalase	+
Urease hydrolise	nd
Nitrate reduction	+
Nitrite reduction	nd
NaCl concentration for growth (%)	None
Utilization of:	
Malate	+
Mannitol	–
Glicerol	–
Acetate	+
Arginine	+
Aspartate	+
Benzoate	–
Butyrate	+
Caproate	+
Citrate	–
Crotonate	–
Ethanol	+
Formate	–
Fructose	–
Fumarate	+
Glucose	–
Glutamate	+
Lactate	+
Methanol	–
Propionate	+
Pyruvate	+
Succinate	+
Tartrate	–

Table 22.23 (continued)

	Rhodospirillum rubrum S1^T
Valerate	+
Predominant ubiquinone	Q-10, RQ-10
Predominant cellular fatty acids	$C_{18:1}$, $C_{16:1}$, $C_{16:0}$
Indol production	nd (it produces 4-hydroxyphenethyl alcohol – a cytokinin-like substance)
DNA G+C (mol%)	65
Genome size	4, 352, 825 bp
Habitat	Fresh water

+, positive; –, negative; nd, not determined

the name has not been validated. The type strains and additional characteristics for these species are listed in *Table 22.25*.

Rhodospira Pfennig et al. 1997

Rho.do.spi'ra. Gr.n. *rhodos*, the rose; Gr.n. *spira*, the spiral; M.L. fem.n., *Rhodospira*, the rose spiral.

Cells are vibrioid to spirilloid with a size of 0.6–0.8 μm; they are motile by means of flagella. Growth is mesophilic. The major fatty acids are $C_{18:1}$, $C_{16:0}$, and $C_{14:0}$. Cells harbor photosynthetic membranes of the vesicular type. The cells grow preferably photoorganotrophically under anaerobic conditions in the light and microaerobically in the dark. Anaerobically grown colonies are beige to peach colored. Bacteriochlorophyll *b* and the carotenoid tetrahydrospirilloxanthin are present. The absorption maxima of living cells for *R. trueperi* type strain are 397, 458, 490, 600, 689, 801, 889, and 986 nm. Major quinone components are Q-7, MK-7, and RQ-7. Cell growth requires reduced sulfur compounds and extracellular sulfur depositions are produced. Biotin, thiamine, and pantothenate are required as growth factors. Cells do not grow in the absence of NaCl. The DNA G+C content is 65.7 mol%. The type species is *Rhodospira trueperi* 8316^T (= ATCC 700224^T), isolated from a peach-colored layer of a laminated microbial mat in a salt marsh at Massachusetts, USA (Pfennig et al. 1997). The type strains and additional characteristics for this species are listed in *Table 22.26*.

Phaeovibrio Lakshmi et al. 2011

Phae.o.vib'ri.o. Gr. adj. *phaeos*, brown; L. v. *vibro*, to set in tremulous motion, move to and fro, vibrate; N.L. masc. n. *vibrio*, that which vibrates, and also a genus name of bacteria possessing a curved rod shape; N.L. masc. n., *Phaeovibrio*, brown vibrio.

Table 22.24

Morphological, physiological and molecular characteristics differentiating species within genus *Pararhodospirillum*

	Pararhodospirillum photometricum 132^T	*Pararhodospirillum sulfurexigens JA143*^{T}	*Pararhodospirillum oryzae* JA318^T
Morphology	Spiral-shaped cells	Spiral-shaped cells	Spiral-shaped cells
Cell size (μm)	1.1–1.5 × 4–7	1.0–1.3 × up to 30	1–3 × 4–16
Flagellation	Polar tufts	Bipolar tufts	Amphitrichous
Motility	+	+	+
N_2 fixation	–	–	–
PHB accumulation or other characteristics	nd	nd	nd
Detection *nifD* and/or *nifH*	+	nd	nd
Temperature for growth (°C):			
Range			25–40
Optimum	25–30	30	30
pH for growth:			
Range			6–8
Optimum	6.5–7.5	7.0	7.0
Oxidase	nd	nd	nd
Catalase	nd	nd	nd
Urease hydrolise	nd	nd	nd
Nitrate reduction	nd	nd	nd
Nitrite reduction	nd	nd	nd
NaCl concentration for growth (%)	None	None	None
Utilization of:			
Malate	+	+	+
Mannitol	+	–	+
Glicerol	+	–	–
Acetate	+	+	+
Arginine	–	–	–
Aspartate	–	–	–
Benzoate	–	–	–
Butyrate	+	+	+
Caproate	–	+	–
Citrate	–	–	–
Crotonate	–	–	+
Ethanol	+	–	+
Formate	–	–	+
Fructose	+	(+)	–
Fumarate	+	+	+
Glucose	+	(+)	(+)
Glutamate	–	(+)	(+)
Lactate	+	(+)	+
Methanol	–	–	(+)
Propionate	+	–	–
Pyruvate	+	+	+
Succinate	+	+	+
Tartrate	–	–	+

Table 22.24 (continued)

	Pararhodospirillum photometricum 132^T	*Pararhodospirillum sulfurexigens JA143*^{T}	*Pararhodospirillum oryzae* JA318^T
Valerate	+	+	+
Predominant ubiquinone	Q-8, RQ-8	Q-8, RQ-8	Q-8, RQ-8
Predominant cellular fatty acids	$C_{18:1}$, $C_{16:1}$, $C_{16:0}$	nd	$C_{16:0}$, $C_{18:1}\omega7c$
Indol production	nd	nd	nd
DNA G+C (mol%)	64.74	64.7	60.2
Genome size	3, 876, 289 bp	nd	nd
Habitat	Fresh water	Fresh water	Soil

+, positive; −, negative; (+), weak growth or microaerobic growth only; nd, not determined

Cells are vibrioid, 0.3–0.5 μm in size. They are motile by polar flagella and multiply by binary fission. They grow obligately phototrophic and strictly anaerobic. Growth is mesophilic. Bacteriochlorophyll a as well as carotenoids of rodopinal series are present in chimeric internal membranes of lamellar stacks and vesicles. The absorption maxima of living cells for *P. sulfidiphilus* type strain are 377, 488, 524, 593, 794, and 863 nm. Cells require biotin and *p*-aminobenzoic acid as growth factors; a limited number of organic substrates can be photoassimilated. Sulfide is required as sulfur source. Major fatty acids are $C_{18:1}\omega7c$ and $C_{16:0}$. The DNA G+C content of the type strain is 67.8–68.8 mol%. Phylogenetic information from 16S rRNA gene sequences differentiates *Phaeovibrio* from other related genera in the family *Rhodospirillaceae*. The type species is *Phaeovibrio sulfidiphilus* and the type strain JA480^T (= KCTC 5825^T = NBRC 106163^T = DSM 23193^T) was isolated from brackish water at Nagapattinam, India (Lakshmi et al. 2011b). The type strains and additional characteristics for this species are listed in *Table 22.27*.

Novispirillum Yoon et al. 2007b

No'vi.spi.ril'lum. L. adj. *novus*, new; N.L. dim. neut. n. *spirillum*, a small spiral; N.L. neut. n., *Novispirillum*, a new small spiral.

Cells are Gram negative and have spirillum, helical, and coccoid forms. The cell size ranges from 0.4 to 0.6 × 2.0 to 7.0 μm (diameter × length). Cells are motile by means of bipolar and fascicles flagella. Positive growth occurs on EMB, MacConkey, TSI, and Seller agars and in MR-VP broth; a predominance of coccoid bodies was observed in older cultures. Colonies are white, circular, and convex with smooth edges. The cells produce water-soluble yellowish green fluorescent or brown pigment, the last in presence of tyrosine and tryptophan. All strains are sensitive to 3.0 % NaCl, negative for hydrolysis of aesculin. They contain as predominant ubiquinone type Q-10. Cells contain esterase (C4) and esterase lipase (C8) activity and hydrolysis of tyrosine; they reduce nitrate if grown anaerobically with KNO_3. The strains grow on malate but only weakly on glycerol. DNA G+C content is 63–65 mol%. The genus *Novispirillum* comprises one species *Novispirillum itersonii* (previously *Aquaspirillum itersonii*) classified into two subspecies, subsp. *nipponicum* and subsp. *itersonii* (Yoon et al. 2007b). The type species of the genus is *Novispirillum itersonii* and the type strain is ATCC 12639 (= CCUG 49447 = CIP 105798 = JCM 21278 = JCM 21494 = LMG 4337 = NBRC 15648). The type strains and additional characteristics for this species are listed in *Table 22.28*.

Marispirillum Lai et al. 2009a

Ma.ri.spi.ril'lum. L. neut. n. *mare*, the sea; N.L. dim. neut. n. *spirillum*, a small spiral; N.L. neut. n., *Marispirillum*, a small spiral of the sea.

Cells are Gram negative, oxidase negative, catalase positive, and helical in shape. Cells are motile by means of polar flagella (three per cell) and moderately halophilic. Growth occurs at salinities of 0.5–12 % and at temperatures of 10–41 °C. The bacteria are capable of denitrification, but they are unable to degrade Tween 80 or gelatin. Major fatty acids are $C_{16:1}\omega7c$, iso-$C_{15:0}$ 2-OH, $C_{16:0}$, $C_{18:1}\omega7c$, $C_{18:0}$, and $C_{19:0}\omega8c$ cyclo. The G+C content of DNA is 67.3 mol%. The type species is *Marispirillum indicum* and the type strain is B142 (= CCTCC AB 208225 = LMG 24627 = MCCC 1A01235). The type strains and additional characteristics for this species are listed in *Table 22.29*.

Insolitispirillum Yoon et al. 2007b

In.so.li'ti.spi.ril'lum. L. adj. *insolitus*, unaccustomed; N.L. dim. neut. n. *spirillum*, a small spiral; N.L. neut. n., *Insolitispirillum*, an unaccustomed small spiral.

The cell form is helical, but in older cultures, coccoid, nonmotile organisms and microcysts predominate. The cell diameter is 0.5–0.7 μm and cell length varies from 5 to 22 μm. Cells are motile by means of bipolar fascicles of flagella that persist even in nonmotile forms. The predominant ubiquinone is Q-9. The bacteria are positive for hydrolysis of urea,

Table 22.25

Morphological, physiological and molecular characteristics differentiating species within genus *Roseospira*

	Roseospira marina CE2105	*Roseospira thiosulfatophila* AT2115	*Roseospira navarrensis* SE3104	*Roseospira mediosalina*	*Roseospira visakhapatnamensis* JA131^T	*Roseospira goensis* JA135^T
Morphology	Spiral to vibriod-shaped	Spiral to vibriod-shaped	Vibriod-rod, often in pairs	Vibriod- to spiral-shaped	Vibriod	Vibriod to crescent-shaped
Cell size (mm)	0.4–0.8 × 1.5–6.0	0.4–0.8 × 2.5–6.5	0.6–0.9 × 3.5–6.5	0.8–1.0 × 2.2–6.0	0.5–0.96 × 2–6	0.8–1.0 × 3.0–8.0
Flagellation	Bipolar tufts of flagella	Polar tufts of flagella	Bipolar tufts of flagella	Polar tufts of flagella	Non-motile	Pair of monopolar flagella
Motility	+	+	+	+	−	+
N_2 fixation	+	+	+	+	−	−
PHB accumulation or other characteristics	nd	nd	nd	nd	nd	nd
Detection *nifD* and/or *nifH*	nd	nd	nd	nd	nd	nd
Main carotenoids	Rhodovibrine, rhodopine	Rhodovibrine, spirilloxanthin	Rhodopine, lycopene	Rhodopine, lycopene	Rhodovibrine	Rhodovibrine
Photolithoautotrophic growth (electron donor)	−	(H_2S, S2O3)	(H_2S)	(H_2S)	–	–
Temperature for growth (°C):						
Range						
Optimum	30−35	30−35	30−35	30−35	30	30
pH for growth:						
Range	5.3–8.4	5.6−8.6	6.0−8.5			
Optimum	6.7–6.8	6.8–7.0	6.8–7.0	7.0	7.0	7.5
Oxidase	nd	nd	nd	nd	nd	nd
Catalase	+	+	+	+	nd	nd
Color of cell suspension	Red	Red	Brown-red	Brown-red	Red-brown	Red-brown
Urease hydrolise	nd	nd	nd	nd	nd	nd
Nitrate reduction	nd	nd	nd	nd	−	−
Nitrite reduction	nd	nd	nd	nd	−	−
NaCl concentration for growth (%)	2–4 (0.5–10)	0.5 (0.2–5)	3–4 (1–10)	4–7 (0.5–15	2 (1–5)	1–3(1–5)
Utilization of:						
Acetate	+	+	+	+	−	weak
Aspartate	+	+	+	+	+	−
Benzoate	−	−	+	−	−	−
Butyrate	+	+	+	+	−	+
Citrate	−	−	+	−	−	−
Crotonate	+	+	+	nd	−	−
N-acetyl-D-glucosamine	nd	nd	nd	nd	nd	nd
meso-inositol	nd	nd	nd	nd	nd	nd
L-rhamnose	nd	nd	nd	nd	nd	nd
meso-erythritol	nd	nd	nd	nd	nd	nd
Adonitol	nd	nd	nd	nd	nd	nd

Table 22.25 (continued)

	Roseospira marina CE2105	*Roseospira thiosulfatophila* AT2115	*Roseospira navarrensis* SE3104	*Roseospira mediosalina*	*Roseospira visakhapatnamensis* JA131^T	*Roseospira goensis* JA135^T
L-arabinose	nd	nd	nd	nd	nd	nd
D-arabinose	nd	nd	nd	nd	nd	nd
Malate	+	–	+	+	–	weak
Sucrose	–	nd	–	nd	nd	nd
D-glucose	–	+	–	–	–	weak
D-fructose	+	–	–	–	–	–
Mannitol	+	–	+	–	+	–
Glycerol	+	+	+	+	–	+
Adipate adipic acid	nd	nd	nd	nd	nd	nd
Azelaic acid	nd	nd	nd	nd	nd	nd
Sebacic acid	nd	nd	nd	nd	nd	nd
Phenol	nd	nd	nd	nd	nd	nd
4-Chlorophenol	nd	nd	nd	nd	nd	nd
Predominant ubiquinone	nd	nd	nd	nd	nd	nd
Predominant cellular fatty acids	nd	nd	nd	nd	nd	nd
Indol production	nd	nd	nd	nd	nd	nd
DNA G+C (mol%)	68.8–69.4	71.9–72.3	66.8	66.6	67	71
Genome size	nd	nd	nd	nd	nd	nd
Habitat	Anoxic sediments; water from coastal and marine environments	Microbial mats in coastal and marine environments	Anoxic sediments exposed to light in inland saline springs	Marine	Marine waters	Marine salterns
Assimilatory SO_4 reduction	+	–	–	+	–	–

utilization of aesculin, and β-glucosidase activity; they are negative for utilization of malate, nitrate reduction, and anaerobic growth with KNO_3. A yellow, water-soluble pigment is formed from phenylalanine, but no pigments are formed from tryptophan or tyrosine. Growth in the presence of 1 % bile was achieved, but not in 1 % glycine. Growth occurs on EMB, TSI, and Seller agars, but not on MacConkey agar or in MR-VP broth. Maximum growth temperature is 39 °C. The pH conducive for growth ranges from pH 5.0 to 8.0. Colonies are finely granular, round, and 2–3 mm in diameter; the color on nutrient agar and potato glucose agar differs from grayish to yellowish, respectively. The G+C content of the DNA is 62–66 mol%. The type species is *Insolitispirillum peregrinum* (Pretorius 1963) and the type strain is ATCC 15387 (= CCUG 13795 = DSM 1839 = JCM 21450 = LMG 4340 = NBRC 14922). This species comprises 2 subspecies based on slightly higher G+C values and the development of cell coccoid forms in older cultures. The subsp. *integrum* (Terasaki 1973 emended Yoon et al. 2007b), basonym *Aquaspirillum peregrinum* subsp. *integrum* (Terasaki 1973; Terasaki 1979), type strain ATCC 33334 (= CCUG 49449 = DSM 11589 = JCM 21428 = LMG 5407 = NBRC 13617), was isolated from oxidation pond water. The type strain for the subsp. *peregrinum* (Pretorius 1963 emend Yoon et al. 2007b), basonym *Aquaspirillum peregrinum* subsp. *peregrinum* (Pretorius 1963; Hylemon et al. 1973), is ATCC 15387 (= CCUG 13795 = DSM 1839 = JCM 21450 = LMG 4340 = NBRC 14922). The type strains and additional characteristics for these species are listed in *Table 22.30*.

Caenispirillum Yoon et al. 2007a

Cae.ni.spi.ril'lum. L. n. *caenum*, sludge, mud; Gr. n. *spira*, a spiral; N.L. dim. neut. n. *spirillum*, a small spiral; N.L. neut. n. *Caenispirillum*, a small spiral isolated from sludge.

Cells are Gram negative, non-spore forming, and motile by means of a single polar flagellum. The cells are helical shaped with a size of 0.5–0.7 × 0.7–7.0 µm. The utilization of various substrates, activities of various enzymes, and other physiological and biochemical properties were tested by using the API 20E, API 20NE, and API 50 CH systems (bioMérieux). In assays with

Table 22.26
Morphological, physiological and molecular characteristics differentiating species within genus *Rhodospira*

	Rhodospira trueperi 8316^T
Morphology	Vibroid to spirilloid
Cell size (μm)	0.6–0.8 × 1.5–3
Flagellation	Bipolar tufts of flagella (two to five fibrils at both ends)
Motility	+
N_2 fixation	nd
PHB accumulation or other characteristics	+
Detection *nifD* and/or *nifH*	nd
Temperature for growth (°C):	
Range	20–35
Optimum	25–30
pH for growth:	
Range	7–7.8
Optimum	7.3–7.5
Oxidase	nd
Catalase	nd
Urease hydrolise	nd
Nitrate reduction	nd
Nitrite reduction	nd
NaCl concentration for growth (%)	0.5–5
Utilization of:	
N-acetyl-D-glucosamine	nd
meso-inositol	nd
L-rhamnose	nd
meso-erythritol	nd
Adonitol	nd
D-arabinose	nd
Malate	+
Sucrose	nd
D-glucose	nd
D-fructose	nd
Mannitol	nd
Glicerol	nd
Adipate adipic acid	nd
Azelaic acid	nd
Sebacic acid	nd
Phenol	nd
4-Chlorophenol	nd
D-galactose	nd
D-xylose	nd
Trehalose	nd
Predominant ubiquinone	Q7, MK7
Predominant cellular fatty acids	C18:1, C16:0, C14:0
Indol production	nd
carotenoids	Tetrahydrospirilloxanthin
DNA G+C (mol%)	65.7
Genome size	nd
Habitat	Salt marsh

Table 22.26 (continued)

nd, not determined

the API ZYM system, alkaline phosphatase, esterase (C4), and esterase lipase (C8) are present and naphthol-AS-BI-phosphohydrolase is weakly present. The type species K92^T is positive for catalase, oxidase, and aesculin hydrolysis and negative for Gram staining, indole production, and hydrolysis of casein and gelatin, while strain AK4^T was positive for oxidase, urease, and DNase activities but negative for gelatinase, catalase, ornithine decarboxylase, lysine decarboxylase, nitrate reduction, indole, and lipase activities. The predominant ubiquinone is Q-10. The major fatty acid is $C_{18:1}\omega7c$. The major respiratory quinone contains Q-10. Phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine, and phosphatidylcholine are the major polar lipids. The DNA G+C content is 70.0–71.0 mol%. The type species is *Caenispirillum bisanense*, type strain K92^T (= KCTC 12839^T = JCM 14346^T). The second species is *C. salinarum*, type strain AK4^T (= JCM 17360 = MTCC 10963), and was isolated from a solar saltern lake (Ritika et al. 2012). The type strains and additional characteristics for these species are listed in *Table 22.31*.

Thalassospira López-López et al. 2002, Emend. Liu et al. 2007

Tha.las'so.spi.ra. Gr. fem. n. *thalassa*, the sea; Gr. fem. n. *spira*, a spire; N.L. fem. n. *Thalassospira*, spiral-shaped organism from the sea.

Bacteria are Gram negative and vibrioid to spiral shapedwith a cell size of 3–5 μm length and 0.6 μm width. Cells are nonmotile and nonflagellated or motile by a single polar flagellum. Some species can grow under anaerobic conditions by reducing nitrate. The bacteria are halophilic, require Na^+ ions for growth, and are able to grow in the presence of up to 12 % NaCl. No requirement exists for organic growth factors. Carbohydrates are used as sole carbon sources and both nitrate and ammonium are used as sole nitrogen sources. Principal fatty acids are $C_{18:1}\omega7c$, $C_{16:0}$, and $C_{18:0}$, while $C_{16:1}\omega7c$, $C_{14:0}$, $C_{16:1}\omega7c$, $C_{14:0}$, $C_{17:0}$, $C_{17:0}$, and $C_{19:0}$ cyclo are variable among species. The G+C content of the genomic DNA ranges from 47 to 61.2 mol%. The following additional species have been described: *T. lucentensis* (López-López et al. 2002), *T. xiamenensis* (Liu et al. 2007), *T. profundimaris* (Liu et al. 2007),

Table 22.27

Morphological, physiological and molecular characteristics differentiating species within genus *Phaeovibrio*

	Phaeovibrio sulfidiphilus JA480^T
Morphology	Vibrioid
Cell size (μm)	0.3–0.5 × 1.2–2.5
Flagellation	0 to 1, polar
Motility	+
N_2 fixation	–
PHB accumulation or other characteristics	nd
Detection *nifD* and/or *nifH*	nd
Temperature for growth (°C):	
Range	20–40
Optimum	25–30
pH for growth:	
Range	7–8
Optimum	7
Oxidase	nd
Catalase	nd
Urease hydrolise	–
Nitrate reduction	–
Nitrite reduction	–
NaCl concentration for growth (%)	0
Utilization of:	
N-acetyl-D-glucosamine	nd
meso-inositol	nd
L-rhamnose	nd
meso-erythritol	nd
Adonitol	nd
D-arabinose	nd
Malate	–
Sucrose	nd
D-glucose	–
D-fructose	–
Mannitol	–
Glicerol	–
Adipate adipic acid	nd
Azelaic acid	nd
Sebacic acid	nd
Phenol	nd
4-Chlorophenol	nd
D-galactose	nd
D-xylose	nd
Trehalose	nd
Predominant ubiquinone	nd
Predominant cellular fatty acids	$C_{18:1}\omega7c$ and $C_{16:0}$
Indol production	nd

Table 22.27 (continued)

	Phaeovibrio sulfidiphilus JA480^T
carotenoids[a]	RP
DNA G+C (mol%)	67.8
Genome size	nd
Habitat	Brackish water

nd, not determined

[a]DHLYDG, Dihydroxylycopene diglucoside; HLG, hydroxylycopene glucoside; LY, lycopene; RP, rhodopin; SP, spirilloxanthin

Table 22.28

Morphological, physiological and molecular characteristics differentiating species within genus *Novispirillum*

	Novispirillum itersonii subsp. *itersonii* ATCC 12639=LMG 4337=CCUG 49447	*Novispirillum itersonii* subsp. *nipponicum* KF8=ATCC 33333=LMG 7370=CCUG 49448
Morphology	Spirilla, helical and coccoid forms	Spirilla, helical and coccoid forms
Cell size (μm)	0.4–0.6 × 2.0–7.0	0.4–0.6 × 2.0–7.0
Flagellation	Bipolar and fascicles flagella	Bipolar and fascicles flagella
Motility	+	+
PHB accumulation or other characteristics	+	+
Detection *nifD* and/or *nifH*	na	na
Temperature for growth (°C):	na	na
Range	43	12–40
Optimum	nd	35
pH for growth:		
Range	5.5–9.0	5.5–9.0
Optimum	nd	nd
Oxidase	+	+
Catalase	+	+
Urease	–	–
Nitrate reduction	+	+
Nitrite reduction	+	+
NaCl concentration for growth (%)	<2.0	<2.0
Utilization of:		
Citrate	–	–
Glycogen	–	–
D-fructose	+	+
Glycerol	–	+

Table 22.28 (continued)

	Novispirillum itersonii subsp. *itersonii* ATCC 12639=LMG 4337=CCUG 49447	*Novispirillum itersonii* subsp. *nipponicum* KF8=ATCC 33333=LMG 7370=CCUG 49448
Malate	+	+
Aesculin	–	–
Pyruvate	–	+
Succinate	+	+
Sucrose	–	–
Glucose	–	–
Aspartic acid	+	nd
Galactose	–	–
L-rhamnose	–	–
α-ketoglutarate	+	nd
Lactose	–	–
Maltose	–	–
D-sorbose	–	–
Tartrate	–	–
N-butyrate	nd	+
γ-hydroxybutyrate	nd	nd
Propionate	+	+
Predominant ubiquinone	Q-10	Q-10
Predominant cellular fatty acids	C18:1ω7c, C16:0	C18:1ω7c, C16:0/ C18:1 2-OH
Indol production	–	–
DNA G+C (mol%)	63.1	64.7
Genome size	4.29	nd
Habitat	Freshwater	Freshwater

+, positive; –, negative; nd, not determined, v, variable; na, not available
Utilization of carbon source was tested using defined basal medium (DBM) vitamine-free containing NH_4Cl as the nitrogen source (0.1%, wt/vol) or using API 20E, API 20NE and API 50CH

T. tepidiphila (Kodama et al. 2008), *T. xianhensis* (Zhao et al. 2010), *T. alkalitolerans* (Tsubouchi et al. 2014), and *T. mesophila* (Tsubouchi et al. 2014). In 2011, the species *T. permensis* was described (Plotnikova et al. 2011). It was isolated from a naphthalene-utilizing bacterial consortium obtained from primitive technogeneous soil in Russia and proposed as a new species within the genus *Thalassospira*. The type strains and additional characteristics for these species are listed in *Table 22.32*.

Magnetospira Williams et al. 2012

Mag.net.o.spi'ra. L. n. *magnes*, fr. Gr. n. Magnes [lithos], "Magnesian stone"[=magnet]; Gr. n. *spira*, the spiral; M.L. fem. n.

Table 22.29
Morphological, physiological and molecular characteristics differentiating species within genus *Marispirillum*

	Marispirillum indicum B142=CCTCC AB 208225=LMG 24627=MCCC 1A01235
Morphology	Helical
Cell size (μm)	0.6–0.7 × 1.9–2.7
Flagellation	1–3, bipolar flagella
Motility	+
N_2 fixation	nd
PHB accumulation or other characteristics	nd
Detection *nifD* and/or *nifH*	nd
Temperature for growth (°C):	
Range	10–41
Optimum	25–37
pH for growth:	
Range	5.0–10.0
Optimum	8.0
Oxidase	–
Catalase	+
Urease	+
Nitrate reduction	+
Nitrite reduction	+
NaCl concentration for growth (%)	0.5–12
Utilization of:	
Citrate	–
Glycogen	+
D-fructose	–
Glycerol	–
Malate	+
Aesculin	na
Pyruvate	+
Succinate	+
Sucrose	–
Glucose	–
Aspartic acid	–
Galactose	–
L-rhamnose	–
α-ketoglutarate	–
Lactose	–
Maltose	–
D-sorbose	nd
Tartrate	nd
N-butyrate	nd
Hydroxybutyrate (α, β and γ)	+
Propionate	+
Predominant ubiquinone	nd

Table 22.29 (continued)

	Marispirillum indicum B142=CCTCC AB 208225=LMG 24627=MCCC 1A01235
Predominant cellular fatty acids	$C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH
Pigment production	nd
Indol production	+
DNA G+C (mol%)	67.3
Genome size	nd
Habitat	Deep water, Marine

+, positive; −, negative; nd, Not determined, v, variable; na, not available
Carbon source test were performed using API 20NE and Biolog GN2 system

Table 22.30
Morphological, physiological and molecular characteristics differentiating species within genus *Insolitispirillum*

	Insolitispirillum peregrinum subsp. *peregrinum* ATCC 15387=LMG 4340=CCUG 13795=DSM 1839	*Insolitispirillum peregrinum* subsp. *Integrum* MF19 = ATCC 33334=LMG 5407=CCUG 49449=DSM 11589
Morphology	Spirilla	Spirilla
Cell size (μm)	0.7–8.5 × 6.0–3.7	0.7–8.5 × 6.0–3.7
Flagellation	single polar flagellum	single polar flagellum
Motility	+	+
N_2 fixation	na	na
PHB accumulation or other characteristics	+	+
Detection *nifD* and/or *nifH*	na	na
Temperature for growth (°C):		
Range	12–39	12–39
Optimum	32	32
pH for growth:		
Range	5.0–8.0	5.0–8.0
Optimum	nd	nd
Oxidase	+	+
Catalase	+	+
Urease	+	+
Nitrate reduction	−	−
Nitrite reduction	−	−

Table 22.30 (continued)

	Insolitispirillum peregrinum subsp. *peregrinum* ATCC 15387=LMG 4340=CCUG 13795=DSM 1839	*Insolitispirillum peregrinum* subsp. *Integrum* MF19 = ATCC 33334=LMG 5407=CCUG 49449=DSM 11589
NaCl concentration for growth (%)	<2.0	<1.5
Utilization of:		
Citrate	−	−
Glycogen	−	−
D-fructose	+	+
Glycerol	−	−
Malate	−	−
Aesculin	+	+
Pyruvate	+	+
Succinate	+	+
Sucrose	−	−
Glucose	−	−
Aspartic acid	nd	nd
Galactose	−	−
L-rhamnose	−	−
α-ketoglutarate	nd	nd
Lactose	−	−
Maltose	−	−
D-sorbose	−	−
Tartrate	−	−
N-butyrate	+	+
γ-hydroxybutyrate	nd	nd
Propionate	+	+
Predominant ubiquinone	Q-9	Q-9
Predominant cellular fatty acids	C18:1ω7c, C16:0/ C18:1 2-OH	C18:1ω7c, C16:0
Pigment production	Yellow in presence of phenilalanine	Brown in presence of tyrosine and tryptophan
Indol production	−	−
DNA G+C (mol%)	62.4–65.6	62.3–63.7
Genome size	nd	nd
Habitat	Oxidation ponds	Freshwater

+, positive; −, negative; nd, not determined, v, variable; na, not available

Magnetospira, the magnetic spiral, which references the spiral morphology and magnetotactic behavior of this bacterium.

Cells are Gram negative and present variable morphology, ranging from truncated spirillum (lima bean shaped) to fully

Table 22.31
Morphological, physiological and molecular characteristics differentiating species within genus *Caenispirillum*

	Caenispirillum bisanense KCTC 12839^T	*Caenispirillum salinarum* AK4^T
Morphology	Helical	Vibrio
Cell size (μm)	0.5–0.7 × 0.7–7.0	0.8–1.2 × 4.0–6.0
Flagellation	1 polar flagellum	Single monopolar
Motility	+	+
N_2 fixation	–	–
PHB accumulation or other characteristics	nd	nd
Detection *nifD* and/or *nifH*	nd	nd
Temperature for growth (°C):		
Range	15–47	15–45
Optimum	37	30–37
pH for growth:	nd	nd
Range	6.5–10.0	6-Oct
Optimum	7.0–8.0	7.5–8.5
Oxidase	+	+
Catalase	+	–
Urease hydrolise	–	+
Nitrate reduction	–	–
Nitrite reduction	nd	nd
NaCl concentration for growth (%)	0.5	2.0–4.0
Utilization of (less than 20):		
4-Chlorophenol	–	nd
Adipate adipic acid	–	nd
Adonitol	–	–
Azelaic acid	–	nd
Citrate	+	+
D-arabinose	–	nd
D-fructose	–	nd
D-glucose	+	nd
Glycerol	–	nd
L-rhamnose	–	nd
Malate	–	–
Mannitol	–	nd
meso-erythritol	+	nd
meso-inositol	+	nd
N-acetyl-D-glucosamine	–	nd
Phenol	–	nd
Sebacic acid	–	nd
Sucrose	–	–
Predominant ubiquinone	Q-10	Q-10
Predominant cellular fatty acids	C18:1v7c	C18:1ω7c and/or C18:103C96c
Indol production	nd	nd
DNA G+C (mol%)	70.0	71
Genome size	nd	nd
Habitat	Sludge	Solar saltern
Biochemical characteristics kit	nd	VITEK 2 GN

+, positive; –, negative; nd, not determined, v, variable; na, not available

helical forms (Meldrum et al. 1993). Cells assimilate inorganic carbon (as CO_2) and grow chemolithoautotrophically with S_2O_3- as the electron donor, using the CBB cycle. The cells harbor form II RuBisCO (CbbM). They are motile by means of bipolar flagella (amphitrichous), with a single flagellum at each pole. *Magnetospira* cells exhibit only polar magnetotaxis and biomineralize a single chain of magnetosomes that contain elongated cuboctahedral magnetite crystals positioned along the long axis of the cell. The G+C content of the DNA was 47.2 mol%. The type species for genus *Magnetospira* is *M. thiophila*, type strain MMS-1 (= ATCC BAA-1438 = JCM 17960). The type strains and additional characteristics for this species are listed in *Table 22.33*.

Magnetovibrio Bazylinski et al. 2013

Ma.gne.to.vi'bri.o. Gr. n. *magnês -êtos*, a magnet; N.L. pref. magneto-, pertaining to a magnet; N.L. masc. n. *vibrio*, a vibrio; N.L. masc. n. *Magnetovibrio*, the magnetic vibrio, which references the vibrioid morphology and magnetotactic behavior of this bacterium.

Cells are Gram negative and vibrioid to helicoid in morphology; they are motile by means of a single polar flagellum. Cells assimilate inorganic carbon (as CO_2) and grow chemolithoautotrophically with thiosulfate and sulfide as the electron donors, using a form II ribulose-1,5-bisphosphate carboxylase/oxygenase (CbbM) and the CBB cycle. Cells of strain MV-1^T exhibit characteristics of both axial and polar magnetotaxis and biomineralize a single chain of magnetosomes that contain magnetite crystals of truncated hexa-octahedral habit, positioned along the long axis of the cell. Major polar lipids identified include phosphatidylethanolamine and phosphatidylglycerol. The G+C content of the DNA is 52.9–53.5 mol%. The type species is *M. blakemorei*, strain MV-1^T. The type strains and additional characteristics for this species are listed in *Table 22.34*.

Table 22.32

Morphological, physiological and molecular characteristics differentiating species within genus Thalassospira

	Thalassospira lucentensis DSM 14000^T	*Thalassospira xiamenensis* DSM 17429^T	*Thalassospira profundimaris* DSM 17530^T	*Thalassospira tepidiphila* DSM 18888^T	*Thalassospira xianhensis* JCM 14850^T	*Thalassospira permensis* NBRC 106175^T	*Thalassospira alkalitolerans* JCM 18968^T	*Thalassospira mesophila* JCM 18969^T
Morphology	Curved rods; spirilla	Curved rods	Curved rods	Vibrioid, spirilla	Curved rods, spirilla	Curved rods	Spirilla	Curved rods
Cell size (μm)	3.0–5.0	0.8–2.3	0.8–2.3	0.9–2.0	1.0–1.6	1.23–1.98	1.3–3.0	1.5–2.9
Flagellation	Single, monopolar	Single, monopolar	–	Single, monopolar	–	Single, monopolar	Single, monopolar	Single, monopolar
Motility	+	+	–	+	–	+	+	+
N_2 fixation	nd	nd	nd	nd	nd	nd	nd	nd
PHB accumulation or other characteristics	nd	nd	nd	nd	nd	nd	nd	nd
Detection *nifD* and/or *nifH*	nd	nd	nd	nd	nd	nd	nd	nd
Temperature for growth (°C):								
Range	4.0–40.0	4.0–40.0	10.0–37.0	8.0–45.0	10.0–42.0	10.0–40.0	4.0–38.0	4.0–36.0
Optimum	20	22	22	43	30	28–30	32	30
pH for growth:								
Range	nd	nd	nd	5.0–10.0	5.5–9.0	nd	4.5–11	4.0–10.5
Optimum	7.2	nd	nd	7	7.2	nd	7.0–8.0	7.0–8.0
Oxidase	+	+	+	+	+	+	–	–
Catalase	+	+	+	+	+	+	–	+
Urease hydrolise	nd	nd	nd	nd	+	–	nd	nd
Nitrate reduction	–	+	+	+	+	+	–	–
Nitrite reduction	–	+	+	–	+	+	–	–
NaCl concentration for growth (%)	2	2.0–4.0	3.0–4.0	3	3.0–6.0	2.0–5.0	2	4
Utilization of:								
N-acetyl-D-glucosamine	+	+	–	–	+	+	–	w
meso-inositol	+	+	–	+	+	+	–	w
L-rhamnose	+	–	–	–	–	–	+	+
meso-erythritol	nd	nd	–	–	+	–	nd	nd
Adonitol	nd	nd	–	–	+	+	nd	nd
D-arabinose	+	+	+	+	+	+	–	–

Table 22.32 (continued)

	Thalassospira lucentensis DSM 14000^T	*Thalassospira xiamenensis* DSM 17429^T	*Thalassospira profundimaris* DSM 17530^T	*Thalassospira tepidiphila* DSM 18888^T	*Thalassospira xianhensis* JCM 14850^T	*Thalassospira permensis* NBRC 106175^T	*Thalassospira alkalitolerans* JCM 18968^T	*Thalassospira mesophila* JCM 18969^T
Malate	+	nd	nd	−	nd	nd	nd	nd
Sucrose	−	+	−	−	+	+	−	+
D-glucose	+	+	+	+	+	+	−	+
D-fructose	+	+	+	+	+	+	−	+
Mannitol	w	+	+	+	+	+	−	+
Glicerol	+	nd	+	+	+	−	nd	nd
Predominant ubiquinone	Q-10	nd	nd	nd	Q-9	Q-10	Q-10	Q-10
Predominant cellular fatty acids	C18:1 w7c; C16:0; C16:1 w7c	C18:1w7c; C16:0; C18:0; C16:1w7c; C14:0	C18:1w7c; C16:0; C18:0; C19:0	C18:1w7c; C16:0; C17:0	C18:1w7c:C16:0; C16:1w7c; C14:0	C18:1w7c; C16:0; C18:0	C18:1 ω7c; C16:0	C10:0; C17:0 cyclo: C18:1 ω7c; C19:0 cyclo ω8c; C10:0
Indol production	nd	nd	nd	nd	nd	nd	−	−
DNA G+C (mol%)	54.7	52.6	47.0	55.1	61.2	53.7	54.4	55.9
Genome size	nd	nd	nd	nd	nd	nd	nd	nd
Habitat	Water	Water	Water	Water	Saline soil	Soil	Plant	Plant

+, positive; −, negative; nd, not determined; w, weak

Table 22.33
Morphological, physiological and molecular characteristics differentiating species within genus *Magnetospira*

	Magnetospira thiophila MMS-1 = ATCC BAA-1438 = JCM 17960
Morphology	Spirillum or lima bean-shaped
	Mostly singly, but also in pairs, chains, and clumps
Cell size (μm)	0.2–0.5 × 1.0–3.0
magnetosome number and structure	Cells produce internal sulfur globules when grown on $S_2O_3^{2-}$
Flagellation	Bipolar flagella (amphitrichous)
Motility	+
N_2 fixation	+
PHB accumulation or other characteristics	nd
Detection *nifD* and/or *nifH*	nd
Temperature for growth (°C):	
Range	5–37
Optimum	25
pH for growth:	
Range	6.9–7.1
Optimum	nd
Oxidase	–
Catalase	–
Urease hydrolise	nd
Nitrate reduction	–
Nitrite reduction	–
NaCl concentration for growth (%)	nd
Utilization of:	
Lactate	–
Acetate	+
Fumarate	+
Malate	+
Peptone	–
Pyruvate	+
Succinate	+
Sucrose	–
Aspartic acid	–
Galactose	–
α-ketoglutarate	–
Lactose	–
Maltose	–
D-sorbose	–
Tartrate	–
N-butyrate	nd
β-hydroxybutyrate	nd
Propionate	–
Predominant ubiquinone	nd
Predominant cellular fatty acids	$C_{16:1}\omega7c$, $C_{16:1}\omega7t$, $C_{16:0}$, $C_{20:0}$
Pigment production	na
Indol production	na
DNA G+C (mol%)	47.2
Genome size (Mb)	nd
Habitat	Marine

+, positive; –, negative; nd, not determined, v, variable; na, not available
Artificial seawater semi-solid agar medium was used for Carbon sources test (final concentration of 0.1%–wt/vol or vol/vol)

Ferrovibrio Sorokina et al. 2012

Fer.ro.vi'bri.o L. n. *ferrum*, iron; L. v. *vibrio*, move to and fro; N. L. masc. n. *vibrio*, which vibrates; N. L. masc. n. *Ferrovibrio*, an iron-oxidizing organism of vibrioid shape.

The cells are vibrioid and motile with one polar flagellum and 0.3 × 0.8–1.3 μm size. Division occurs by binary fission. The cell wall is of Gram-negative type. The cells have a facultative anaerobic metabolism. Growth occurs within the ranges of 5–45 °C and pH 5.5–8.0. Oxidase activity and low catalase activity are present. Organotrophic, mixotrophic, or lithoheterotrophic growth is possible owing to oxidation of Fe(II) coupled to reduction of NO_3^- or N_2O, with accumulation of Fe(III) oxides on the cell surface. Fe(II) may be used as an electron donor for anaerobic mixotrophic or lithoheterotrophic growth. Aerobic organotrophic growth occurs with acetate, butyrate, citrate, fumarate, glycerol, lactate, malate, propanol, propionate, pyruvate, succinate, peptone, and yeast extract as carbon and energy sources. Weak growth occurs on amino acids alanine, histidine, aspartate, and glutamate. Sugars, asparagine, benzoate, butanol, ethanol, formate, glutamine, leucine, oxalate, phenylalanine, proline, tryptophan, and casein hydrolysate are not utilized. Ammonium salts, NO_3^-, N_2O, urea, yeast extract, and peptone may be used as nitrogen sources. NO_2, histidine, aspartate, and casein hydrolysate are not used. Anaerobic growth does not occur with ClO_4^-, SO_4^{2-}, $S_2O_3^{2-}$, or $Fe(OH)_3$ as electron acceptor. In mineral medium with nitrate, H_2 is not used as an electron donor. The DNA G+C content is 64.2 mol%. The type species is *F. denitrificans* and the type strain is Sp-1^T (= LMG 25817 ^{T} = VKMB-2673 ^{T}) – isolated from a moderately thermal, iron-sulfide mineral spring of the Psekups mineral water deposit (Northern Caucasus, Russia). The type strains and additional characteristics for this species are listed in *Table 22.35*.

Table 22.34
Morphological, physiological and molecular characteristics differentiating species within genus *Magnetovibrio*

	Magnetovibrio blakemorei MV-1 = ATCC BAA-1436 = DSM 18854
Morphology	Vibroid to helical
Cell size (μm)	0.2–0.4 × 1.0–3.0
magnetosome number and structure	Single chain of magnetosomes that contain magnetite crystals of truncated hexa-octahedral habit. Produce internal sulfur globules when grown on $S_2O_3^{2-}$
Flagellation	Single polar flagellum
Motility	+
N_2 fixation	+
PHB accumulation or other characteristics	nd
Detection *nifD* and/or *nifH*	nd
Temperature for growth (°C):	
Range	4–31
Optimum	27
pH for growth:	
Range	7.0–7.5
Optimum	7.0
Oxidase	+
Catalase	−
Urease hydrolise	nd
Nitrate reduction	+
Nitrite reduction	+
NaCl concentration for growth (%)	nd
Utilization of:	
Lactate	+
Acetate	+
Fumarate	+
Malate	+
Peptone	+
Pyruvate	+
Succinate	+
Sucrose	−
Aspartic acid	+
Galactose	−
α-ketoglutarate	+
Lactose	−
Maltose	−
D-sorbose	−
Tartrate	−
N-butyrate	−
β-hydroxybutyrate	nd
Propionate	+
Predominant ubiquinone	nd
Predominant cellular fatty acids	$C_{18:1}\omega7$, $C_{16:1}\omega7$, $C_{16:0}$, and $C_{16:1}\omega5$
Pigment production	na
Indol production	na
DNA G+C (mol%)	52.9–53.5
Genome size (Mb)	3.7
Habitat	Marine

+, positive; −, negative; nd, not determined, v, variable; na, not available
Carbon sources at a concentration of 0.1% (w/v) were tested for microaerobic growth in semi-solid media using N_2O as the terminal electron acceptor

Isolation, Enrichment, and Maintenance Procedures

Azospirillum

The isolation of *A. lipoferum* and *A. brasilense* is based on the use of N-free semisolid medium, containing agar (1.75 g L^{-1}). The recipe contains (g L^{-1}) the following: malic acid, 5.0; K_2HPO_4, 0.5; $MgSO_4.7H_2O$, 0.2; NaCl, 0.1; $CaCl_2.2H_2O$, 0.02; micronutrient solution A, 2 mL; bromothymol blue (0.5 % in 0.2 N KOH), 2 mL; Fe-EDTA (solution 1.64 %), 4 mL; vitamin solution B, 1 mL; and KOH, 4.5 g; complete volume to 1,000 mL and adjust pH to 6.5–6.8. To semisolid medium, add 1.75–1.80 g agar L^{-1}. To solid medium, add 15 g agar L^{-1}. Micronutrient solution (g L^{-1}): $CuSO_4.5H_2O$, 0.04; $ZnSO_4.7H_2O$, 0.12; H_3BO_3, 1.40; $Na_2MoO_4.2H_2O$, 1.0; $MnSO_4.H_2O$, 1.175. Complete volume to 1,000 mL with distilled water. Store the solution in the refrigerator. Vitamin solution: biotin, 10 mg; pyridoxal HCl, 20 mg. Dissolve in hot-water bath. Complete to 100 mL adding distilled water. Store the solution in refrigerator.

These microaerobically nitrogen-fixing (diazotrophic) bacteria are selectively enriched because they can grow with N_2 as nitrogen source. Because azospirilla do not harbor powerful oxygen-protective mechanisms for the oxygen-sensitive nitrogen-fixing system, they are unable to grow on N_2 as sole N-source in N-free agar plates or liquid media because of too high oxygen levels in air. Microaerobic diazotrophs are aerotactic, and as a result, the nitrogen-fixing population collects in zones of reduced oxygen concentration. There, they form a thin pellicle or veil that moves upward as it becomes thicker (Döbereiner and Pedrosa 1987). After characteristic pellicles have formed, N_2 fixation can be checked by acetylene reduction activity, and active cultures are transferred to new vials

Table 22.35
Morphological, physiological and molecular characteristicas differentiating species within genus *Ferrovibrio*

	Ferrovibrio denitrificans LMG 25817^T
Morphology	Vibrioid
Cell size (μm)	0.3 × 0.8–1.3
Flagellation	Single polar
Motility	+
N_2 fixation	nd
PHB accumulation or other characteristics	Accumulation of Fe(III) oxides on the cell surface.
Detection *nifD* and/or *nifH*	nd
Temperature for growth (°C):	
Range	5.0–45.0
Optimum	35.0
pH for growth:	
Range	5.5–8
Optimum	6.2
Oxidase	+
Catalase	+
Urease hydrolise	nd
Nitrate reduction	+
Nitrite reduction	–
NaCl concentration for growth (%)	0–2.5
Utilization of (less than 20):	
4-Chlorophenol	nd
Adipate adipic acid	nd
Adonitol	–
Azelaic acid	nd
Citrate	+
D-arabinose	nd
D-fructose	nd
D-glucose	nd
Glycerol	+
L-rhamnose	–
Malate	nd
Mannitol	nd
meso-erythritol	nd
meso-inositol	nd
N-acetyl-D-glucosamine	nd
Phenol	nd
Sebacic acid	nd
Sucrose	nd
Predominant ubiquinone	Q-10
Predominant cellular fatty acids	18:1w7c, 19:0 cyc, 16:0
Indol production	nd
DNA G+C (mol%)	64.2
Genome size	nd
Habitat	Iron–sulfide mineral spring
Biochemical characteristics kit	na

+, positive; –, negative; nd, Not determined, v, variable; na, not available

containing the same medium. As soon as a new pellicle is visible, the cultures are streaked out on agar plates containing the same medium with yeast extract (20 mg L^{-1}) added. The small amount of yeast extract permits the growth of small colonies on the surface of plates. Characteristic individual colonies are then transferred again to N-free semisolid media, and those that grow well are streaked out on potato agar for final purification.

The species *A. irakense* (Khammas et al. 1989) can also be isolated using the semisolid NFb medium containing up to 0.3 % NaCl, pH adjusted to 7.0–8.5 and incubation at 33 °C. Similarly, *A. doebereinerae* (Eckert et al. 2001) can be isolated using the NFb semisolid medium after incubation for 3–5 days at 30 °C. Further purification is done on NFb (supplemented with 50 mg yeast extract L^{-1}).

The species *A. oryzae* can be isolated using the M (malate) medium with the following composition in g L^{-1}: sodium malate, 5.0; $CaCl_2.2H_2O$, 0.02; $MgSO_4.7H_2O$, 0.2; K_2HPO_4, 0.1; KH_2PO_4, 0.4; NaCl, 0.1; $FeCl_3$,0.010, $Na_2MoO_4.2H_2O$, 0.002; yeast extract, 0.1; and biotin, 2 μg. Complete to 1,000 mL with distilled water and adjust the pH to 6.8. The NFG medium can also be used with the composition (g L^{-1}): glucose, 10.0; $CaCl_2.2H_2O$, 0.020; $MgSO_4.7H_2O$, 0.2; K_2HPO_4, 1.0; $CaCO_3$, 5.0; $FeSO_4.7H_2O$, 0.050; and $Na_2MoO_4.2H_2O$, 0.001. Complete to 1,000 mL with distilled water and adjust the pH to 7.3. Similarly, *A. zeae* and *A. canadense* can be isolated using the M medium by omitting the addition of biotin, and the pH of the medium is adjusted to 7.2–7.4. (Xie and Yokota 2005; Mehnaz et al. 2007a, b). Sub-cultivation is done on the same medium at 30 °C for 48–72 h.

The species *A. amazonense* (Magalhães et al. 1983) is isolated in a semisolid sucrose medium (LGI or Fam). Composition of LGI medium (g L^{-1}): sucrose, 5.0; K_2HPO_4, 0.2; KH_2PO_4, 0.6; $MgSO_4.7H_2O$, 0.2; $CaCl_2.2H_2O$, 0.02; $Na_2MoO_4.2H_2O$, 0.002; bromothymol blue (0.5 % in 0.2 N KOH), 5 mL; Fe-EDTA (solution 1.64 %), 4 mL; vitamin solution (see above), 1 mL. Complete volume to 1,000 mL with distilled water. Adjust pH to 6.0–6.2 with H_2SO_4. For semisolid medium, add 1.75–1.80 agar L^{-1} and 15 g agar L^{-1} for solid medium. FAM medium has the composition (g L^{-1}): sucrose, 5.0; KH_2PO_4, 0.12; K_2HPO_4, 0.03; $MgSO_4.7H_2O$, 0.2; $CaCl_2$, 0.02; Fe-EDTA, 0.066; NaCl, 0.1; $Na_2MoO_4.2H_2O$, 0.002; $MnSO_4$, 0.00235; H_3BO_3, 0.0028; $CuSO_4.5H_2O$, 0.00008; $ZnSO_4.7H_2O$, 0.00024; biotin, 0.0001; and pyridoxine-HCl, 0.0002 g. Complete volume to 1,000 mL with distilled water. For semisolid medium, add 1.75 g agar L^{-1} and adjust pH to 6.0.

The species *A. melinis* (Peng et al. 2006) can also be isolated in a semisolid LGI medium or a semisolid NFB medium after incubation at 28 °C for 3–5 days. Purification can be done by repeatedly streaking the isolates on plates of solid LGI or NFb medium.

The species *A. halopraeferans* (Reinhold et al. 1987) can be isolated in the semisolid SM medium supplemented with 1.5 % NaCl, pH adjusted to 8.5 and vials incubated at 41oC. Composition (g L^{-1}): DL-malic acid, 5.0; KOH, 4.8; NaCl, 1.2; $NaSO_4$, 2.4; $NaHCO_3$, 0.5; $CaCl_2$, 0.22; $MgSO_4.7H_2O$, 0.25; K_2SO_4, 0.17;

Na_2CO_3, 0.09; Fe-EDTA, 0.077; K_2HPO_4, 0.13; biotin, 0.0001; $MnCl_2.4H_2O$, 0.0002; H_3BO_3, 0.0002; $ZnCl_2$, 0.00015; $CuCl_2.2H_2O$, 0.000002; $Na_2MoO_4.2H_2O$, 0.002; distilled water, completed to 1,000 mL. The final pH of the medium is 8.5. Cells may grow to 1.2 µm length and 0.7–1.4 µm thick if the pH turns alkaline.

The non-nitrogen-fixing *A. palatum* (Zhou et al. 2009) can be isolated using a TYB medium containing 0.3 % yeast extract, 0.2 % beef extract, 0.6 % tryptone, 0.3 % NaCl, and 001 % $FeCl_3$, pH 7.0.

For rapid multiplication, many *Azospirillum* species can be grown in liquid media to which a combined nitrogen source has been added (NH_4Cl (1 g/l), KNO_3 (1 g/l), or yeast extract (0.4 g L^{-1})). Alternatively, complex media such as nutrient broth (NB) or 1/2 DYGS medium (D,L-malate (1 g L^{-1}), yeast extract (2 g L^{-1}), glucose (1 g L^{-1}), glutamate (1.5 g L^{-1}), peptone (1.5 g L^{-1}), $MgSO_4.7H_2O$ (0.5 g L^{-1})) can be applied (Rodrigues Neto et al. 1986). In such media, with rapid stirring or shaking, cell concentrations of 10^8 per ml are reached after 24–48 h. To stabilize the pH at the desired value upon prolonged growth, the addition of 50 mM MOPS (3-(N-morpholino)propanesulfonic acid) buffer (pH 6.8) or MES (2-(N-morpholino)ethanesulfonic acid) buffer (pH 6.0; for *A. amazonense*) is recommended. Alternatively, the *Azospirillum* minimal medium of Okon et al. (1977), which also contains high phosphate levels, can be used.

Storage of the cultures for many years at −80 °C or in liquid N_2 is also possible after adding 50 % glycerin or dimethyl sulfoxide (DMSO) to an exponentially growing culture. The cells can also be preserved by lyophilization according to the following protocol (Döbereiner et al. 1995). The cultures are grown to late log phase in the following medium: K_2HPO_4, 0.5 g; $MgSO_4.7H_2O$, 0.2 g; NaCl, 0.1 g; K-DL-malate (*A. brasilense*) or glucose (*A. lipoferum*), 5 g; yeast extract, 0.4 g; and 1 l of distilled water. The cells must then be collected by centrifugation and resuspended to a dense cell suspension with 10 % sucrose solution containing 5 % peptone. Then 0.1 ml portions are transferred into lyophilization ampoules, which are frozen and lyophilized according to the procedures recommended for *Rhizobium* spp. (Vincent 1970).

Caenispirillum, *Conglomeromonas*, *Constrictibacter*, *Defluviicoccus*, *Desertibacter*, *Dongia*, *Elstera*, *Ferrovibrio*, *Fodinicurvata*, and *Inquilinus*

Caenispirillum bisanense (Yoon et al. 2007a) was isolated from a sludge sample collected from the wastewater treatment plant of a dye works at Daegu, Korea. The type strains K92 and K93 were isolated on nutrient agar (Difco) and trypticase soy agar (TSA; Difco) at 30 °C, using standard dilution plating technique. Colonies on TSA are circular, raised, smooth, glistening, grayish yellow in color, and 1.5–2.5 mm in diameter after incubation for 2 days at 37 °C. *Caenispirillum salinarum* AK4T was isolated from a solar saltern at Kakinada, Andhra Pradesh, India (Ritika et al. 2012) using the same medium.

Constrictibacter antarcticus (Yamada et al. 2011), strain 262-8^T, was obtained from the white rock sample from the Skallen region in Antarctica. The white rock collected by the summer party of the 46th Japanese Antarctic Research Expedition in 2004–2005 was stored at 4 °C for 6 months. To screen for autotrophic bacteria, rock samples were crushed, added to BG-11 liquid medium (ATCC medium 616), and incubated at 25 °C in the light. Strain 262-8^T was able to grow in 0.25 × LB/MA, 0.25 × LB/ASW and marine broth 2216. After growth on 0.25 × LB/MA medium for 2 weeks, colonies were white and circular, with a diameter of 0.2 mm. Strain 262-8^T was able to form colonies microaerobically, but anaerobic growth could not be observed after 2 weeks in either light or dark conditions. No growth was observed in synthetic media. The type strain could be stored as a 20 % (v/v) glycerol suspension at −80 °C for at least 2 years.

Defluvicoccus vanus (Maszenan et al. 2005), a tetrad-forming organism, was isolated by micromanipulation from a sample of activated sludge biomass from an enhanced biological phosphorus removal (EBPR) plant in Pilsen, Czech Republic, in 1997 (Maszenan et al. 1997). For growth, freshly prepared GS medium (Williams and Unz 1985) was the most successful in supporting growth of this organism from activated sludge. Strain Ben 114^T grew very slowly on GS agar, taking 2–3 weeks to produce visible mucoid beige colonies of < 5 mm diameter. In GS broth dispersed growth was seen. Strain Ben 114^T was stored at −80 °C for 8 years.

Desertibacter roseus (Liu et al. 2011), gamma radiation-resistant bacterium, was isolated from the Taklimakan desert, Xinjiang, China. Sand was sampled from the Taklimakan desert, and 1-g samples were exposed to 10-kGy radiation at a dose of 300 Gy min at room temperature. After exposure, the samples were serially diluted in water (0.85 %, w/v, NaCl) and plated on different media TSA (Difco), nutrient agar (Difco), and R_2A agar (Difco). After incubation at 30 °C for 20 days, the type strain 2622^T was isolated on R_2A agar. Colonies were pink, circular, and convex with regular margins after growth on R_2A agar at 37 °C for 4 days at pH 8. Strain 2622^T was stored by lyophilization.

Dongia mobilis, strain LM22^T, was isolated during an investigation of the culturable microbial diversity in the activated sludge of a sequencing batch reactor for the treatment of malachite green effluent. A sludge sample was suspended in normal saline by vigorous vortexing, and 0.1 ml suspension was spread onto 1/10-diluted trypticase soy agar (TSA; Difco) and incubated at 30 °C for 1 week. A pure culture of strain LM22T was obtained after subcultivation on YP agar (Difco). Colonies on YP agar are white, transparent, smooth, circular, convex, and 0.5–1 mm in diameter after incubation at 30 °C for 3 days. Abundant growth was observed on R2A. No growth was seen on LB agar, NA, or TSA (Liu et al. 2010). Strain LM22^T was maintained on YP agar and stored in 15 % (w/v) glycerol at −80 °C.

Elstera litoralis (Rahalkar et al. 2012), a biofilm-associated bacterium, was isolated from the stones of the littoral zone (20–30-cm water depth) of Lake Constance, Germany, on 2006. The biofilm material was diluted and vortexed vigorously to disperse the bacteria. Strain Dia-1^T was isolated from the final plated dilution of the biofilm sample where EPS was used as the carbon source. It also grew well in 1:2-diluted nutrient broth supplemented with 10 mM glucose and in VM medium (pH 6.5–7.0) with ethanol as sole carbon source at room temperature (20–23 °C), both in liquid medium and on solid medium plates. In liquid VM media without shaking, the strain initially formed small aggregates or white flocks. On agar plates, milky white to cream-colored colonies were formed within 3–4 days, which turned light yellow at the periphery after extended incubations. Strain Dia-1^T grew well in VM-ethanol medium and did not grow in nutrient broth only, i.e., a sugar or ethanol was required for growth.

Ferrovibrio denitrificans (Sorokina et al. 2012) was isolated from freshly precipitated sediments from the redox zone at the FeS–Fe(OH)3 boundary in the bottom sediments of the Marka low-salinity iron-rich spring at its confluence with a sulfide spring located at the groundwater discharge zone of the Psekups mineral water deposit, Northern Caucasus (Krasnodar Krai, Russia). The cultivation medium was described by Sorokina et al. (2012) and contained 0.2 mL of a freshly prepared FeS suspension (Hanert 1981) that was added to each tube per 10 mL of the medium. The incubation time was 2–3 weeks. In agar medium, the bacteria formed small (2–3 mm in diameter), loose spherical colonies. The colonies are orange colored because of the presence of iron oxides. In liquid medium of the same composition, an ochreous precipitate is formed at the bottom of the vials. FeS, $FeSO_4$, and $FeCO_3$ are used as Fe(II) sources for lithotrophic growth.

Fodinicurvata sediminis (Wang et al. 2009) was isolated during the course of a study of the microbial diversity of the Fenggang salt mine in Yunnan, southwest China. The type strains YIM D82^T and YIM D812^T were isolated from a sediment sample collected from the salt mine by using a standard dilution-plating technique at 28 °C on Difco marine agar 2216 (MA; pH 7.2), supplemented with 3 % (w/v) NaCl. Pure cultures are maintained on nutrient agar (NA; Difco) supplemented with 5 % NaCl. Colonies are cream–white, circular, convex, and opaque with irregular margins after growth on NA supplemented with 5 % at 28 °C for 5 days. Growth occurred under anaerobic conditions. Pure cultures are maintained on nutrient agar (NA; Difco) supplemented with 5 % NaCl and stored as 20 % (v/v) glycerol suspensions at −80 °C.

Inquilinus limosus was isolated from respiratory secretions of cystic fibrosis patients in the USA in 1995 (Pitulle et al. 1999) and described by Coenye et al. (2002). The type strain AU0476^T grows on BCSA at 32 °C. During the course of a study on the culturable aerobic and facultatively anaerobic bacterial community of ginseng field soil in Pocheon Province, South Korea, a large number of bacteria were isolated (Im et al. 2005). One of these isolates, Gsoil 080^T, was identified as *I. limosus*. It was one of the several isolates that appeared on modified R_2A agar plates under aerobic conditions and was routinely cultured on R_2A agar (Difco) at 30 °C. After 2 days of incubation on R_2A agar, colonies are creamy white, round to slightly irregular, and 1.0–5.0 mm in diameter. It was routinely cultured on R_2A agar (Difco) at 30 °C and maintained as a glycerol suspension (20 %, w/v) at −70 °C.

Insolitispirillum–Limimonas–Magnetospira–Magnetospirillum–Magnetovibrio–Marispirillum–Nisaea–Novispirillum–Oceanibaculum

Magnetospirillum gryphiswaldense was isolated from water and the muddy upper layers of sediment collected from the eutrophic river Ryck near Greifswald, Germany, after magnetotactic enrichment collected from jars. Jars were filled with 100 ml mud and 200 ml water. Magnetotactic bacteria found near the magnetic pole were collected with a pipette over several weeks. After centrifugation, a drop of the cell concentrate was placed on one edge of a 5-cm-long and 1-mm-wide strip of sterile soft agar (2 g agar/I tap water) processed as describe by Scheifer et al. (1991) and used as inocula for the isolation medium consisting of 50 ml mud, 100 ml water, and 1.5 mg disodium succinate. It was filled into 200-ml bottles and sterilized at 121 °C for 20 min. The bottles were tightly sealed by rubber stoppers. The inoculated medium was incubated at 30 °C for 10 days.

Magnetospirillum magnetotacticum strain MS-1 was isolated from sediments collected in Cedar Swamp (Woods Hole, MA, USA) after enrichment of sampled material by application of steady, nonuniform magnetic fields as described by (Blakemore et al. 1979). The semisolid isolation medium consisted of (per 90 ml of distilled water) 10 ml of filtered swamp or bog water, 1 ml of vitamin elixir (23), 1 ml of mineral elixir (Wolin et al. 1963), and 0.5 mM potassium phosphate buffer (pH 6.7). To this mixture were added 5 g of vitamin B12, 25 mg of NH_4Cl, 10 mg of sodium acetate (anhydrous), 0.2 mg of resazurin, and 90 mg of Ionagar no. 2 (Oxoid). The pH was adjusted to 6.7 with NaOH. A well-isolated area of growth was homogenized, and cells were cloned by serial dilution into tubes containing molten, prereduced isolation medium containing 0.85 % (wt/vol) Ionagar no. 2. Well-isolated colonies which appeared in these tubes after 1 week at 30 °C were homogeneous as evidenced by microscopy. Strain MS-1 was maintained at 30 °C with weekly transfers in screw-capped culture tubes containing a semisolid growth medium consisting of (per 98 ml of distilled water) 1 ml of vitamin elixir, 1 ml of mineral elixir, 5 mM KH_2PO_4, 25 MuM ferric quinate, and 0.2 mg of resazurin. To this mixture were added (per 100 ml) 0.1 g of succinic acid, 20 mg of sodium acetate (anhydrous), 10 mg of $NaNO_3$, 5 mg of sodium thioglycolate, and 130 mg of agar (GIBCO Laboratories). The ferric quinate solution was prepared by combining 2.7 g of $FeCI_3$ and 1.9 g of quinic acid with 1 l of distilled water. Before adding the agar, the pH of the medium was adjusted to 6.7 with NaOH. The medium was boiled, and 12 ml was added to each

screw-capped tube (16 by 125 mm) containing approximately 0.1 ml of 5 % (wt/vol) sodium thioglycolate in distilled water. After autoclaving, the medium stands overnight for the establishment of O_2 gradients.

M. magneticum AMB-1^T is a magnetic bacterium capable of growing aerobically, isolated from freshwater sludges and sediments obtained from ponds at Koganei in Tokyo (Matsunaga et al. 1991). Separation of magnetic bacteria from sediment and water samples was achieved using an apparatus adapted from Matsunaga and Kamiya (1987). This apparatus allowed that magnetic bacteria migrated through the cotton plug toward the south pole of a samarium–cobalt (Sin-Co) magnet (produced by TDK, Tokyo, Japan) placed on the side of the sterile solution. Dark gray suspension around the magnet was sampled with a pipette and inoculated into the isolation medium. The isolation medium contained (per liter of distilled water) 2 ml Wolfe's mineral solution (Wolin et al. 1963), 0.2 g potassium dihydrogen phosphate, 0.12 g sodium nitrate, 0.02 g yeast extract, 0.02 g malt extract, and 0.05 g L-cysteine HC1. H_2O, 10 M ferric gallate (prepared in 100 ml distilled water containing 0.27 g $FeCI_3$ and 0.19 g gallic acid.), and 0.5 mg biotin. The medium was adjusted to pH 7.0, and after sterilization, 0.6 ml of 10 % glucose filter sterilized (pore size 0.45 m) solution was added to the medium.

The *Magnetospirillum* strains can be routinely grown microaerobically in semisolid (1.5 g l^{-1} agar Noble; Difco Laboratories) revised Magnetic Spirillum Growth Medium (MSGM) (American Type Culture Collection, 1989 – available at http://www.atcc.org). Bazilinski et al. (2000) proposed the following modifications to MSGM during a study of nitrogen fixation in *Magnetospirillum* strains: tartaric acid was omitted and the concentration of succinic acid was raised to 5 mM; ascorbic acid was replaced by 0.1 g l^{-1} sodium thioglycolate; the Wolfe's mineral solution added at 5 ml l^{-1} was modified by increases in the amounts of $Na_2MoO_4.2H_2O$ (from 0.01 g to 0.4 g l^{-1}) and $CuSO_4.5H_2O$ (from 0.01 g to 0.02 g l^{-1}) and by the addition of 0.01 g l^{-1} $NiCl_2.6H_2O$. They grow in the presence of NH_4Cl (4 mM), $NaNO_3$ (8 mM), and N_2 as sole nitrogen sources.

Magnetospirillum bellicus, the second dissimilatory perchlorate-reducing bacteria (DPRB), was isolated from the surface of a working electrode in an active perchlorate-reducing bioelectrical reactor (BER) that was inoculated with water from Strawberry Creek on the University of California, Berkeley, campus (Thrash et al. 2007). Perchlorate-reducing enrichments were established by transferring 1 g of electrode surface scrapings into 9 mL of prepared anoxic medium as indicated by Miller and Wolin (1974) under a gas stream of N_2-CO_2 (80:20; v/v). Acetate (590 mg L^{-1}) was the electron donor and perchlorate (990 mg L^{-1}) was the electron acceptor. Incubations were done at 30 °C in the dark. Positive enrichments were identified by visual increase in optical density and by microscopic examination. Once a positive enrichment was established, the perchlorate-reducing culture was transferred (10 % inoculum) into 9 mL of fresh anoxic medium. Isolated colonies were obtained from transfers of positive enrichments by the standard agar shake-tube technique with acetate (590 g L^{-1}) as the sole electron donor and perchlorate (990 mg L^{-1}) as the sole electron acceptor.

Magnetospirillum aberrantis was isolated from the coastal bottom sediment of the Ol'khovka River in the city of Kislovodsk (Gorlenko et al. 2011). Enrichment cultures were obtained by microaerobic incubation of the medium inoculated with the bottom sediments. The medium contained the following (g/l): KH_2PO_4, 0.4; NH_4Cl, 0.33; KCl, 0.33; $MgCl_2$, 0.33; Na_2SO_4, 0.25; $Na_2S_2O_3$, 0.25; $NaNO_3$, 0.33; $NaHCO_3$, 0.25; sodium acetate, 1.0; and yeast extract, 0.1, as well as Fe(III) citrate, 30 μM; resazurin, 0.5 mg/l; sodium thioglycolate, 50 mg/l; vitamin B12, 15 μg/l; and trace elements, 1 ml/l. The optimal oxygen concentration determined by cultivation in sealed Hungate tubes was from 1 % to 20 %. Magnetic separation was used to obtain pure bacterial cultures as described by Gorlenko et al. (2011). Bacteria were grown in 5-ml syringes under microaerobic conditions with a small air bubble at 30°C, pH 6.7. The same medium was used for the subsequent cultivation of the isolates.

Magnetospira thiophila strain MMS-1 was obtained from mud and water samples using the capillary magnetic racetrack technique (Wolfe et al. 1987). Concentrated magnetotactic cells were inoculated into ASW medium containing 5 ml of modified Wolfe's mineral elixir (Frankel et al. 1997), 0.25 g of NH_4Cl, and 100 μL of 0.2 % (wt/vol) aqueous resazurin. To produce an oxygen gradient, the medium was modified into semisolid by addition of 2.0 g of Agar Noble. Cultures were incubated at 25–28 °C and cells grew as a microaerophilic band at the oxic–anoxic transition zone of the tubes (pink/colorless interface). Cells also grew in this same medium when 3.7 mM sodium succinate replaced the thiosulfate. Separate colonies were obtained in a serial dilution of a culture in ASW solid medium shake tubes with succinate as the electron donor. Colonies were removed aseptically and the process was repeated three times and the purity of the cultures was determined using light microscopy as described in Williams et al. (2012). To achieve a sufficient yield of biomass cells, these authors grew MMS-1 chemolithoautotrophically in 2L glass bottles containing 850 ml using the same medium modified by the addition of thiosulfate ($S_2O_3^{2-}$) as the electron donor and O_2 as the terminal electron acceptor. After sterilization, the medium was cooled to room temperature, and the following solutions were injected (per liter) into the medium bottles, in order, from oxygen-free stocks (except for the cysteine, which was made fresh and filter sterilized directly into the medium): 1.5 ml of 0.5 M Potassium phosphate buffer, pH 6.9, neutralized cysteine HCl.H_2O to give a final concentration of 0.04 g l1, 10 ml of 25 % (wt/vol) $Na_2S_2O_3.5H_2O$, and 0.5 ml of vitamin solution (Frankel et al. 1997). The medium was allowed to become reduced (= colorless), after which 2.5 ml of 0.01 M $FeSO_4$ dissolved in 0.2 N HCl was injected. The medium was inoculated with several bands of cells from semisolid medium, after which sterile O_2 was introduced (0.4 % of the final headspace), and carefully placed at 25 °C for O_2 gradient establishment, indicated by pink color at the surface while the remaining medium

remained colorless. Growth initiated at the oxic–anoxic interface near the surface, and as growth increased, O_2 in the headspace was replenished up to a maximum of 4 % of the headspace every 24–48 h during 7–10 days.

Magnetovibrio blakemorei was isolated from shallow, brackish, salt-marsh pools near the Neponset River estuary in Milton, MA, USA. Samples were placed under dim light at room temperature, and after several days, formation of a horizontal "plate" of microorganisms in the water column was observed in one of the bottles. The characterization of the environmental conditions that favored the enrichment of this bacterium suggested that the gradient of sulfide was in the presence of an opposing gradient of oxygen diffusing from the surface to the bottom and thus the plate probably formed at the oxic–anoxic interface within the bottle at pH 7.5. Cells removed from the plate were used to inoculate sulfide–O_2 concentration gradient medium, prepared following the recipe of Nelson and Jannasch (1983) but modified by using diluted artificial seawater (ASW) solution rather than natural seawater and by the addition of 25 mM ferric quinate (Blakemore et al. 1979) and 200 µl 0.2 % aqueous resazurin per liter. The ASW was adjusted to approximately 23 % and consisted of (g l^{-1}) NaCl, 16.4; $MgCl_2.6H_2O$, 3.5; Na_2SO_4, 2.7; KCl, 0.47; and $CaCl_2.2H_2O$, 0.39. After enrichment into ASW modified medium, small amounts of magnetotactic cells were observed in a low percentage of the cultures forming microaerophilic bands of cells. For isolation of the strain, cells from these enrichment gradient cultures were inoculated in a dilution series of solid agar (13 g Agar Noble l^{-1}; Difco Laboratories) shake tubes of ASW [O_2]-gradient medium containing 5 ml modified Wolfe's mineral elixir containing 0.5 ml vitamin solution as described in Bazylinski et al. (2013). Anoxic conditions and the use of nitrous oxide (N_2O) at a pressure of 2 atm (202.7 kPa) were necessary to avoid contamination by nonmagnetic bacteria. After 2–3 weeks, shake tube black, lens-shaped colonies consisting of the magnetotactic vibrio individual colonies were removed and used as inocula for a second series of shake tubes, and the process was repeated once more to ensure purity of the culture. Since then, cells of *M. blakemorei* are routinely grown in oxygen-free liquid cultures of ASW modified medium containing 5 ml modified Wolfe's mineral elixir and N_2O at 1 atm as the terminal electron acceptor.

Species of the genera *Novispirillum* and *Insolitispirillum* are routinely grown on LMG medium no. 8 (composition per liter: 1 g succinic acid, 10 g peptone, 1 g $(NH_4)_2SO_4$, 1 g $MgSO_4.7H_2O$, 2 mg $FeCl_3.6H_2O$, 2 mg $MnSO_4.H_2O$, and 15 g agar, pH 7.0).

Nisaea spp. were isolated from one of the major sites of water-column denitrification among the world's oceans using filter-sterilized seawater from the isolation site for the preparation of media and dilution to extinction as described by Schut et al. (1993). After 1 month at 20 °C, positive cultures were plated on seawater R2A agar (Difco) and incubated at 20 °C for 1 week. After subculturing, two isolates forming cream-colored colonies on Marine Broth 2216 medium (MB; Difco) were obtained and designated as *N. denitrificans* DR41_21^T and *N. nitritireducens* DR41_18^T. According to genus description (Urios et al. 2008), growth occurs at 15–44 °C (optimum, 30 °C), at pH 5.0–9.0 (optimum, pH 6.0), and at salinities in the range 0–60 g l^{-1} (optimum, 20 g l^{-1}).

Marispirillum indicum was isolated from the seawater of the Southwest Indian Ridge, Indian Ocean (Lai et al. 2009a). Seawater sample was added with crude oil, as carbon and energy source, for enrichment of oil-degrading bacteria. After 2 months, 1 ml enrichment culture was transferred into 100 ml fresh MM medium as described in Lai et al. (2009b). Sequential transfers were performed three times at intervals of 2 weeks and incubation at 28 °C with shaking at 160 r.p.m. Bacteria were isolated using the plate screening method on 216 L medium (containing, per liter seawater: CH_3COONa, 1.0 g; tryptone, 10.0 g; yeast extract, 2.0 g; sodium citrate, 0.5 g; NH_4NO_3, 0.2 g; pH 7.5). The 216 L medium was used for all studies of strain B142^T.

Oceanibaculum species were isolated from deep seawater during a survey for PAH-degrading bacteria (Lai et al. 2009b; Dong et al. 2010). PAH-degrading bacteria media containing 1 % (v/v) sterilized crude oil and two different PAH mixtures were used for enrichment. *O. indicum* P24^{T}was isolated from the Southwest Indian Ridge, using PAH mixture containing naphthalene, phenanthrene, anthracene, and pyrene, at 200 p.p.m each, dissolved in crude oil as the carbon and energy source. *O. pacificum* strain LMC2up-L3^T was isolated from a hydrothermal field of the southwest Pacific Ocean, using PAH mixture containing naphthalene and phenanthrene at a final concentration of 100 p.p.m. and 20 p.p.m. of pyrene. After 2 months, 1 mL of each enrichment culture was transferred into 100 ml fresh seawater medium containing per liter 1.0 g NH_4NO_3, 0.5 g KH_2PO_4, and 2.8 mg $FeSO_4.7H_2O$, using the PAH mixture respective to each species, as the sole carbon and energy source. After 3 weeks of incubation at 28 °C with shaking at 160 r.p.m., each culture was transferred repeatedly to the same medium for further enrichment every 4 weeks three times. Bacteria were isolated by using the plate screening method on 216 L medium (per liter seawater: 1.0 g CH_3COONa, 10.0 g tryptone, 2.0 g yeast extract, 0.5 g sodium citrate, and 0.2 g NH_4NO_3; pH 7.5). For further studies of these species, the same medium has been used as described by Lai et al. (2009b) and Dong et al. (2010).

Limimonas halophila (Amoozegar et al. 2013) designated strain IA16^T was isolated using the modified growth medium (MGM) with 24 % (w/v) total salt concentration as described (Dyall-Smith 2008): 5 g peptone (Oxoid), 1 g yeast extract, and 200 ml pure water with 767 ml of a stock salt solution that contained (L^{-1}) 240 g NaCl, 35 g $MgSO_4.7H_2O$, 30 g $MgCl_2.6H_2O$, 7 g KCl, and 1 g $CaCl_2$. The pH of the medium was adjusted to pH 7.2–7.4 with Tris base, and agar was added to the medium to give a final concentration of 1.5 % (w/v). The isolation procedure consisted of spreading mud sample serial dilution in sterile 20 % (w/v) on plates of MGM agar. After growth development, achieved at 40 °C after 2 months under aerobic conditions, successive cultivation leads to pure isolate of IA16^T.

Pelagibius–Phaeospirillum–Phaeovibrio–Rhodocista–Rhodospira

Pelagibius litoralis was isolated from coastal seawater off the east coast of Korea. Autoclaved seawater (500 ml) supplemented with urea (100 mM) was inoculated with seawater (100 µl) and incubated at 20 °C in the dark for about 8 months. Incubated material was spread on a Marine Agar 2216 (Difco) plate (https://www.bd.com/europe/regulatory/Assets/IFU/Difco_BBL/212185.pdf) following incubation aerobically at 30 °C for 2 weeks. Purified cultures were obtained by subsequently streaking the isolated strain onto fresh MA plates at 30 °C under aerobic conditions. Pure cultures were stored in Marine Agar 2216 at 30 + C and in Marine Broth 2216 (Difco) supplemented with 30 % (v/v) glycerol at −80 °C (Choi et al. 2009).

In general, media and growth conditions for *Phaeospirillum* species can be the same applied for other freshwater photosynthetic non-sulfur bacteria such as *Rhodospirillum*, considering the need to establish and maintain reduced oxygen partial pressure. Cultures can be cryopreserved in liquid nitrogen or at −80 °C through standard techniques.

Phaeospirillum fulvum and *Phaeospirillum molischianum* were both isolated from an enrichment culture using mud or surface water as inoculum in a glass stoppered bottle. Carprylate or pelargonate (up to 0.04 % at pH 7.5) were added to the mineral salt media used to isolate *P. fulvum* (van Niel 1944; Imhoff et al. 1998) and can provide selective growth conditions for *P. fulvum* and *P. molischianum*. Hay was used as organic substrate to isolate *P. molischianum* (Giesberger 1947; Imhoff et al. 1998). Pure cultures were obtained by using successive dilutions of enrichment culture in agar medium under anaerobic conditions according to van Niel (1944).

Phaeospirillum chandramohanii was isolated from a photolithoheterotrophic enrichment of a water sample from a freshwater reservoir sampled at Mudasarlova (India), using mineral medium (Biebl and Pfennig 1981) supplemented with $Na_2S.9H_2O$ (1 mM) plus 0.3 % pyruvate (w/v), in anaerobiose (Kumar et al. 2009). Pure cultures were obtained by using the repeated agar shake dilution method (Pfennig and Truper 1992; Imhoff 1988) using the medium described by Pfennig and Truper (1974) supplemented with $Na_2S_2O_3$ (4 mM).

Phaeospirillum oryzae was isolated from an enrichment culture of the rhizosphere soil of a paddy (Nadergul, India), using a photoheterotrophic medium (Biebl and Pfennig 1981), pH 7.0, incubated at 2,400 lx, 28–30 °C for 7 days in fully filled screw-capped bottles (Lakshmi et al. 2011a). Pure cultures were obtained by repeated streaking on agar slants in test tubes (25 × 150 mm) sealed with butyl rubber corks and replacing the gas phase with argon to achieve anaerobic conditions. Purification media contained (g l^{-1}) KH_2PO_4 (0.5), $MgSO_4.7H_2O$ (0.2), NaCl (0.4), NH_4Cl (0.6), $CaCl_2.2H_2O$ (0.05), sodium pyruvate (0.5), sodium succinate (0.5), sodium acetate (0.5), yeast extract (0.3), ferric citrate (5 ml l^{-1} forms a 0.1 % w/v stock solution), and trace element solution SL 7 (1 ml l^{-1}; Biebl and Pfennig 1981).

Phaeospirillum tilakii was isolated from an enrichment culture of aquatic sediment (Nelapattu, India) using photoheterotrophic medium prepared according to Lakshmi et al. (2011b) containing the following: NH_4Cl (18 mM), $MgSO_4.7H_2O$ (1.2 mM), $CaCl_2.2H_2O$ (1.3 mM), KH_2PO_4 (3.6 mM), NaCl (17 mM), sodium succinate (7.4 mM), yeast extract (2.0 g l^{-1}), and Na_2HPO_4 (2 mM), pH 7.0. Enrichment cultures were incubated at 2,400 lx, 28–30 °C for 7 days in fully filled screw-capped bottles (Raj et al. 2012). Pure cultures were obtained by repeated streaking on agar slants in test tubes (25 × 150 mm) sealed with butyl rubber corks and replacing the gas phase with argon to achieve anaerobic conditions. Purification media contained (g l^{-1}) KH_2PO_4 (0.5), $MgSO_4.7H_2O$ (0.2), NaCl (0.4), NH_4Cl (0.6), $CaCl_2.2H_2O$ (0.05), sodium pyruvate (0.5), sodium succinate (0.5), sodium acetate (0.5), yeast extract (0.3), ferric citrate (5 ml l^{-1} forms a 0.1 % w/v stock solution), and trace element solution SL 7 (1 ml l^{-1}; Biebl and Pfennig 1981).

Phaeovibrio sulfidiphilus was isolated from sediment of a brackish shrimp pond (pH 8.2) at Vadkku Poigainallur (India). Enrichment culture was obtained in a photoheterotrophic medium containing the following: NH_4Cl (18 mM), $MgSO_4.7H_2O$ (1.2 mM), $CaCl_2.2H_2O$ (1.3 mM), KH_2PO_4 (3.6 mM), NaCl (17 mM), sodium succinate (7.4 mM), yeast extract (2.0 g l^{-1}), and Na_2HPO_4 (2 mM), pH 8.2. Cultures were incubated at 2,400 lx, 28–30 °C for 7 days in fully filled screw-capped bottles (Lakshmi et al. 2011b). Pure cultures were obtained by repeated streaking on agar slants in test tubes (25 × 150 mm) sealed with butyl rubber corks and replacing the gas phase with argon to achieve anaerobic conditions. Purification media contained KH_2PO_4 (3.6 mM), $MgSO_4.7H_2O$ (0.8 mM), NaCl (6.8 mM), NH_4Cl (11 mM), $CaCl_2.2H_2O$ (0.34 mM), sodium pyruvate (4.5 mM), sodium succinate (1.8 mM), sodium acetate (3.6 mM), yeast extract (0.3 g l^{-1}), Na_2S (1 mM), $NaHCO_3$ (100 mM), ferric citrate (0.2 mM), and trace element solution SL 7 (1 ml l^{-1}; Biebl and Pfennig 1981).

Rhodocista centenaria was isolated from a water sample collected at the edge of a hot spring (55 °C) at Wyoming (USA) (Favinger et al. 1989). Enrichment culture was at 40 °C using a procedure selective for anoxygenic N_2-fixing photosynthetic bacteria according to Guest et al. (1985). Maintenance of pure cultures can be achieved in SA agar medium kept at 10 °C in the dark (Kawasaki et al. 1992).

Rhodocista pekingensis originates from activated sludge from a municipal wastewater treatment plant in Beijing (China). Dilluted samples were inoculated in soft-agar (0.7 % agar) tubes using the following media modified from the AT medium (Imhoff and Trüper 1992): ATB medium with butyrate as sole carbon source, ATY medium with 0.05 % w/v yeast extract and removal of sodium hydrogen carbonate, and ATYP medium with addition of 0.03 % w/v peptone to ATY medium. Inoculated tubes were incubated anaerobically at 34–41 °C under incandescent illumination of 1,000–2,000 lx for 1 week. After incubation period, pink-reddish colonies were picked and streak onto agar plates (1.5 % agar) with the same medium and incubation conditions (Zhang et al. 2003).

Rhodospira trueperi was isolated from the peach-colored layer of a laminated microbial mat in Massachusetts (USA). Material was suspended in sterile seawater and inoculated in a deep-agar dilution series. Cultures were grown phototrophically in 100-ml screw-capped bottles with rubber seals, at 20–22 °C and a light intensity of 300–500 lx, using basal medium containing (g l^{-1}) KH_2PO_4 (0.25), NH_4Cl (0.4), KCl (0.35), NaCl (20.0), $MgSO_4.7H_2O$ (2.8), $CaCl_2.2H_2O$ (0.25), $NaHCO_3$ (1.5), $Na_2S.9H_2O$ (0.3), 1 ml vitamin solution (Pfennig and Trüper 1981), 1 ml trace element solution SL 12 (Overmann et al. 1992), 3 mM acetate, and 1 % w/v washed agar. Pure cultures were obtained after repeated deep-agar dilution series (Pfennig et al. 1997). Pure cultures were stored at 4 °C in the dark.

Rhodospirillum–Pararhodospirillum–Rhodovibrio

A number of media have been used for the isolation and enrichment of *Rhodospirillaceae* species (Biebl and Pfennig 1981; Imhoff and Trüper 1992). Among these, a mineral medium has been used for culturing the majority of "purple non-sulfur bacteria" (J. F. Imhoff 2005b): AT medium contains 1.0 g·L^{-1} KH_2PO_4, 0.5 g·L^{-1} $MgCl_2{\cdot}6H_2O$, 0.1 g·L^{-1} $CaCl_2{\cdot}2H_2O$, 1.0 g·L^{-1} NH_4Cl, 3.0 g·L^{-1} $NaHCO_3$, 0.7 g·L^{-1} Na_2SO_4, 1.0 g·L^{-1} NaCl, 1 mL of sulfate-free trace element solution SLA (Imhoff and Trüper 1977; Imhoff 1992), and 1 mL of vitamin solution VA (Imhoff and Trüper 1977; Imhoff 1992). Organic carbon sources include (routinely 10 mM) sodium malate, sodium succinate, sodium pyruvate, or sodium acetate and also, for oxygen-sensitive strains, 0.5 g·L^{-1} of sodium ascorbate or 0.25 g·L^{-1} thioglycolate, added separately. The initial pH is adjusted to 6.9. Vitamin solution VA, prepared in double distilled water, contains 0.01 % biotin, 0.035 % niacinamide, 0.03 % thiamine dichloride, 0.02 % p-aminobenzoic acid, 0.01 % pyridoxal hydrochloride, 0.01 % calcium pantothenate, and 0.005 % vitamin B12. The trace element solution SLA has the following composition: 1.8 g·L^{-1} $FeCl_2{\cdot}4H_2O$, 250 mg·L^{-1} $CoCl_2{\cdot}6H_2O$, 10 mg·L^{-1} $NiCl_2{\cdot}6H_2O$, 10 mg·L^{-1} $CuCl_2{\cdot}5H_2O$, 70 mg·L^{-1} $MnCl_2{\cdot}4H_2O$, 100 mg·L^{-1} $ZnCl_2$, 500 mg·L^{-1} H_3BO_3, 30 mg·L^{-1} $Na_2MoO_4{\cdot}2H_2O$, and 10 mg·L^{-1} $Na_2SeO_3{\cdot}5H_2O$; the pH of the solution is adjusted with HCl to 2–3.

Bacteria of the *Rhodospirillum* species can be isolated through standard techniques for anaerobes in agar dilution series and on agar plates, keeping oxygen-free conditions, especially for oxygen-sensitive species (Biebl and Pfennig 1981; Imhoff and Trüper 1992). This can be accomplished by adding 0.5 g·L^{-1} of sodium ascorbate or 0.25 g·L^{-1} thioglycolate to the growth medium in completely filled screw-capped bottles. Cell cultures can be maintained by standard techniques in liquid nitrogen or at −80°C (Imhoff 2005b).

Bacteria of the *Pararhodospirillum* species can be isolated through standard techniques for anaerobes in agar dilution series and on agar plates, keeping oxygen-free conditions, as these are oxygen sensitive (Biebl and Pfennig 1981; Imhoff and Trüper 1992). This can be accomplished by adding 0.5 g·L^{-1} of sodium ascorbate or 0.25 g·L^{-1} thioglycolate to the growth medium in completely filled screw-capped bottles. Cell cultures can be maintained by standard techniques in liquid nitrogen or at −80°C (Imhoff 2005b).

Rhodovibrio species require high salt concentrations and complex nutrients for growth (Imhoff 2005a). Thus, complex media with salt concentrations of ∼10 % and anaerobic incubation in the light constitute selective conditions for the enrichment of *Rhodovibrio* species. A suitable medium for both *Rhodovibrio* species, named DSIC$^-$ or SAL (Mack et al. 1993), contains per liter: 1 g yeast extract, 1 g sodium acetate, 125 g NaCl, 10 g $MgCl_2{\cdot}6H_2O$, 0.2 g $CaCl_2{\cdot}2H_2O$, 0.5 g NH_4Cl, 0.6 g KH_2PO_4, 2.5 g K_2SO_4, 1 g $NaHCO_3$, 0.1 g $Na_2S_2O_3{\cdot}5H_2O$, 2.1 g MOPS buffer, 20 µg vitamin B12, 1 ml trace element solution SLA (see above), and pH 7. To avoid precipitation, the magnesium and calcium salts, as well as the $NaHCO_3$, are autoclaved as separate solutions. *Rhodovibrio* species can be isolated through standard techniques for anaerobes in agar dilution series and on agar plates, keeping oxygen-free conditions (J. F. Imhoff and Trüper 1992; Imhoff 2005a). This can be achieved by photosynthetic growth, in completely filled screw-capped illuminated tubes, at 37°C. Cell cultures can be maintained by standard techniques in liquid nitrogen, by lyophilization, or storage at −80°C (Imhoff 2005a).

Roseospira–Skermanella–Telmatospirillum

Roseospira marina strain CE2105 was isolated from brackish Certes Fishponds (Arcachon Bay, French Atlantic coast), which are periodically flooded with seawater. Liquid enrichment cultures were prepared from the upper layer of the anoxic sediments (Guyoneaud et al. 2002). Enrichment and isolation of strain CE2105 were obtained by using a basal medium containing filtered (0.2-µm pore size) seawater, 750 ml; distilled water, 250 ml; NH_4Cl, 0.035 % (w/v); yeast extract, 0.04 % (w/v); and Fe citrate, 0.001 % (w/v). The medium was autoclaved and cooled under a gas mixture of N_2/CO_2 (90/10, v/v). Vitamin V7 solution (Pfennig and Truper 1992; 1 ml.l^{-1}), phosphate buffer (0.1 M, pH 6.8, 36 ml.l^{-1}), and Na ascorbate/cysteine HCl (0.25 % (w/v)/0.5 % (w/v) solution at pH 7.0, 0.2 ml.l^{-1}) were then aseptically added to the medium.

Roseospira navarrensis strain SE3104 was isolated from the surface of sulfide-rich sediment from a small saline pond in the Spanish Pyrenees, formed from the outflow of a saline spring (Salinas de Oro, Navarra, Spain) with salinity varying from 2 % to 10 % (total salinity) (Guyoneaud et al. 2002). This spring water is rich in chloride (46 % w/v), sodium (28 % w/v), sulfate (15 % w/v), calcium (5 % w/v), and potassium (4 % w/v). For enrichment and isolation of strain SE3104, the culture medium was prepared according to the method of Pfennig and Trüper (1992) which contained (per liter water) 0.35 g KH_2PO_4, 0.05 g $CaCl_2.2H_2O$, 0.5 g NH_4Cl, 10 g NaCl, 0.7 g $MgCl_2.6H_2O$, 0.35 g $MgSO_4.7H_2O$, 1.5 g $NaHCO_3$, 1 ml vitamin solution V7 (Pfennig and Trüper 1981), 1 ml trace element solution SL12B containing (per liter of deionized water) 3 g $Na_2EDTA.2H_2O$,

1.1 g $FeSO_4.7H_2O$, 0.3 g H_3BO_3, 0.19 g $CoCl_2.6H_2O$, 0.05 g $MnCl_2.4H_2O$, 0.042 g $ZnCl_2$, 0.024 g $NiCl_2.2H_2O$, 0.018 g $NaMoO_4.2H_2O$, 0.002 g $CuCl_2.2H_2O$ (Overmann et al. 1992), 0.5 g yeast extract, 1.35 g (5 mM) disodium succinate, 0.68 g (5 mM) sodium acetate, and pH 6.8 and supplemented with 5 % (w/v) NaCl and 1 % (w/v) $MgCl_2.6H_2O$.

Roseospira thiosulfatophila strain AT2115 was isolated from microbial mats in French Polynesia (Tetiaroa Atoll, Society Islands) (Guyoneaud et al. 2002). The culture medium used for enrichment and isolation of strain AT2115 contained filtered (0.2-µm pore size) seawater, 1,000 ml; NH_4Cl, 0.05 % (w/v); KH_2PO_4, 0.02 % (w/v); and yeast extract, 0.05 % (w/v). The medium was autoclaved and cooled under N_2/CO_2 (90/10, v/v). Vitamin V7 solution (1 ml.l–1), $NaHCO_3$ (0.15 % w/v), and $Na_2S.9H_2O$ (0.02 % w/v) were then aseptically added to the medium. The final pH for all media was adjusted to 6.8. The media were dispensed into sterile 50-ml screw-capped bottles. Organic substrates (5 mM sodium acetate and 5 mM disodium succinate) were added just before utilization. Pure cultures were obtained by repeated application of the deep-agar dilution method (Pfennig 1978). Deep-agar tubes were incubated at 25 °C under a light/dark cycle (16 h light/8 h dark) using tungsten lamps.

The pure cultures of *Roseospira marina, Roseospira navarrensis*, and *Roseospira thiosulfatophila* are cultivated, characterized and maintained in the synthetic media with the composition (per liter of distilled water): KH_2PO_4, 0.03 % (w/v); NH_4Cl, 0.05 % (w/v); $CaCl_2.2H_2O$, 0.005 % (w/v); $MgCl_2.6H_20$, 0.1 % (w/v) (0.3 % w/v for strain SE3104); $MgSO_4.7H_2O$, 0.05 % (w/v) (0.2 % w/v for strain SE3104); NaCl, 2 % (w/v) (5 % w/v for strain SE3104); trace element solution SL12 (Overmann et al. 1992), 1 ml; and yeast extract, 0.05 % (w/v). Media were autoclaved and cooled under N_2/CO_2 (90/10, v/v). Vitamin V7 solution (1 ml.l–1), Na ascorbate (0.05 % w/v), and $NaHCO_3$ (0.15 % w/v) were then aseptically added to the medium. The final pH was adjusted to 6.8–7.0 and the medium was dispensed into sterile 50-ml screw-capped bottles. Organic substrates (5 mM Na acetate and/or 5 mM di-Na succinate) were added as substrates before use. In addition, for strains SE3104 and AT2115, $Na_2S.9H2O$ (0.02 % w/v) was also added to the medium prior to utilization. Pure cultures were grown in 50-ml screw-capped bottles and stored at +4 °C in the dark for preservation.

Roseospira visakhapatnamensis strain JA131 was isolated from a water sample (pH ~6.8, 30°C, 2–3 % (w/v) salinity) collected on 25 March 2004 from the fishing harbor at Visakhapatnam, India (17° 41′ N 83° 18 E). *Roseospira goensis* strain JA135 was isolated from a sediment sample (pH ~6.8; 30°C, 6–7 % (w/v) salinity) collected on 12 February 2005 from Kurka saltern, Goa, India (15° 29′ N 73° 49 E). Original enrichments of both strains were from photolithoheterotrophic media (anaerobic, 1 mM $Na_2S.9H_2O$ + 0.3 % (w/v) pyruvate/malate). Strain JA131 was isolated from an enriched culture containing 2 % NaCl, and strain JA135 was isolated from an enrichment containing 8 % NaCl. Subsequent culturing, purification, and characterization were as described by Biebl and Pfennig (1981) medium with the following modifications (g per liter): 1 g $MgSO_4.7H_2O$, 0.15 g $CaCl_2.2H_2O$, and 20 g NaCl and supplemented with $Na_2S_2O_3.5H_2O$ (2 mM) (Chakravarthy et al. 2007).

Skermanella parooensis was isolated from the water of the Paroo Channel in southwest Queensland, Australia (Skerman et al. 1983). A drop of water was inoculated on the surface of lake water agar (LWA) (Franzmann and Skerman 1981) plates, air-dried, and incubated for periods of up to 3 weeks. Multicellular bodies developed on some plates were transferred by micromanipulation to fresh LWA, in which each conglomerate transformed after periods of 4–8 h to actively motile cells containing highly refractive granules. Further incubation led these cells to produce water-clear colonies. Single cells selected and cultured on LWA produced this colony form. After prolonged incubation, a few of the multicellular forms appeared among the dense population.

Skermanella aerolata was isolated from air samples (20–1,000 ml) collected with a MAS-100 air sampler (a single-stage, multiple-hole impactor; Merck) on the roof of Taean Lily Experimental Station (Chungnam Provincial Agricultural Research and Extension Services in the Taean district of Korea) on 16 April 2005 (Weon et al. 2007). The sampler contained Petri dishes with R2A agar (BBL) supplemented with 200 micrograms/milliliter cycloheximide and incubated in the dark at 28 ° C for 5 days.

Skermanella xinjiangensis was isolated from sand soil sample collected from Xinjiang (An et al. 2009). Strain 10-1-101^T was isolated after dilution and plating on 0.1 × trypticase soy broth (TSB) agar plates (Difco) at 28 °C. The isolate could also grow on R2A (Difco).

Skermanella stibiiresistens was isolated from soil collected from Jixi coal mine (45° 18′ N 130° 57′ E) of Jixi City, Heilongjiang Province, Northeast China (Luo et al. 2012). The soil texture was sandy with a pH of 7.2, and total As, Sb, Fe, and Cu concentrations were 0.04, 0.01, 18.0, and 0.09 $g.kg^{-1}$, respectively. Total C, N, P, S, and nitrate concentrations were 303.0, 3.8, 0.6, 0.2, and 0.04 g kg^{-1}, respectively. Sb-resistant bacteria were isolated using CDM medium (per liter): $MgSO_4.7H_2O$, 2.0 g; NH_4Cl, 1.0 g; Na_2SO_4, 1.0 g; K_2HPO_4, 0.013 g; $CaCl_2.2H_2O$, 0.067 g; sodium lactate, 5.0 g; $Fe_2SO_4.7H_2O$, 0.033 g; $NaHCO_3$, 0.798 g; and 15.0 g agar, pH 7.2 (Weeger et al. 1999) containing 0.1 mM $C_8H_4K_2O_{12}Sb_2.3H_2O$ (potassium antimony tartrate trihydrate).

Telmatospirillum siberiense was isolated by plating of acidotolerant methanogenic consortia from northern acidic peatlands on 1 % agarose N-free mineral medium (g per liter): K_2HPO_4, 0.25; KH_2PO_4, 1.0; $CaCl_2$, 0.1; $MgSO_4$, 0.4; Na-EDTA, 0.01; $FeCl_3 \cdot 6H_2O$, 1×10^{-3}; KI, 2×10^{-4}; $CoCl \cdot 6H_2O$, 2×10^{-4}; $MnCl_2 \cdot 4H_2O$, 8×10^{-4}; $ZnSO_4$, 8×10^{-4}; H_3BO_2, 1×10^{-4}; $Na_2MoO_4 \cdot 2H_2O$, 1×10^{-4}; $CuCl_2$, 1×10^{-4}; and $NiCl_2 \cdot 6H_2O$, 2×10^{-4}, with 0.6 mM Ti(III) citrate as reducing agent and H_2:CO_2:N_2 in the headspace. Brown colonies of non-methanogenic microorganisms formed after 5–7 months of anaerobic incubation. Intensive growth occurred in liquid Na citrate medium after complete utilization of citrate in 4–7 days.

Small brown, beige, and pink colonies formed on citrate-agar medium in 10–14 days. Colonies were subcultured at 28 °C on the same liquid medium supplemented with $(NH_4)_2SO_4$ and Na citrate instead of Ti(III) citrate under N_2 (Sizova et al. 2007).

Thalassobaculum

For isolating *Thalassobaculum litoreum* CL-GR58^T, coastal seawater and sediment samples were incubated in a 150-mm-diameter glass Petri dish for around 15 months at room temperature. Without disturbing the sediment, a 100-ml sample of seawater was removed from the surface and spread on a Marine Agar 2216 (MA; Difco) plate, which was then incubated at 30 °C for 1 week. Strain CL-GR58^T was isolated and subsequently purified on MA at 30 °C four times. The strain was maintained both on MA at 30 °C and in Marine Broth 2216 (MB; Difco) supplemented with 30% (v/v) glycerol at −80 °C (Zhang et al. 2008).

Thalassobaculum salexigens CZ41-10a^T was isolated from seawater samples. Subsamples were spread on nutrient agar plates (Bio-Rad) prepared with filtered seawater and incubated at 25 °C for 2 weeks. Colonies were picked and purified by three subcultures. Among these colonies, an isolate forming cream-colored colonies was obtained and designated strain CZ41-10a^T (Urios et al. 2010).

Thalassospira

For isolating *Thalassospira lucentensis*, the culture medium consisted of autoclaved and filtered seawater supplemented with cocktail FRV (at 0.01 g l^{-1}), which contains *Spirulina* (Sigma), fish meal, and *Artemia salina* (1 :1: 1). The pH of the medium was adjusted to 7.2. A 2-l bioreactor was completely filled up with the sample and incubated at 13 °C (in situ temperature) and with slow magnetic stirring. After 24 h, a flow rate was established to obtain a dilution rate of 0.0004 h^{-1}. The setup was maintained for three months. Weekly, 100 µl of enrichment was plated onto solidified FRV medium and incubated at 13 °C. Initially, bacteria grow as very small colonies on the complex oligotrophic FRV medium. After subculturing, they are able to grow in media containing a higher nutrient content than that of the medium used for initial isolation. In fact, the peptone-yeast extract-based media, marine agar, or YEA routinely used to culture fast-growing, copiotrophic marine bacteria allow fairly good growth.

For isolating *Thalassospira xiamenensis* M-5^T and *Thalassospira profundimaris* WP0211^T, bacteria were enriched by culturing in artificial seawater medium (ASM; Liu and Shao 2005), supplemented with 10 g diesel fuel l^{-1} (strain M-5^T) or 5 g pyrene l^{-1} (strain WP0211^T) as the sole carbon source. HLB medium (modified from Luria–Bertani medium by increasing the NaCl concentration to 30 g l^{-1}; Liu and Shao 2005) was used for routine cultivation of the isolates and for most of the phenotypic tests. All cultures were incubated at 28 °C with rotation at 200 r.p.m. unless noted otherwise. As was previously found for *Thalassospira lucentensis* QMT2^T (López-López et al. 2002), both strains formed very small colonies in the oligotrophic medium, but showed fairly good growth in the HLB media (Liu et al. 2007).

Thalassospira tepidiphila 1-1B^T was isolated from petroleum-contaminated seawater during a bioremediation experiment (Kodama et al. 2008). Seawater was collected from a bioremediation tank, serially diluted, and spread onto 1 % (w/v) Gelrite plates containing the artificial seawater medium ONR7a (Dyksterhouse et al. 1995). The plates were then coated with heat-treated Arabian light crude oil (0.2 %, w/v) (Kasai et al. 2002) and incubated at 20 °C. After incubation for 3 weeks, small colonies appearing on these plates were picked and streaked onto solid plates containing Marine Broth 2216 (MB; Difco) and 1 % (w/v) Gelrite for purification. MB was used for routine cultivation. Cells of strain 1-1B^T were stored at −80 °C in MB supplemented with 15 % (v/v) glycerol.

To isolate *Thalassospira xianhensis* P-4^T (Zhao et al. 2010), 5 % sea-salt defined medium (5 % SSDM; Zhao et al. 2009) and 5 % SSDM with 0.5 % yeast extract (5 % SSDMY) were used. Solid 5 % SSDMY medium was prepared with 1.5 % agar. A sample of oil-polluted saline soil (1 g) was added to 100 ml 5 % SSDM medium supplemented with phenanthrene (100 mg ml^{-1}) in a 300-ml Erlenmeyer flask. The culture was aerobically incubated at 30 °C in darkness on a rotary shaker operating at 200 r.p.m. After 2 weeks, 10 ml culture was transferred to 100 ml 5 % SSDM medium and incubated under the conditions described above. The enrichment was performed five or six times. Next, a culture broth dilution series was spread on 5 % SSDMY agar. After incubation for 2 days, single colonies were picked and cultivated in 5 ml 5 % SSDM using phenanthrene as the sole source of carbon and energy. These isolates developed a yellowish-orange or reddish-brown color, which is an indication of ring cleavage of polycyclic aromatic hydrocarbons (Guerin and Jones 1988) and, thus, phenanthrene-degrading activity.

For isolating *Thalassospira permensis* SMB34^T (Plotnikova et al. 2011), the enrichment culture was incubated aerobically at 28 °C with shaking in Raymond's mineral medium (RMM), containing (g l^{-1}) NH_4NO_3 (2.0), $MgSO_4{\cdot}7H_2O$ (0.2), KH_2PO_4 (2), Na_2HPO_4 (3), $CaCl_2{\cdot}6H_2O$ (0.01), Na_2CO_3 (0.1), 2 ml of 1 % $MnSO_4{\cdot}5H_2O$, and 2 ml of 1 % $FeSO_4{\cdot}7H_2O$ that was supplemented with naphthalene (0.1 %, w/v) and NaCl (6 %, w/v). Strain SMB34^T was isolated by plating the enrichment onto RMM agar supplemented with 0.5 % (w/v) tryptone, 0.25 % (w/v) yeast extract, and 3 % (w/v) NaCl (designated complete Raymond's medium, CRM). The strain was routinely cultured on CRM agar and Marine Agar 2216 (MA; Difco) at 28 or 30 °C (Plotnikova et al. 2011).

Thalassospira alkalitolerans MBE#61^T and *Thalassospira mesophila* MBE#74^T were isolated from a piece of sunken bamboo in the coastal area of Japan (Tsubouchi et al. 2014). Bamboo is a fast-growing plant and significant bioresource in the east and south area of Asia. A portion (approximately 1 g) of sinker was soaked in 2 ml of sterile artificial seawater (ASW; Nihon Pharmaceuticals, Japan) and shaken briefly on

a vortex at room temperature. The immersion fluid was incubated at 25 °C for 1 day and then spread on 1.5 % (w/v) agar containing milled Japanese timber bamboo (MJTB; 2 % (w/v) milled Japanese timber bamboo and 0.5 × ASW). Both strains were isolated after incubation at 25 °C for 10 days. After incubation, small colonies appearing on MJTB plates were picked and streaked onto solid plate containing Marine Broth (MB; BD Difco) for purification. For routine cultivation, MB was used.

Tistrella

Tistrella mobilis was isolated from samples of wastewater in mineral salt medium prepared with 0.81 mM $MgSO_4$, 0.58 mM $CaSO_4$, 18 mM $FeSO_4$, 1.0 mM $NaMoO_4$ in 5 mM potassium phosphate, 50 mM ferric citrate, 3 % glucose, and 15 mM ammonium acetate (pH 7.1) (Shi et al. 2002). The polyhydroxyalkanoate (PHA) content in bacterial colonies can be determined qualitatively by observing the presence of visible, intracellular granules using a phase-contrast microscope. To recognize PHA-rich colonies, colonies grown on nitrogen-deficient agar after 5-day incubation at 30 °C are stained with Sudan Black B (0.02 % in 96 % ethanol). The dye is removed after 20 min, and the plates are then treated for 1 min with 10 ml of 96 % ethanol. The colonies of PHA-rich cells retain the dye and appear dark blue, whereas those of PHA-deficient cells decolorize and appear light gray.

Tristella bauzanensis BZ78^T was isolated from soil containing high levels of heavy oil and heavy metals (Zhang et al. 2011). For that, 10-g soil was shaken with 90 ml of sterile 1 % sodium pyrophosphate for 20 min at 150 r.p.m. Appropriate dilutions, prepared with sterile saline solution (0.9 % NaCl), were plated (0.1 ml) on R2A agar (0.05 % yeast extract, 0.05 % peptone, 0.05 % casamino acids, 0.05 % glucose, 0.05 % starch, 0.03 % sodium pyruvate, 0.03 % K_2HPO_4, 0.005 % $MgSO_4$, 1.5 % agar; pH 7; Reasoner and Geldreich 1985) and incubated at 20 °C. Strain BZ78^T was routinely cultured in R2A liquid medium at 20 °C and maintained as a suspension in skimmed milk (10 %, w/v) at −80 °C.

Tistlia

For isolating *Tistlia consotensis*, water samples were collected aseptically from the Salado de Consotá spring in 2006 by filling sterile glass containers to the brim (Díaz-Cárdenas et al. 2010). Enrichments were initiated by inoculating a 2-ml water sample in 10-ml filter-sterilized saline spring water which had been amended with 0.1 % (w/v) starch (Sigma) and 0.02 % (w/v) yeast extract (Sigma). Turbidity was observed after 10 days incubation at 37 °C. Subsequent phase-contrast microscopy (Eclipse 50i; Nikon) revealed the presence of curved and rod-shaped cells. Several colonies developed from serial dilutions of the enrichment culture streaked onto the same medium fortified with 2 % (w/v) Noble Agar (Sigma) after 3 days of incubation at 37 °C. A beige colour and slightly raised, circular, mucoid colony (1-mm diameter) was selected and the culture derived from this, designated strain USBA 355^T. Then it was routinely cultured in a basal medium (BM) supplemented with 20 mM D-glucose and 0.1 % (v/v) yeast extract. BM contained (l^{-1} deionized water) 1 g NH_4Cl, 0.3 g K_2HPO_4, 0.3 g KH_2PO_4, 3 g $MgCl_2.6H_2O$, 0.1 g $CaCl_2.2H_2O$, 0.1 g KCl, 23 g NaCl, and 1 ml Zeikus' trace element solution (Zeikus et al. 1979); the pH of the medium was adjusted to 7.1 with 1 M NaOH. Cells were preserved at −20 °C in BM supplemented with 20 % (v/v) glycerol.

Ecology

The species belonging to the family *Rhodospirillaceae* present wide range of habitats. For example, *Azospirillum* genus was first described as bacterial colonizing plant tissues, but more recently has been reported in broad range of niches, such as oil-contaminated soil and discarded road and fermentative tank. Species from the other genera have the aquatic environment (freshwater, stagnant, anoxic, acid, or saline, petroleum-contamined seawater), ocean, and saline soil as the common habitats although the species *I. limosus* was isolated from respiratory secretions of cystic fibrosis patients and from ginseng field soil.

Azospirillum–Skermanella–Desertibacter–Rhodocista–Dongia–Elstera–Inquilinus

The nitrogen-fixing genera are widespread in agricultural soils, where they are frequently associated with grasses, cereals, and crops (Bally et al. 1983; Day and Dobereiner 1976; Kirchhof et al. 1997; Ladha et al. 1987; Patriquin et al. 1983; Baldani and Baldani 2005) grown especially in soils of tropical and subtropical and temperate regions (Lavrinenko et al. 2010). More detailed, *A. lipoferum* and *A. brasilense* followed by *A. amazonense* were the first three species described and found associated with many cereals and other grasses grown in different regions of Brazil (Magalhães et al. 1983; Baldani and Baldani 2005), while the species named *A. halopraeferens* was found exclusively associated with kallar grass (*Leptochloa fusca*) grown in saline soils in Pakistan (Reinhold et al. 1987). In 1989, Khammas and collaborators isolated the species *A. irakense* using root samples of rice grown in Iraq. Many other species were also found associated with plants: *A. doebereinerae* with washed roots and rhizosphere soil of *Miscanthus sinensis* cv. Giganteus and *Miscanthus sacchariflorus* grown in Germany (Eckert et al. 2001), *A. oryzae* (Xie and Yokota 2005) with rice roots, and *A. melinis* (Peng et al. 2006) with subtropical molasses grass plants collected in China. *A. canadense* (Mehnaz et al. 2007a) and *A. zeae* (Mehnaz et al. 2007b) had its origin in the rhizosphere of corn (Zea mays) plants grown in Canada. In contrast, *A. rugosum* (Young et al. 2008) and *A. picis* (Lin et al. 2009) were enriched from contaminated soils and discarded road tar collected in Taiwan. *A. palatum* (Zhou et al. 2009) was isolated from forest soil in Zhejiang province, China, while *A. thiophilum* had its origin from a sulfide spring in Russia

(Lavrinenko et al. 2010). *A. formosense* was isolated from agricultural soil collected in Taiwan (Lin et al. 2012) and the species *A. humicireducens* from microbial fuel cell in Guangdong, China. On the other hand, the *A. largomobile* (formerly *Conglomeromonas largomobilis* subsp. *largomobilis*) was enriched from a freshwater sample collected in Australia (Sly and Stackebrandt 1999), while *A. fermentarium* was isolated from a fermentative tank in Taiwan (Lin et al. 2013). All species of the *Azospirillum* genus fix nitrogen, except *A. palatum*, a nonvalidated species. The species described more recently are less studied, and the knowledge about their ecological distributions is restricted to the original description of the type species. Therefore, more new data on the ecology of *Azospirillum* species are needed.

The genus *Azospirillum* is widely known as containing free-living nitrogen-fixing plant-growth-promoting rhizobacteria (Okon and Labandera-Gonzalez 1994, Okon and Itzigsohn 1992, and many others), and the carbon source and N-metabolism in this genus make it well adapted to several conditions of the soil and competent to colonize the rhizosphere and in some cases the inner part of the plant tissue (Döbereiner 1992; Steenhoudt and Vanderleyden 2000). They predominantly colonize the root surface, and only a few strains are able to infect plants (Patriquin et al. 1983; Döbereiner et al. 1995). Some *Azospirillum* strains have specific mechanisms to interact with roots and colonize even the root interior, while others colonize the mucigel layer or injured root cortical cell (Baldani et al. 1986). The physiological basis for the observed invasiveness of *A. brasilense* and others is not known. Even species are known which possess enzymes degrading carbon polymer structures of plant host cells, such as in the case of *A. irakense*; a conclusive model of invasiveness is not established (Khammas et al. 1989). Usually, bacteria enter in inner part of the plant using opportunities such as disrupted cortical tissue at lateral root junction, lysed root hairs, or natural cracks on the plant tissue (Steenhoudt and Vanderleyden 2000). Mainly the data on *Azospirillum* interaction with plants are based on a single species and the most studied one: *A. brasilense*. Chemotaxis is the basis of attraction and primary interaction with the host and flagella and frimbriae are involved in the adhesion, anchoring phase and irreversible adsorption that involves polar flagellum (Croes et al. (1993) and extracellular polysaccharide production (Michiels et al. 1991; Skvortsov and Ignatov 1998).

Skermanella species seems to have a large range of habits, being isolated from air, freshwater, and soil. *S. aerolata* was isolated from air samples in Korea (Weon et al. 2007), whereas *S. parooensis* was isolated in a microbial survey of waters of the Paroo Channel, Queensland, Australia. The two other species, *S. xinjiangensis* and *S. stibiiresistens*, were isolated from soil. The former was isolated from desert soil sample from Xinjiang, China (An et al. 2009), while the latter was from coal mine soil and exhibits resistance up to 4 mM Sb(III) in R2A broth (Liu et al. 2011).

Desertibacter roseus was isolated from gamma-irradiated sand sample from the Taklimakan desert, Xinjiang, China. The high resistance of this organism against the lethal actions of DNA-damaging agents including ionizing radiation and ultraviolet light (UV) has been widely reported (Zhang et al. 2007). Radiation-resistant bacteria can survive severe damage from gamma radiation, which implies that they have high DNA repair efficiency (Sghaier et al. 2008) and are adept at detoxifying reactive oxygen species (ROS) (Zhang et al. 2007).

The species of the genus *Rhodocista* have been mainly found in freshwater ecosystems; *Rhodocista* sp. T4 (tentatively *Rhodocista hanoiensis*) was isolated from an enrichment culture from a wastewater pond in Vietnam (Do et al. 2007a). An additional record at NCBI taxonomy browser assigned *Rhodocista* to include *R. xerospirillum* (no standing in the taxonomy) isolated from an upland paddy soil in India (accession no. AM072288, 98.7 % sequence similarity to *R. centenaria*). *Rhodocista* sp. strain JA353 (AM999778, 99.3 % sequence similarity to *R. peckingensis*) was indeed isolated from rhizosphere soil in India. *Rhodocista* sp. strains AR2107 and AT2107 (AJ401217 and AJ401204, respectively) were isolated from microbial mats in Rangiroa Atoll at French Polynesia and share 99.6 % and 99.3 % sequence similarities to *R. centenaria*, respectively. *Rhodocista* sp. strain W38 (KC248056, 96.3 % and 95.7 % sequence similarities to *Azospirillum irakense* and *R. peckingensis*, respectively) was obtained from a water sample, although no further information about this isolate is available. Isolation of *Rhodocista* sp. strain CAJ2-2 from the digestive organ of the Asian lady beetle *Harmonia axyridis* (HQ876734, 92.6 % and 90.7 % sequence similarities to *R. peckingensis* and *Azospirillum irakense*, respectively) is intriguing, although this genus is phylogenetically close to some *Azospirillum*, which are found in association with several plant species. In addition, sequences assigned as *Rhodocista* isolates from saline/marine environments (EU374900, EF650482, and DQ658977) are rather related to other species. In this same sense, the isolate *Rhodocista* sp. M71 (KC464867) originating from rhizosphere soil is more closely related to *Azospirillum*/*Skermanella* genus than to *Rhodocista*. Environmental 16S rRNA clone sequences related to *Rhodocista* are scarce, nevertheless reinforce the preference of this organism to inhabit freshwater environments. Few environmental sequences have >97 % 16S rRNA gene sequence similarity to *R. centenaria*; these are JF739669 (97.5 % sequence similarity) retrieved from soil from peatlands at Indonesia and JF278043 (97.8 % sequence similarity) retrieved from a biofilm grown in flow cell of an urban water canal at Singapore. Environmental clones with 16S rRNA sequences that have low similarity to *Rhodocista* reference sequences include FJ572031 (95.5 % similarity) retrieved from lake water in China. The clone sequences JF412910, JF412944, JF413199, JF413867, JF413890, JF413920, and JQ700914 (88.7–92.1 % 16S rRNA gene sequence similarities to *R. pekingensis*) were retrieved from the aquatic microbial community from the enclosed Cuatro Cienegas Basin in Mexico. Clone sequences retrieved from the vaginal microbiota of HIV-infected women were unusually assigned as *Rhodocista* sp. (JF475184, JF478845, JF487499, JF487508, JF487548, JF487561, JF487568, JF487584, JF487604, JF487608, JF487611, JF487613); nevertheless, such sequences present ~50 % coberture and 81.6–93.3 % similarity

to *R. pekingensis* that has been more closely related to *Rhodovulum*, *Ancalomicrobium*, and *Yangia* species.

The species *Dongia mobilis* LM22^T was found associated with a batch reactor for the treatment of malachite green effluent. In addition, sequences of 16S rRNA with high similarity (94–99 %) with *D. mobilis* were retrieved from a marine hot spring from Kalianda Island, Indonesia (JX047098), in soils, and in the associated Fe–Mn nodules of four regions in China (JX493260, JX493549, JX493661, JX493873, JX494044) and marine coastal sediment in India (KF465352, KF465359).

Elstera litoralis Dia-1^T was isolated from biofilms on stones in the littoral zone of Lake Constance, Germany (Rahalkar et al. 2012). *E. litoralis* was found living associated with diatoms in photic biofilms. It is possible that the extracellular polysaccharides (EPS) produced by the diatoms are an important source of organic carbon for heterotrophic bacteria in such biofilms (Bruckner et al. 2008). So far, there are no environmental clones known closely related to *Elstera.*

The genus *Inquilinus* comprises the species *I. limosus* (Coenye et al. 2002) and *I. ginsengisoli* (Jung et al. 2011). *I. limosus* was isolated from respiratory secretions of cystic fibrosis patients in the USA while *I. ginsengisoli* from ginseng field soil in Pocheon Province, South Korea. Sequences very closely related to *Inquilinus* have been retrieved from polluted soil contaminated by Zn and Cd (Gomez-Balderas et al. 2014), tufa core sample formation (FM177000) water from long-term experimental oligotrophic mesocosms in Cuatro Cienegas in Mexico (JQ701641), soil (JN64589, EF662791, GU300421), and bronchoalveolar lavage fluid from human with cystic fibrosis (DQ188295).

Magnetospirillum–Nisaea–Thalassobaculum–Oceanibaculum–Fodinicurvata–Pelagibius–Tistlia–Phaeospirillum–Telmatospirillum–Defluviicoccus–Tistrella–Constrictibacter–Rhodovibrio–Limimonas

The occurrence of MTB, surprisingly, appears to not be dependent on particularly high concentrations of iron in the environment but on the presence of an oxygen/anoxic interface that represents, in most environments, opposing gradients of oxygen from the surface and reduced compounds (usually reduced sulfur species) in sediments or water columns (reviewed by Lefévre et al. 2013a). Siderophore production was not detected in the culture supernatants of *Magnetospirillum gryphiswaldense* (Schüler and Baeuerlein 1996). However, during the growth of *M. magnetotacticum* MS-1 and *M. magneticum* AMB-1 under iron-rich condition, iron is rapidly assimilated from the medium, and the initial high concentration is reduced to levels comparable to iron-deficient cultures, thereby triggering siderophore production and excretion (Paoletti and Blakemore 1986; Calugay et al. 2003; Calugay et al. 2006). Based on this characteristic and their participation in other biogeochemical processes, Simmons and Edwards (2007) suggested that representatives of this group show a great potential for iron, nitrogen, sulfur, and carbon cycling in natural environments. Since the discovery of MTB, several morphologically and metabolically diverse types of magnetotactic bacteria are detected worldwide and ubiquitously in sediments of freshwater, brackish, marine, and hypersaline habitats as well as in chemically stratified water columns of these environments (reviewed by Frankel 2009; Lefèvre and Bazylinski 2013). Although widely spread in different ecosystems worldwide, only few axenic cultures mostly isolated from aquatic environment are available.

The chemoheterotrophic bacterium *Thalassobaculum litoreum* CL-GR58^T was isolated from coastal seawater in Gori, Korea (Zhang et al. 2008). *Thalassobaculum salexigens* CZ41-10a^T was obtained from seawater samples collected at the SOLA station located in the bay of Banyuls-sur-Mer, France, at a depth of 3 m (Urios et al. 2010). *Nisaea* species were detected in marine denitrification sites (Urios et al. 2008). No further data about their distribution and ecological importance is available.

Both *Oceanibaculum* species were isolated from a PAH-degrading consortium from deep-seawater sample collected from the Indian Ocean and Pacific Ocean. A study to determine the compositions of the bacterial community associated with oil and water phases in a mesothermic oil field showed that sequences representative of *Oceanibaculum* associated to the pooled microbial community are associated with the aqueous phase (Kryachko et al. 2012). Noteworthy, *Oceanibaculum* strains are reported to consume both water-insoluble aromatic hydrocarbons and water-soluble substances, but their primary metabolic substrate(s) must be water soluble, as suggested for other microorganisms found associated with the aqueous phase. However, further studies are necessary to elucidate the ecology of this microorganism under environmental conditions.

Two *Fodinicurvata* strains, designated YIM D82^T and YIM D812^T, were isolated from a Fenggang salt mine in Yunnan, southwest China. No environmental 16S rRNA gene sequences resembling the genus *Fodinicurvata* were deposited in the public databases.

Species of *Pelagibius* genus have been cultured exclusively from marine environment, such as coastal seawater (Korea) or in association with the coral reef *Eunicea fusca* in the USA (accession no. KC545308, 93 % sequence similarity). Environmental clones with 16S rRNA gene sequences assigned as *Pelagibius* have been identified associated to the coral reef *Montastraea faveolata* in Puerto Rico (JQ516378, JQ515728, JQ515699, JQ515532), sediments of the Baltic Sea in Finland (FR820363), groundwater contaminated with chlorinated aliphatic hydrocarbon in the southwest of North China Plain (JQ279035), microbial fuel cell (JF522342), and oil sands tailings pond in Canada (HQ043938). Indeed, DGGE bands of 16S rRNA gene sequences retrieved from a study of diet-induced lesions in the shell of lobsters (JF297201, JF297191) were also assigned as *Pelagibius.* Although most of clone sequences indicate marine environment as the natural niche of *Pelagibius*, the sequences closely related retrieved from groundwater and from a microbial fuel cell suggest that this organism can also inhabit nonmarine

environments. Further information on ecological function of members of this genus is needed.

The aerobic, chemoheterotrophic, nitrogen-fixing bacterium *Tistlia consotensis* USBA 355^T was isolated from samples collected from the saline spring, Salado de Consotá, located in the Colombian Andes (64° 40′ 43.1″ N, 75° 31′ 34.3″ W) (Díaz-Cárdenas et al. 2010). Salado de Consotá is a neutral pH spring with a salt content of 4.5 % (w/v), close to seawater, and the dominant ions are Na^+, Ca^{2+}, and Cl^-.

The habitat of the genus *Phaeospirillum* appears to be freshwater-rich environments, since all type strains were isolated from mud and rhizosphere soil. Additionally, *P. fulvum* was isolated from lake mud in Russia and from the top layer of aquatic sediments in the Netherlands. Information regarding the occurrence of additional isolation of *P. molischianum* strains is scarce, although Paterek and Paynter (1988) identified the presence of *P. molischianum* – by using morphological and physiological approaches – in the anaerobic photosynthetic bacterial community of salt-marsh sediment in the USA. Despite the need for further evidence, Paterek and Paynter (1988) findings suggest the possibility that the bacteria of this genus can also colonize saline environments. The species *P. molischianum* has an additional record at NCBI taxonomy browser assigned as *Phaeospirillum* sp. strain AK-42, isolated from sediment from a lake in India (unpublished results, accession number HF562217, 98.2 % 16S rRNA gene sequence similarity). A single record of a *Phaeospirillum* sp. strain closely related to *P. oryzae* is available at NCBI taxonomy browser, in addition to the isolated strains obtained by Lakshmi et al. (2011b) from paddy soil. *Phaeospirillum* sp. strain MPA1 was obtained in a survey to characterize spiral-shaped purple non-sulfur bacteria from New Zealand thermal environments (unpublished results, accession number AF487433, 98.8 % 16S rRNA gene sequence similarity to *P. oryzae* type strain). Additional records of *Phaeospirillum* strains related to *P. tilakii* at NCBI taxonomy browser were presented by Hisada et al. (2007), who isolated *Phaeospirillum* sp. strain TUT3101 from the microbial community associated to *Chloroflexus* and cyanobacterial mats developing at 50–65 °C from a hot spring in Japan (accession number AB250624, 98.7 % sequence similarity to *P. tilakii* type strain). Environmental 16S rRNA clone sequences listed in the NCBI taxonomy browser – as well as some isolates such as *Phaeospirillum* sp. JA795 (accession no. HF559003) – related to *Phaeospirillum* include ambiguous assignments, as their relateness to *Phaeospirillum* reference sequences is lower than to other bacterial species. In this sense, although low identity to *Phaeospirillum* type species is mostly observed, few environmental sequences will be presented as putative members of this genus. These include KC994874 (identical to KC994669), retrieved from a metagenome survey of biofilms associated with the microalgae *Chlorella vulgaris* and *Scenedesmus obliquus* in a photobioreactor in Germany (Krohn-Molt et al. 2013), with a sequence similarity of 89.2 % to *P. chandramohanii*. Sequences KF523288 and JF340073 were obtained from agricultural soil in China and share 94.7 % sequence similarity to *P. chandramohanii* and 100 % sequence similarity to *P. fulvum*, respectively, even though the similarity to other few reference sequences was the same, probably due to the short size of these sequences (169 and 172 bases, respectively). The sequences JF278044 and HM371256, with 90.3 % and 90.7 % sequence similarities to *P. tilakii*, respectively, were retrieved from biofilm grown on polyethylene terephthalate water canal in Singapore and from the estuarine belt of Narmada River (India), respectively. Sequences DQ252395 and EU682492 with 94.5 % and 91.0 % sequence similarities to *P. fulvum*, respectively, were cloned from the indigenous microflora associated with the decomposition of jute stalks in India and from the surface of coastal marine sediments in Hong Kong, respectively. Sequence GQ257682 with 98.8 % sequence similarity to *P. oryzae* was retrieved from groundwater contaminated with the explosive compound RDX in the USA. In addition, Rudney et al. (2010) performed a metaproteomic survey from the human salivary microbiota and assigned one peptide to *P. molischianum*. Lenchi et al. (2013) used pyrosequencing analysis to assign 16S rRNA gene sequences to *Phaeospirillum* as part of the bacterial community associated with production waters from flooded wells and in injection waters used for flooding Algerian oilfields (temperature range 51–96 °C, salinity range 0.58–21.18 g NaCl l^{-1}). It is clear from the above data that *Phaeospirillum* species have a preference for freshwater environments and that its inhabitation in marine environments needs further experimental evidence.

Telmatospirillum siberiense was isolated from northern acidic waters from peatlands under *Sphagnum* moss mats (Sizova et al. 2007).

Defluvicoccus genus with only one species, *D. vanus* Ben 114^T, was isolated from a sample of biomass from an enhanced biological phosphorus removal activated sludge plant in the Czech Republic. Several clones closely related to *Defluvicoccus* were detected in marine samples (JQ516397, JQ793263, FN687095, and JN210804); others are from activated sludge (KC797679) or contaminated soils (HE974846) and gypsum-treated oil sands tailings pond (HQ044218, HQ041215, HQ042479, HQ042046); some are also related to human diseases as cystic fibrosis sinuses (JQ794646) and cutaneous microbiome (KF509289).

The type strain *Tistrella mobilis* IAM 14872^T was isolated from wastewater in Thailand and showed a good ability in producing polyhydroxyalkanoates (PHA) efficiently (Shi et al. 2002). *Tistrella bauzanensis* BZ78^T was isolated from soil containing high levels of heavy oil and heavy metals located in Bozen, South Tyrol, Italy (Zhang et al. 2011).

The unique species of *Constrictibacter*, *C. antarcticus* 262-8^T, was isolated from a cavity within white rock collected in the Skallen region of Antarctica. Environmental 16S rRNA clone sequences related to the genus *Constrictibacter* have not been deposited.

Limimonas is a genus of extremely halophilic microorganism in this family isolated from a saline mud sample collected from the hypersaline Lake Aran-Bidgol in Iran, and its characterization led to the description of the species

Limimonas halophila (Amoozegar et al. 2013). This microorganism is able to tolerate salt concentrations in the range of 15–30 % (w/v).

Rhodospirillum–Pararhodospirillum–Phaeovibrio–Roseospira–Rhodospira–Novispirillum–Marispirillum–Insolitispirillum–Caenispirillum–Thalassospira–Magnetospira–Magnetovibrio–Ferrovibrio

Stagnant and anoxic freshwater that is exposed to the light is the habitat of *Rhodospirillum* and most *Pararhodospirillum* species (Imhoff 2005b; Kumar et al. 2008; Lakshmi et al. 2014). A number of culture-dependent and culture-independent studies have shown that anoxic zones of saline or hypersaline environments such as seawater, brines, and salt lakes that are exposed to the light are the habitat of *Rhodovibrio* species (Nissen and Dundas 1984; Mack et al. 1993; Sørensen et al. 2005; Blazejak et al. 2006; Maturrano et al. 2006; Boutaiba et al. 2011; Atanasova et al. 2012; Makhdoumi-Kakhki et al. 2012; Liu and Liu 2013; Ntougias 2014; Schneider et al. 2013). Additional information on strains of *Phaeovibrio* is lacking, and the description of the species is the only source of ecological distribution (Lakshmi et al. 2011b). Environmental 16S rRNA sequences related to *Phaeovibrio* genus have not been deposited in the databases. Most closely related sequences are from *Rhodospirillum* (~93 % 16S sequence similarity).

Roseospira species are spiral-shaped purple nonsulfur bacteria which are slightly to moderately halophilic. The main habitat seems to be anoxic sediments in coastal waters such as brackish lagoons and saline springs. *R. navarrensis* was isolated from a microbial mat in Tetiaroa Atoll, Society Islands (Guyoneaud et al. 2002).

The description of the type species of *Rhodospira mediosalina* is the only source of ecological distribution (Pfennig et al. 1997), and information on additional strains of *Rhodospira* is lacking since environmental 16S rRNA sequences related closely to the genus *Rhodospira* have not been deposited in the databases. The available 16S rRNA gene sequences are HE797786 (94.5 % similarity), retrieved from anoxic photosynthetic biofilm from brackish water in France, and AM691104 (94.3 % similarity), retrieved from a hypersaline spring in Canada.

Marispirillum indicum strain B142^T is a marine bacterium isolated after enrichment from crude oil-contaminated seawater (Lai et al. 2009a). At the time of characterization, it showed the highest 16S rRNA gene sequence similarity (97.1 %) with an uncultured proteobacterial clone isolated from subsurface water from the Kalahari Shield in South Africa, but no further data about species distribution is available.

Species of *Novispirillum* and *Insolitispirillum* were isolated from primary oxidation pond water (Pretorius 1963; Hylemon et al. 1973).

The species of the genus *Caenispirillum* have been isolated from aquatic ecosystems; the species *C. bisanense* was isolated from sludge sample collected from the wastewater treatment plant of a dye works at Daegu, Korea, while *C. salinarum* from a sediment sample collected from a solar saltern at Kakinada, Andhra Pradesh, India. Uncultured 16S rRNA clone sequences related to *C. bisanense* K93 have been deposited in the NCBI databases. KF500423 (96 % sequence similarity) was obtained from the shrimp culture pond sediment in India and JF421153 (98 % sequence similarity) from a petroleum-contaminated saline–alkali soil with phytoremediation in China (unpublished).

Thalassospira lucentensis was isolated from offshore seawater samples obtained at 38° 06′87″ N, 0° 05′ 23″ W (21 nautical miles off Alicante, Spain) from a depth of 100 m by means of a Niskin bottle. For its isolation, enrichments in a continuous culture reactor designed to maintain extremely oligotrophic conditions such as those assumed to be found in the open ocean were carried out (López-López et al. 2002). *Thalassospira xiamenensis* M-5^T was isolated from surface water collected from a waste-oil pool at an oil storage dock in the city of Xiamen, Fujian Province, on the eastern coast of China (Liu et al. 2007). This seawater-based waste-oil pool had suffered long-term pollution with crude oil. *Thalassospira profundimaris* WP0211^T was retrieved from a deep-sea sediment sample, which was collected by a multi-core sampler from the West Pacific (region 973, station WP02-3; 147° 58′ 55″ E, 12° 59′ 54″ N; water depth 4,480 m) (Liu et al. 2007). *Thalassospira tepidiphila* 1-1B^T was isolated from petroleum-contaminated seawater in a beach-simulation tank during a bioremediation experiment (Kodama et al. 2008). *Thalassospira xianhensis* P-4^T originated from a saline soil contaminated by crude oil, collected from Xianhe, Shandong Province, China (Zhao et al. 2010). In 2011, Hütz et al. reported that bacteria affiliated with the genus *Thalassospira* were found to constitute a regular, low-abundance member of the bacterioplankton that can be detected throughout the water column of the Eastern Mediterranean Sea. A representative (strain EM) was isolated in pure culture and exposed to a strong positive chemotaxis toward inorganic phosphate that was induced exclusively in phosphate-starved cultures. Although *Thalassospira* sp. represents only up to 1.2 % of the total bacterioplankton community in the water column of the Eastern Mediterranean Sea, its chemotactic behavior potentially leads to an acceleration of nutrient cycling and may also explain the persistence of marine copiotrophs in this extremely nutrient-limited environment (Hütz et al. 2011). The halotolerant bacterium *Thalassospira permensis* SMB34^T was isolated from a naphthalene-utilizing bacterial consortium obtained from primitive technogeneous soil formed on salt-mine spoils at the Verchnekamsk deposit of potassium–magnesium salts (Berezniki, Perm region, Russia); this was located at the place of the ancient Permian sea about 280 Ma ago. In contrast to the majority of organisms of the genus *Thalassospira* which are marine inhabitants, it might be speculated that *Thalassospira permensis* or its ancestor also originally inhabited the ocean and then survived, being trapped within salt crystals, subsequently evolving as a terrestrial bacterium, together with other members of the local microbial community (Plotnikova et al. 2011). Recently, two new species

of marine bacteria were isolated from a piece of sunken bamboo in the marine environment in Japan (Tsubouchi et al. 2014): *Thalassospira alkalitolerans* MBE#61^T and *Thalassospira mesophila* MBE#74^T. These isolations were the result of searching for microbes that show high metabolizing activity against lignin-related compounds; indeed, they metabolize effectively lignin-related aromatic compounds.

For example, *Magnetovibrio blakemorei* strain MV-1 was isolated from sulfide-rich sediments in a salt marsh near Boston, MA (Bazylinski et al. 1988). Like many marine bacteria, strain MV-1 is euryhaline but has a growth requirement for salts, as it will not grow at very low concentrations of ASW or in freshwater media (Kaye and Baross 2004; Bazylinski et al. 2013). Magnetospira type strain "MV-4" (magnetic vibrio number 4) was isolated from a salt marsh in Woods Hole (Meldrum et al. 1993; Williams et al. 2012), but in contrast to their closest characterized magnetotactic relative, *M. blakemorei* and *M. thiophila* can use only a relatively small number of organic acids as carbon and energy sources.

The species *Ferrovibrio denitrificans* is able to use FeS, $FeSO_4$, and $FeCO_3$ as Fe(II) sources for lithotrophic growth and unable to use NO_2, ClO_4^-, S^0, $S_2O_3^{-2}$, and $Fe(OH)_3$ as electron acceptors for anaerobic growth. The incrustation phenomenon, which occurs not only at laboratory conditions but also in the natural habitats, indicates that this is a natural way of living under anaerobic conditions (Sorokina et al. 2012). No environmental 16S rRNA gene sequences resembling the genus *Ferrovibrio* are available yet.

Pathogenicity and Clinical Relevance

Analysis of the species belonging to the family *Rhodospirillaceae* indicates the presence of species that have been detected as opportunistic human pathogens. On the other hand, no plant related pathogenicity has been reported for the genera. Despite the large spectrum of antibiotic resistance among the species, the majority of them are nonpathogenic to human or no information is available.

Opportunistic Human Pathogen

Azospirillum – Recent analyses of 16S rRNA gene sequences and phenotypic characteristics suggested that *R. fauriae* was closely related to *Azospirillum brasilense* (Cohen et al. 2004; Han et al. 2003; Weyant and Whitney 2005). *Roseomonas* species are opportunistic pathogens and have been isolated from a range of human infections including septicemia, occurring primarily in patients with underlying medical conditions (Dé et al. 2004; Struthers et al. 1996). One single report was based on four phenotypically similar bacterial strains isolated from fungal, plant, and human sources that were identified as *Azospirillum* species (Cohen et al. 2004). Strains RC1 and LOD4 were isolated from the mycelium of the apple root pathogen *Rhizoctonia solani* AG 5 and from the rhizosphere of wheat grown in apple orchard soil, respectively. Strains C610 and F4626 isolated from human wounds were previously misclassified as *Roseomonas* genomospecies 3 and 6. All four strains demonstrated close similarities in 16S rRNA gene sequences, having greater than or equal to 97 % identity to *A. brasilense* type strain ATCC 29145 and <90 % identity to *Roseomonas gilardii*, the *Roseomonas* type strain. Authors also report that their results indicate a wide range of potential sources for *Azospirillum* spp. with the isolation of *Azospirillum* spp. from human wounds warranting further investigation. In 2013 another case was described related to *Azospirillum* infection in an immunocompetent middle-aged female manifesting as granulomatous tenosynovitis on the right hand (Serelis et al. 2013). A reclassification of *Roseomonas fauriae* and R. genomospecies 6 into the *A. brasilense* species has been suggested, mostly based on very close 16S rRNA gene similarities (Helsel et al. 2006). A case of peritonitis in a 65-year-old woman with ESRD was published in 2000 (Bibashi et al. 2000). McLean et al. (2006) described a 3-month-old girl suffering from stage III neuroblastoma who had experienced a 2-day history of fever, vomiting, and diarrhea. Blood cultures drawn through her catheter recovered *R. fauriae*. These cases may represent the first reported opportunistic human infections caused by the *Azospirillum*-related bacteria. Hogue et al. (2007) described that the overall mortality rate associated with these pink-pigmented bacteria (similar to *A. brasilense*) is essentially negligible and although a few deaths have been recorded (most notably in association with *Roseomonas*), these bacteria have not been conclusively demonstrated to be involved in the patient's demise. The relatedness of *R. fauriae* and *A. brasilense* is still a matter of debate, and whole genome comparisons are in progress to clarify this issue (A. Hartmann, unpublished results).

Inquilinus – *I. limosus* has been documented mainly in CF patients and was sometimes accompanied with exacerbation or respiratory decline. The pathogenic potential, the impact on respiratory function, and the risk of patient-to-patient transmission of *I. limosus* are still unclear, and the environmental habitat of this bacterium is unknown. It has been isolated in the USA, Germany, France, the UK, and Spain (Pitulle et al. 1999; Coenye et al. 2002; Schmoldt et al. 2006; Cooke et al. 2007; Wellinghausen et al. 2005; Salvador-García et al. 2013). *I. limosus* may represent a new threat to CF patients, as it has a mucoid phenotype (i.e., production of EPS), multiresistance to a wide number of antibiotics, and the ability to persist in the respiratory tract. In agreement with Kuttel et al. (2012), *I. limosus* secretes two uniques exopolysaccharides (EPS), α-(1→2)-linked mannan and β-(1→3)-linked glucan. They demonstrated that the mixture of these two EPS is able to inhibit the lytic action of antimicrobial peptides of the innate immune system and it is possible that the coexistence of the two different secondary structures could enhance this biological activity. Additionally, these EPS form an effective barrier to penetration of chemically reactive biocides, antibiotics, and antimicrobial agents.

Antibiotic Sensitivity

Azospirillum – Wild-type strains of *Azospirillum lipoferum* and *Azospirillum brasilense* were found to be naturally resistant to penicillin, a resistance that was attributed to β-lactamase activity (Franche and Elmerich 1981). Antibiotic resistance is also studied in *Azospirillum*, for example, the β-lactam antibiotics that cause cell envelope stress by inhibiting peptidoglycan biosynthesis, and nalidixic acid that inhibits DNA gyrase activity is present in *A. brasilense*. *Azospirillum lipoferum* RG20 was found to be naturally resistant to penicillins and cephalosporins (Boggio et al. 1989). This strain showed high resistance to benzylpenicillin, ampicillin, carbenicillin, cephalosporin C, cephaloridine, cephalothin, and cefotaxime (MIC 1,000 μg ml^{-1}), whereas it was more susceptible to oxacillin and cloxacillin (MIC = 200 μg ml^{-1}) (Boggio and Roveri 2003). *A. amazonense* strains showed similar resistance to *A. lipoferum* and *A. brasilense* such as penicillin and relative tolerance to chloramphenicol and tetracycline (Magalhães et al. 1983).

Inquilinus – *I. limosus* is sensitive to imipenem, ciprofloxacin, and meropenem, but resistant to aminoglycosides, piperacillin–tazobactam, cefotaxime, ceftazidime, cefepime, aztreonam, kanamycin, gentamicin, amikacin, fosfomycin, doxycycline, and colistin (Bittar et al. 2008; Chiron et al. 2005). This bacterium is multiresistant to several antimicrobial drugs, particularly colistin, which is widely used for treatment for *P. aeruginosa* colonization (as was the case for our four patients). Bittar et al. (2008) hypothesize that this bacterium is selected during the evolution of the disease.

Constrictibacter – According to ATB VET system (bioMérieux), the type strain of the genus is resistant to erythromycin, lincomycin, pristinamycin, tylosin, co-trimoxazole, sulfamethizole, nitrofurantoin, fusidic acid, metronidazole, penicillin, amoxicillin, oxacillin, cephalothin, cefoperazone, chloramphenicol, and tetracycline, but sensitive to colistin, flumequine, oxolinic acid, enrofloxacin, rifampicin, amoxicillin/clavulanic acid, streptomycin, spectinomycin, kanamycin, gentamicin, apramycin, and doxycycline.

Desertibacter–Dongia–Ferrovibrio–Desertibacter roseus is susceptible to erythromycin, vancomycin, streptomycin, acheomycin, and penicillin. *Dongia mobilis* is susceptible to vancomycin, gentamicin, carbenicillin, polymyxin B, streptomycin, kanamycin, ampicillin, neomycin, chloramphenicol, and penicillin and weakly sensitive to tetracycline, erythromycin, novobiocin and rifampicin, while the species *Ferrovibrio denitrificans* is sensitive to amikacin, lincomycin, neomycin, polymyxin, streptomycin, rifampicin, and nalidixic acid. The last species is resistant to ampicillin, bacitracin, vancomycin, gentamicin, kanamycin, mycostatin, novobiocin, penicillin, and tetracycline.

Novispirillum and *Insolitispirillum* – Strains from these species show susceptibility to streptomycin, chloramphenicol, gentamicin, tetracycline, kanamycin, and neomycin, but not to penicillin G, ampicillin, cephalothin, and oleandomycin (Ding and Yokota 2002; Yoon et al. 2007b). In addition, representatives of the genus *Novispirillum* are susceptible to novobiocin, only weakly susceptible to polymyxin B, and resistant to carbenicillin, but each subspecies can be differentiated based on their characteristic lincomycin susceptibility. Both species of the genus *Insolitispirillum* present susceptibility to polymyxin B and carbenicillin.

Marispirillum – *M. indicum* is susceptible to carbenicillin, chloramphenicol, ciprofloxacin, erythromycin, gentamicin, kanamycin, minocycline, neomycin, norfloxacin, ofloxacin, rifampicin, ceftriaxone, streptomycin, and doxycycline and resistant to ampicillin, cefalexin, cefazolin, cefoperazone, cefradine, clindamycin, co-trimoxazole, furazolidone, lincomycin, metronidazole, oxacillin, penicillin G, piperacillin, polymyxin B, tetracycline, and vancomycin (Lai et al. 2009a).

Oceanibaculum – The *Oceanibaculum* species differ from each other according to their characteristic sensitivity or resistance to several antibiotics (Lai et al. 2009b; Dong et al. 2010). The species are resistant to cefalexin, cefazolin, cefradine, clindamycin, erythromycin, furazolidone, lincomycin, metronidazole, norfloxacin, ofloxacin, oxacillin, and vancomycin. *Oceanibaculum indicum* is susceptible to ciprofloxacin, co-trimoxazole, kanamycin, neomycin, polymyxin B, and streptomycin, while *O. pacificum* is susceptible to ampicillin, carbenicillin, chloromycetin, ciprofloxacin, gentamicin, kanamycin, neomycin, penicillin G, polymyxin B, rifampicin, rocephin, streptomycin, tetracycline, and vibramycin.

Limimonas – *L. halophila* is susceptible to nitrofurantoin, novobiocin, and rifampicin, but resistant to amikacin, amoxicillin, bacitracin, carbenicillin, chloramphenicol, erythromycin, gentamicin, kanamycin, polymyxin B, streptomycin, tetracycline, cephalothin, nalidixic acid, tobramycin, and penicillin G (Amoozegar et al. 2013).

Rhodospirillum–Rhodovibrio–Pararhodospirillum – *R. rubrum* is resistant to penicillin, ampicillin, carbenicillin, and nalidixic acid, while it is sensitive to triclosan, chloramphenicol, tetracycline, kanamycin, streptomycin, and gentamicin (Weaver et al. 1975; Pycke et al. 2010), while *Rhodovibrio salinarum* is sensitive to chloramphenicol, tetracycline, kanamycin, streptomycin, rifampicin, and spectinomycin (Borghese et al. 2001). *Rhodovibrio* sp. (isolates GV-2 and GV-3) are sensitive to antimicrobial substances produced by the halophilic archaea *Haloferax* (Atanasova et al. 2013). *Pararhodospirillum photometricum* is sensitive to penicillin (Imhoff 2005b).

Thalassospira – The antibiotic sensitivity varies among the *Thalassospira* species. *T. xiamenensis* is sensitive to chloramphenicol, norfloxacin, furazolidone, co-trimoxazole, ofloxacin, midecamycin, ceftriaxone, polymyxin B, doxycycline, tetracycline, neomycin, kanamycin, gentamicin, amikacin, erythromycin, minocycline, carbenicillin, cefalexin, cefradine, ciprofloxacin, and cefuroxime, but resistant to cefoperazone, clindamycin, vancomycin, ceftazidime, cefazolin, penicillin, oxacillin, ampicillin, and piperacillin (Liu et al. 2007). *T. profundimaris* is sensitive to chloramphenicol, norfloxacin, furazolidone, co-trimoxazole, ofloxacin, midecamycin, ceftriaxone, polymyxin B, doxycycline, tetracycline, neomycin, kanamycin, gentamicin, amikacin, erythromycin, minocycline, carbenicillin, cefalexin, ciprofloxacin, cefoperazone, vancomycin, ceftazidime, cefazolin, penicillin, oxacillin, ampicillin, and piperacillin but

resistant to clindamycin, cefuroxime, and cefradine (Liu et al. 2007). *T. xianhensis* is sensitive to ampicillin, cephalothin, clarithromycin, clindamycin, erythromycin, penicillin, and vancomycin, but resistant to ceftriaxone, cefotaxime, ciprofloxacin, gentamicin, streptomycin, and tetracycline (Zhao et al. 2010). *T. alkalitolerans* is sensitive to kanamycin, gentamicin, chloramphenicol, penicillin G, polymyxin B, and carbenicillin, slightly sensitive to tetracycline, streptomycin, novobiocin, rifampicin, and erythromycin, but resistant to bacteriocin, vancomycin, neomycin, ampicillin, and lincomycin (Tsubouchi et al. 2014). *T. mesophila is* sensitive to kanamycin, neomycin, novobiocin, gentamicin, chloramphenicol, ampicillin, penicillin G, and carbenicillin, slightly sensitive to tetracycline, streptomycin, rifampicin, erythromycin, and polymyxin B, and resistant to bacteriocin, vancomycin, and lincomycin (Tsubouchi et al. 2014).

Thalassobaculum–Tistrella–Tistlia – Thalassobaculum litoreum is sensitive to streptomycin, gentamicin, vancomycin, kanamycin, penicillin, erythromycin, tetracycline, chloramphenicol, ciprofloxacin, and ampicillin (Zhang et al. 2008). *Tistrella bauzanensis* is sensitive to kanamycin, amikacin, rifampicin, and neomycin but resistant to chloramphenicol, tetracycline, and erythromycin (Zhang et al. 2011), while *Tistlia consotensis* is sensitive to ampicillin, streptomycin, chloramphenicol, tetracycline, and penicillin (Díaz-Cárdenas et al. 2010).

Application

The *Rhodospirillaceae* family is formed by bacterial species with very diverse metabolic functions, but so far, only *Azospirillum* species have already been applied as biofertilizer in the agriculture, while other genera have been exploited for industry and environmental application. Many species of other genera have shown biotechnological potential as suggested by the genome sequencing analyses, while others have not been exploited yet.

Agricultural Application

Members of the genus *Azospirillum* are commonly used in the field as biofertilizers and other field tests in many countries such as (alphabetical order): Argentina, Brazil, Colombia, Egypt, France, Israel, Turkey, South Africa, and many others (Okon and Labandera-Gonzalez 1994; Dobbelaere et al. 2001; Turan et al. 2012). Most of these applications are linked to the oldest species described, especially the commercial inoculants based on *A. brasilense* strains. At the beginning, only cereals were tested but recently its application is spread in co-inoculation of rhizobia in legumes such as soybean (Juge et al. 2012), *Vicia sativa* (Star et al. 2012), and beans. Also its single application was extended to other cultures such as lettuce (Fasciglione et al. 2012), cactus (Lopez et al. 2012) trees (Leyva and Bashan 2008), and even in microalgae (Choix et al. 2012).

Species from the *Azospirillum* genus has the capacity to produce several plant growth regulators such as abscisic acid, ethylene, gibberellic acid, indole 3-acetic acid, zeatin (Tien et al. 1979; Bashan et al. 2004), nitric oxide (NO) (Steenhoudt et al. 2001), polyamides (Thuler et al. 2003), and siderophores (Saxena et al. 1986). However, the bacteria also produce a wide variety of these signaling molecules and influence plant growth. *Azospirillum* is a well known bacterium that produces high amounts of auxins "in vitro," and different pathways are involved in this production but not in all species the indole production was determined such as *A. humicireducens* (Zhou et al. 2013). The best characterized pathway in *Azospirillum* auxin production is via indole-3-acetamide (IAM) and indole-3-pyruvate (IPyA) intermediates, both well described by Spaepen et al. (2007) to generate osmotic stress response in plants (Aziz et al. 1997) and solubilize phosphates (Seshadri et al. 2000). Several modifications of plant architecture and physiology of the cells were recorded: increased respiration rates of root cell in plants inoculated with *Azospirillum* such as maize and sorghum (Okon and Kapulnik 1986; Sarig et al. 1992); membrane proton efflux in wheat; increased mineral uptake (Bashan et al. 1989); and hydrolysis of conjugated phytohormones and flavonoids (Volpin et al. 1996). The main effect visually observed after inoculation is the root proliferation. This effect causes enhancement of the root surface activity to the plant that increases mineral nutrients and water (Spaepen et al. 2007). *Azospirillum* can contribute some nitrogen in cellulose-decomposing mixed cultures with Cellulomonas gelida (Halsall and Gibson 1985) or through the ability of straw decomposition by some specific N_2-fixing *Azospirillum* sp. isolates (Halsall et al. 1985). Another feature of *Azospirillum* is related to the accumulation of polybetahydroxyalkanoates (PHA) in the cells. It appears to be an important trait for root colonization and plant growth promotion, especially for A. brasilense inoculants where cells with high amounts of PHA play a better capacity of plant growth in field experiments carried out in South America with maize and wheat. These assays revealed that increased crop yields were consistently obtained using inoculants prepared with PHA-rich *Azospirillum* cells (Dobbelaere et al. 2001; Helman et al. 2011).

In the last years, *Azospirillum* spp. have been applied in consortium with other PGPR bacteria such as *Pseudomonas* in maize (Salamone et al. 2012), other nitrogen-fixing bacteria in sugarcane (Oliveira et al. 2006), or with mycorrhizal fungi (Couillerot et al. 2013). It has also been used as a biocontrol component in cotton inoculation (Marimuthu et al. 2013), or reported its activity in stressed conditions such as drought (Abbasi and Zahedi 2013; Bano et al. 2013) and saline conditions (Nakade 2013).

Two recently reported new species of the genus *Thalassospira*, *T. alkalitolerans* and *T. mesophila*, metabolize effectively lignin-related aromatic compounds, and therefore, their use is expected by the biochemical industries in the degradation of plant biomass (Tsubouchi et al. 2014).

Industrial Application

The species *Rhodospirillum rubrum* provides several potential biotechnological applications for the industry. It produces

polyhydroxyalkanoates (PHAs) composed of both short- and medium-chain-length monomers, and it can produce up to 50 % (dry weight) of the cell in PHAs (Brandl et al. 1989; Liebergesell et al. 1991; Ulmer et al. 1994). Because of its metabolic versatility, R. rubrum offers the potential for converting many different carbon sources to PHA (Do et al. 2007b; Smith et al. 2008). *R. rubrum* also provides the potential for hydrogen fuel production. It may produce H_2 growing photoheterotrophically using, for example, malate as carbon source and electron donor; growing anaerobically using fructose, lactate, acetate, or succinate as carbon source, and dimethyl sulfoxide (DMSO) or trimethylamine-N-oxide (TMAO) as electron donor; or fermenting fructose or pyruvate when an external electron acceptor is absent (Gest and Kamen 1949; Uffen 1973; Schultz and Weaver 1982). *R. rubrum* has been genetically modified in order to increase its capacity to produce hydrogen (Kars and Gündüz 2010); in this way, a Hup plus (uptake hydrogenase) mutant has been generated, and this showed a significant increase in H_2 production (Kern et al. 1994). Through an applied approach, *R. rubrum* has been grown on synthesis gas in order to produce both H_2 and PHA (Do et al. 2007b). *R. rubrum* offers the potential for production of the food additive coenzyme Q10 (CoQ10) and the carotenoid lycopene, which is also a natural colorant (Tiana et al. 2010; Wang et al. 2012). R. rubrum has been used as a heterologous expression system of membrane proteins (Butzin et al. 2010). No data are available for application of neither *Pararhodospirillum* nor *Rhodovibrio* species.

The biologically derived magneto-functional inorganic nanocrystals of magnetite have been used as carriers for enzymes and in immunoassay methods including those involving nucleic acids, antibodies, and targeted delivery of anticancer drugs (Naresh et al. 2012). The process of magnetosome synthesis has been used to develop novel tools for ligand–receptor interaction analysis, such as those applied for TRAb immunoassay in Graves' disease patients (Sugamata et al. 2013).

Ghosh et al. (2012) reported on lactose hydrolysis of milk by crude enzyme extracted from deep marine psychrophilic strain *Thalassospira* sp. 3SC-21. They showed that 80.18 % of lactose was hydrolyzed after 43 min of incubation at 20 °C, within a pH range of 6.5–7.5. This activity was also observed at 10 °C (65 %), indicating that this enzyme is useful at refrigerated temperature. From these findings, they conclude that *Thalassospira* sp. 3SC-21 is a source for the production of cold active β-galactosidase enzyme that can be applicable at cold temperature and might be considered in food industry including dairy industry on a larger scale.

Tistrella mobilis and *Tistrella bauzanensis* produce didemnins (antineoplastic agents) via unique post-assembly line maturation process (Xu et al. 2012). Complete genome sequence analysis of *T. mobilis* strain KA081020-065 revealed the putative didemnin biosynthetic gene cluster (*did*) on the megaplasmid pTM3. The *did* locus encodes a 13-module hybrid non-ribosomal peptide synthetase–polyketide synthase enzyme complex organized in a collinear arrangement for the synthesis of the fatty acylglutamine ester derivatives didemnins X and Y rather than didemnin B. Imaging mass spectrometry of *T. mobilis* bacterial colonies captured the time-dependent extracellular conversion of the didemnin X and Y precursors to didemnin B, in support of an unusual post-synthetase activation mechanism. Significantly, the discovery of the didemnin biosynthetic gene cluster may provide a long-term solution to the supply problem that presently hinders this group of natural products and pave the way for the genetic engineering of new didemnin congeners.

The bacterium *Tistlia consotensis*, isolated from a saline spring in the Colombian Andes, represents an interesting environmental model to be compared with extremophiles or other moderate organisms (Díaz-Cárdenas et al. 2010). To explore the halotolerance molecular mechanisms of the *T. consotensis*, through a proteogenomic approach, a large number of proteins were found to be produced in greater amounts when cells were cultivated in either hypo-osmotic or hyper-osmotic conditions (Rubiano-Labrador et al. 2014).

Other genera present biotechnological potential such as the *Phaeospirillum* species that have the photosynthetic apparatus of phototrophic bacteria (e.g., Mascle-Allemand et al. 2010) and evolutionary importance due its high amount of genes related to signal transduction (Duquesne et al. 2012). The close phylogenetic relationship of *Rhodocista centenaria* and *Rhodocista pekingensis* to *Azospirillum irakense/Azospirillum amazonense* also indicates scientific importance to this genus due to cyst-forming ability (Lu et al. 2010).

Environmental Application

New features are arising on the environmental application for the genus *Azospirillum*. *A. brasilense* strains Sp 7 and Sp245 are able to reduce selenium (IV) to elementary selenium (Tugarova et al. 2013), while *A. thiophilum* has the capacity for lithotrophic growth coupled oxidation for reduced sulfur compounds (Frolov et al. 2013).

Defluvicoccus vanus has been identified as glycogen-accumulating organisms (GAOs) (Meyer et al. 2006; Burow et al. 2007). According to Burow et al. (2007), the activity of glycogen-accumulating organisms (GAOs) in enhanced biological phosphorus removal (EBPR) wastewater treatment plants has been proposed as one cause of deterioration of EBPR. GAOs possess the ability to take up volatile fatty acids (VFA) under anaerobic conditions and convert them to polyhydroxyalkanoates (PHA), which are stored until the following aerobic period and then oxidized to CO_2 or transformed to glycogen.

Magnetotaxis and magnetosome productions are special traits shared by some magnetotactic and magnetosome bacteria that have been extensively employed for the development of new technology in the nanotechnology field. Besides the important role of nanobiotechnology to medicine, its application has already become a matter of study into many other applied sciences, such as environmental engineering and agricultural science. Ginet et al. (2011) demonstrated that functionalized magnetic nanoparticles efficient as a reusable nanobiocatalyst

for pesticide bioremediation in contaminated effluents can be produced by genetically modified magnetotactic bacteria.

The strains *Thalassospira xiamenensis* M-5^T and *Thalassospira profundimaris* WP0211^T were isolated separately from bacterial consortia that used hydrocarbons as their sole carbon sources; neither strain could degrade any of the hydrocarbons tested in their characterization tests (Liu et al. 2007). However, the analysis of 16S rRNA gene sequences obtained from various samples from marine environments has revealed that these two species and their close relatives were frequently detected in petrol-oil-degrading consortia. This suggests they may play some role in the degradation of petroleum hydrocarbons (Liu et al. 2007). However, no further study was done.

The strain T. profundimaris WP0211^T cannot use pyrene as the sole carbon source for growth, though it was isolated from a pyrene-degrading consortium. Its genome sequence analysis revealed a gene encoding a ring hydroxylating dioxygenase and therefore supports further characterization (Lai and Shao 2012b).

Thalassospira tepidiphila is considered to play important roles in marine spilled-oil bioremediation (Kodama et al. 2008). Polycyclic aromatic hydrocarbons (PAHs), hydrocarbons containing two or more fused aromatic rings, are released into the marine environment as a result of various anthropogenic activities such as marine seepage and accidental discharges during the transport and disposal of petroleum products and the use of fossil fuels (Sohn et al. 2004). Some PAHs are highly carcinogenic, genotoxic, and cytotoxic to marine organisms and may be transferred to humans through seafood consumption (Menzie et al. 1992). Therefore, removal of PAHs from contaminated marine environments is of considerable importance, hence the importance of isolating PAH-degrading bacteria such as *Thalassospira xianhensis* (Zhao et al. 2010). Recently, Um et al. (2013) reported that chemical investigation on the marine unicellular bacterium *Thalassospira* sp. led to the discovery of a new peptide, thalassospiramide G, along with thalassospiramides A and D. The peptides are structurally unique, with unusual γ-amino acids, such as 4-amino-5-hydroxy-penta-2-enoic acid (AHPEA) and 4-amino-3,5-dihydroxy-pentanoic acid (ADPA). In addition, thalassospiramide G bears a 2-amino-1-(1*H*-indol-3-yl) ethanone (AIEN) moiety, which is quite rare in a natural product. In the LPS-induced NO production assay, thalassospiramide D displayed more significant inhibition of NO production than thalassospiramide A, indicating its potential as an anti-inflammatory agent. The structural novelty and the biological activity of the secondary metabolites isolated from this marine α-proteobacterial taxonomic group suggest that marine unicellular bacteria, particularly α-proteobacteria, which have been overlooked in the search for new bioactive compounds, could potentially provide a rich source of chemically and pharmaceutically interesting natural products (Um et al. 2013).

Tistrella mobilis IAM 14872^T produces polyhydroxyalkanoates (PHAs) as intracellular granules (Shi et al. 2002). PHA is a biodegradable, biocompatible, and thermoplastic material, which has a potential role as a so-called biomass transducer, i.e., it can be used for the microbial transformation of carbohydrate feedstock via PHA into chiral depolymerization products (Seebach and Zuger 1984) or small-molecule organic chemicals by pyrolysis (Anderson and Dawes 1990). So far, these biodegradable PHAs, however, are not priced competitively mainly because of the high cost, which lies in both the use of glucose as a fermentation feedstock and the low product yield.

Tistrella sp. strain ZP5, isolated from soil samples contaminated with polycyclic aromatic hydrocarbon (PAH)-containing waste (Zhao et al. 2008), cannot degrade phenanthrene individually, but it can increase the speed of phenanthrene degradation together with *Sphingomonas* sp. ZP1. Such two strains may be useful for bioremediation applications.

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