12 The Family *Hyphomonadaceae*

Wolf-Rainer Abraham[1] · *Manfred Rohde*[2]
[1]Chemical Microbiology, Helmholtz Centre for Infection Research, Braunschweig, Germany
[2]Medical Microbiology, Helmholtz Centre for Infection Research, Braunschweig, Germany

Abstract
The family *Hyphomonadaceae* comprises strict aerobic and stalked and non-stalked species. Cells in this family can have two differing cell cycles. Species of the genera *Algimonas*, *Maricaulis*, *Oceanicaulis*, *Ponticaulis*, *Robiginitomaculum*, and *Woodsholea* divide by binary fission of the cell leading to flagellated offsprings. Species of the genera *Hellea*, *Hirschia*, *Hyphomonas*, and *Litorimonas* divide by budding, forming a flagellated cell at the tip of the prosthecum. All flagellated cells are mobile but later develop a stalk, shed the flagella, and become sessile. These two-cell different cycles are both optimal for living in oligotrophic habitats where the competition for food is fierce and should be minimized between siblings. The genus *Henriciella* is currently the only genus with species lacking any stalks. With the possible exception of *Hyphomonas polymorpha*, all species are marine isolates and require sodium chloride for optimal growth. Species of *Hyphomonadaceae* occupy various habitats in the oceans where they are usually found in oligotrophic niches from the Antarctic to deep sea vents. Only few isolates can grow on minimal media with defined carbon sources; however, most species require complex growth factors for growth. Polar lipids for most species are monoglycosyl and monoglucuronosyl diacylglycerols. Common phospholipids are phosphatidylglycerols and many species possess sulfoquinovosyl diacylglycerols and glucuronopyranosyl diacylglycerol taurine amides as well. Most species reduce nitrate and are alkaline phosphatase and leucine arylamidase positive.

Taxonomy, Historical and Current

For many decades, dimorphic, prosthecate bacteria which reproduce by the separation of two cells that are morphologically and behaviorally distinct from each other were called *Caulobacter*. One of these cells is sessile and nonmotile and prosthecate, possessing at least one elongated, cylindrical appendage, a prostheca (Staley 1968). The other cell is flagellated, bearing one polar flagellum, by which it is motile. The motile cell later develops a stalk, losses it flagellum, and becomes sessile. The mode of reproduction of the dimorphic, prosthecate bacteria is unique as a regular feature of a prokaryotic reproductive cycle. It helps to disperse the population at each generation, minimizing competition among siblings for resources. Loeffler was the first to report on the isolation of these bacteria (Loeffler 1890), but only in 1935, Henrici and Johnson described the genus *Caulobacter*, with *Caulobacter vibrioides* as the type species (Henrici and Johnson 1935). Three decades later, Poindexter (1964) described *Caulobacter maris* and *Caulobacter halobacteroides*, the first two caulobacteria from seawater. At that time, the taxonomy of the members of the genus was based almost exclusively on the characteristic morphology and mode of reproduction (Poindexter 1981). Stahl et al. (1992) sequenced the 16S rRNA of several isolates belonging to *Caulobacter*, revealing that members of *Caulobacter* actually formed three distinct lineages, one comprising the freshwater and brackish water *Caulobacter* strains and the other comprising the marine *Caulobacter* strains. Bacteria of the third lineage belong to the genus *Sphingomonas* with *S. leidyi* (Poindexter) as the only prosthecate *Sphingomonas* species so far described (Chen et al. 2012a). In 1999 Abraham et al. transferred *Caulobacter maris* to the new genus *Maricaulis* as the type

E. Rosenberg et al. (eds.), *The Prokaryotes – Alphaproteobacteria and Betaproteobacteria*, DOI 10.1007/978-3-642-30197-1_260,

species *M. maris*. For prosthecate species also proliferating by fission but being in several aspect differing from species belonging to *Maricaulis*, the novel genera *Oceanicaulis* (Strömpl et al. 2003) and *Woodsholea* (Abraham et al. 2004) were described.

In 1957, Pongratz reported the isolation of a prosthecate, but budding bacterium from the nasal mucous of a patient with sinusitis (Pongratz 1957). Because this organism grew on complex media, such as blood agar, and was unable to grow on simple mineral salt media containing only one-carbon compounds as the sole sources of carbon and energy, a new genus was proposed to accommodate this organism, and the organism was named *Hyphomonas polymorpha*. Another budding species, *Hirschia baltica*, was described in 1990, which is still the only known species of the *Hyphomonadaceae* coming from brackish water (Schlesner et al. 1990). Only with the advent of gene sequencing it became apparent that *Maricaulis*, *Oceanicaulis*, and *Woodsholea*, reproducing by fission, and *Hyphomonas* and *Hirschia*, reproducing by budding, belong to the same family which was described in 2005 as *Hyphomonadaceae* (Lee et al. 2005). This family grew considerable in size when the genera *Robiginitomaculum* (Lee et al. 2007), *Hellea* (Alain et al. 2008), *Henriciella* (Quan et al. 2009; emend. Lee et al. 2011), *Ponticaulis* (Kang and Lee 2009a), *Litorimonas* (Jung et al. 2011), and *Algimonas* (Fukui et al. 2012) were established.

Hy.pho.mo.na.da'ceae. N.L. fem. n. *Hyphomonas* type genus of the family; -aceae ending to denote a family (N.L. fem. pl. n. *Hyphomonadaceae* the *Hyphomonas* family).

The description of the family is an emended version of the description given by Lee et al. (2005): Phylogenetically together with the *Caulobacteraceae* (Henrici and Johnson 1935) and the *Rhodobacteraceae* nom. ill. (Garrity et al. 2005), a member of the order *Caulobacterales* (Henrici and Johnson 1935), phylum Proteobacteria, class *Alphaproteobacteria* (Garrity et al. 2005). Gram-negative, rod-shaped bacteria, motile. Cells do not form spores. Chemoorganotrophic. Some species require peptone or B vitamins and amino acids. Species are aerobic or facultatively anaerobic. Some species denitrify. In most species, the major isoprenoid quinone is Q-10. Most species contain the polar lipids α-D-glucopyranosyl diacylglycerol, α-D-glucuronopyranosyl diacylglycerol, and sulfoquinovosyl diacylglycerol; in some genera α-D-glucuronopyranosyl diacylglycerol taurine amide and phosphatidyl diacylglycerol are also present. Many species show a reduction of the relative amount of phospholipids, sometimes down to <1 %, in favor of glyco- and sulfolipids (Abraham et al. 1997; Batrakov et al. 1996a). Members of the family have been isolated from seawater. The family comprises the type genus *Hyphomonas* (Pongratz 1957; emend. by Moore et al. 1984) and the genera *Hirschia* (Schlesner et al. 1990), *Maricaulis* (Abraham et al. 1999), *Oceanicaulis* (Strömpl et al. 2003), *Woodsholea* (Abraham et al. 2004), *Robiginitomaculum* (Lee et al. 2007), *Hellea* (Alain et al. 2008), *Henriciella* (Quan et al. 2009; emend. Lee et al. 2011), *Ponticaulis* (Kang and Lee 2009a), *Litorimonas* (Jung et al. 2011), and *Algimonas* (Fukui et al. 2012) (*Table 12.1*).

Molecular Analyses

The four genomes of *Hyphomonadaceae* sequenced and annotated so far contain between 3.17 and 3.71 megabases. They are therefore somewhat smaller than most genomes of *Caulobacteraceae*. Usually one chromosome is present but cells of *Hirschia baltica* also harbor a plasmid of 84.492 bp. These genomes contain between 3.077 and 3.568 genes coding 3.029–3.505 proteins. Most genomes have two identical copies of ribosomal RNA operons; only *Hyphomonas neptunium* ATCC 15444^T has only one. The number of tRNA genes is also pretty uniform, and 43–45 tRNA genes were predicted from the genome sequences (*Table 12.2*).

To thrive in oligotrophic environment, highly efficient mechanisms for nutrient uptake are essential in *Hyphomonadaceae*. For the uptake of compounds which are too large for uptake by simple passive diffusion, active transports by TonB-dependent receptors are used which are both anchored in the outer membrane and energy dependent. The known genomes of *Caulobacterales* and *Hyphomonadaceae* contain a large number of TonB-dependent receptors, e.g., *Caulobacter crescentus* has 63 and *Hyphomonas neptunium* and *Hirschia baltica* 46 of these genes (Chertkov et al. 2011). However, only 24 TonB-genes were identified in the *Oceanicaulis* sp. HTCC2633 genome (Oh et al. 2011), but far too little is known to draw any conclusions from these differences.

All species of *Hyphomonadaceae* have a very characteristic cell cycle similar to most species of *Caulobacterales*. Bioinformatic analysis of genes controlling the dimorphic cell cycle revealed the conservation of 14 key proteins that function in the regulation of the cell cycle (Brilli et al. 2010). There is, however, one notable exception: DivJ, a histidine kinase, is absent in the *Hyphomonas neptunium* and *Hirschia baltica* genomes but present in the *Oceanicaulis* sp. HTCC2633 and *Maricaulis maris* MCS 10 genomes. Bacteria belonging to the *Caulobacterales* and *Hyphomonadaceae* are also known for the ability to produce a holdfast, a polar polysaccharide, required for strong adhesion to surfaces. The genes required for the synthesis (Smith et al. 2003) and anchoring (Hardy et al. 2010) of the holdfast have been identified and characterized both in *Caulobacter crescentus* and *Hirschia baltica* but were largely absent from the genome of *Hyphomonas neptunium*, which does not produce a polar holdfast. The observation that most developmental regulators and holdfast genes are conserved in the budding bacteria belonging to the *Hyphomonadaceae* as well as the non-budding bacteria belonging both to the *Caulobacterales* and the *Hyphomonadaceae* can be interpreted that this regulation of the cell cycle evolved prior to the separation of the *Hyphomonadaceae* and *Caulobacterales*.

Quite different results came from studies of the control of chromosome replication. The dimorphic growth of *Hyphomonadaceae* requires cell-type-specific controls of chromosome replication preventing replication in swarmer cells but enabling the progeny stalked cell to perform chromosome replication. This is achieved by the CtrA protein which

Table 12.1
Characteristics of genera of *Hyphomonadaceae*

Genus	Replication	Polar lipids	Major fatty acids	G+C content	Ubiquinone
Algimonas[a]	Binary fission	MGDG[a], GUDG, PG	C18:1ω7c	58.5	
			2-OH C18:1		
Hellea	Budding	MGDG, GUDG, PG[b]	C18:1ω7c, C19:18c	47.9	
			2-OH iC19:1		
Hirschia	Budding		C18:1ω7c, C16:0	43.7–44.5	Q-10
			3-OH C12:1		
Henriciella	Binary fission	MGDG, GUDG, Tau, PG	C18:1ω7c, C16:0	55.2–61.0	Q-10
			3-OH C12:0		
Hyphomonas	Budding	MGDG, GUDG, Tau, PG	C18:1w7,	57–64	Q-11 (Q-10)
			3-OH C12:1		
Litorimonas	Budding	MGDG, GUDG, PG[b]	C18:1ω7c, C17:0	63.6	Q-10
			2-OH C18:1	47.1[a]	
Maricaulis	Binary fission	MGDG, GUDG, Tau, SQDG, PG	C18:1ω7		
			3-OH C12:0		
Oceanicaulis	Binary fission	MGDG, GUDG, SQDG, PG	C18:1ω7, C18:0	61–62	Q-10, Q-9
			3-OH C12:0		
Ponticaulis	Binary fission		C18:1ω7c, C16:0	53.3	Q-10
Robiginitomaculum	Budding		C18:1ω7, C17:1ω8	60.3	Q-10
			3-OH C11:0		
Woodsholea	Binary fission	MGDG, GUDG, Tau, SQDG	C18:1ω7	65	
			3-OH C12:0		

[a]*MGDG* monoglucosyl diacylglycerol, *GUDG* α-D-glucopyranuronosyl diacylglycerol, *PG* phosphatidyl glycerol, *Tau* 1,2-diacyl-3-α-D-glucuronopyranosyl-sn-glycerol taurine amide, *SQDG* 1,2-diacyl-3-O-sulfoquinovosylglycerol
[b]Fukui et al. (2012)

Table 12.2
Characteristics of annotated genomes of *Hyphomonadaceae*

Strain	Size (Mb)		rRNA operons	tRNAs	Genes	Proteins
Hirschia baltica ATCC 49814^T (Chertkov et al. 2011)	3.54	1 chromosome	2	44	3.269	3.187
	0.08	1 plasmid				
Hyphomonas neptunium ATCC 15444^T (Badger et al. 2006)	3.71	1 chromosome	1	43	3.568	3.505
Maricaulis maris MCS 10 (Copeland et al. 2011)	3.37	1 chromosome	2	45	3.133	3.063
Oceanicaulis sp. HTCC 2633 (Oh et al. 2011)	3.17	1 chromosome	2	44	3.077	3.029

binds to the chromosome origin and prevents its replication. As a consequence the CtrA protein is present in swarmer cells but absent in stalked cells (Bowers et al. 2008). A study directed to the replication origins of *Caulobacter*, *Oceanicaulis*, and *Maricaulis* strains revealed that the genera of *Caulobacteraceae* and *Hyphomonadaceae* developed differently. While all these replication origins use CtrA to repress replication, the study showed that CtrA usage evolved separately among these two families confirming the phylogeny derived from their 16S rRNA gene sequences (Shaheen et al. 2009).

Further differences between *Caulobacter* and *Hyphomonas* were found in their selenoproteins. While *Hyphomonas neptunium* encodes an L-seryl-tRNA selenium transferase, a selenocysteine-specific translation elongation factor, and a selenoprotein of unknown function, all these selenoproteins are absent in *C. crescentus*. Another argument for a clear distinction between *Caulobacteraceae* and *Hyphomonadaceae* comes from the biosyntheses of holdfasts. While *H. neptunium* and *C. crescentus* both synthesize holdfasts, none of the genes required for its synthesis have orthologs encoded in the

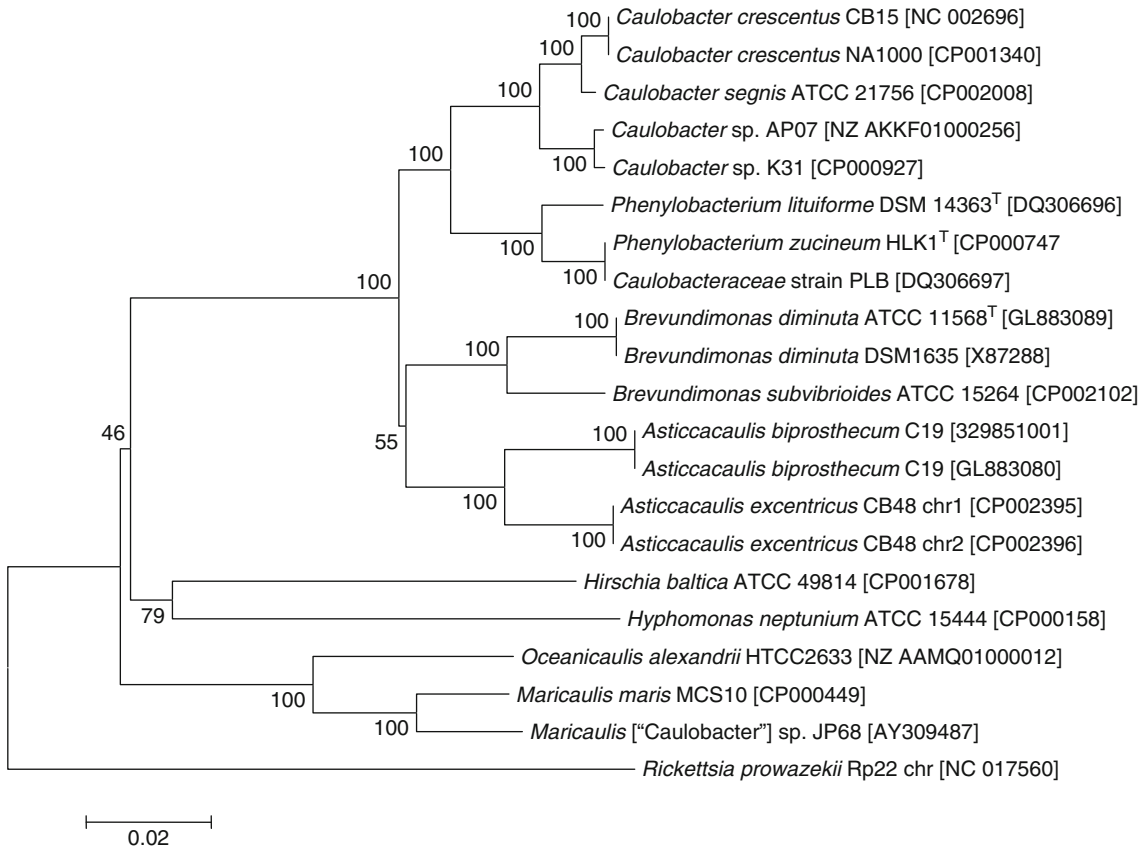

Fig. 12.1
Phylogenetic tree based on LSU rRNA gene sequences (neighbor joining, 1,000 bootstrap replication, *Rickettsia prowazekii* served as outgroup)

corresponding other genome (Badger et al. 2006). Therefore, it seems that holdfast synthesis and attachment must be achieved differently in both species. In line with these observation is also the gene organization for flagella formation in both species. While in *H. neptunium* most of the genes required for the flagellum and its synthesis are organized in one region comprising 35 contiguous open reading frames, the corresponding genes are scattered over the entire genome of *C. crescentus*. Furthermore, transcription of many flagellar genes in *C. crescentus* requires a σ^{54}-RNA polymerase holoenzyme which seems not to be involved in the corresponding transcriptions in *H. neptunium*.

Badger et al. (2005) reported that phylogenetic trees based on the 23S rRNA gene sequences differ remarkably from those calculated using the 16S rRNA gene sequences. However, using a much larger set of data, we could not find this discrepancy but a clear distinction between *Caulobacteraceae* and *Hyphomonadaceae* in phylogenetic trees based either on the 16S or the 23S rRNA gene sequences (*Figs. 12.1* and *12.2*).

DNA–DNA hybridizations between *Hyphomonas* isolates (Weiner et al. 2000), between *Maricaulis* isolates (Abraham et al. 2002), and between Henriciella isolates (Lee et al. 2011) have been reported. Applying the definition of species being distinct if DNA–DNA hybridization values are 70 % or less (Rosselló-Mora and Amann 2001) and comparing these results with the phylogenetic tree based on the 16S rRNA gene sequences, it can be concluded that only a few base pairs difference between two isolates of these genera is usually sufficient to assume two distinct species. This situation is similar to the one found in the *Caulobacteraceae* family (Abraham et al. 1999) (*Tables 12.3* and *12.4*).

This statement is corroborated by the DNA–DNA relatednesses reported between *Henriciella litoralis* SD10^T and *H. marina* Iso4^T of 12.0 % and between *H. marina* Iso4^T and *H. aquamarina* LMG 24711^T of 22.9 %.

Phenotypic Analyses

All species are oligotrophic organisms and the cells can be deformed, damaged, or even killed by higher substrate concentrations. Most strains have complex nutrient and growth factor requirements which cannot be fulfilled by simple addition of vitamins or amino acids. As a consequence cultivation on complex media is usually the only way to grow them. Most strains form biofilms and the formation of rosettes by attachment of the holdfast is a characteristic feature easily seen under the microscope.

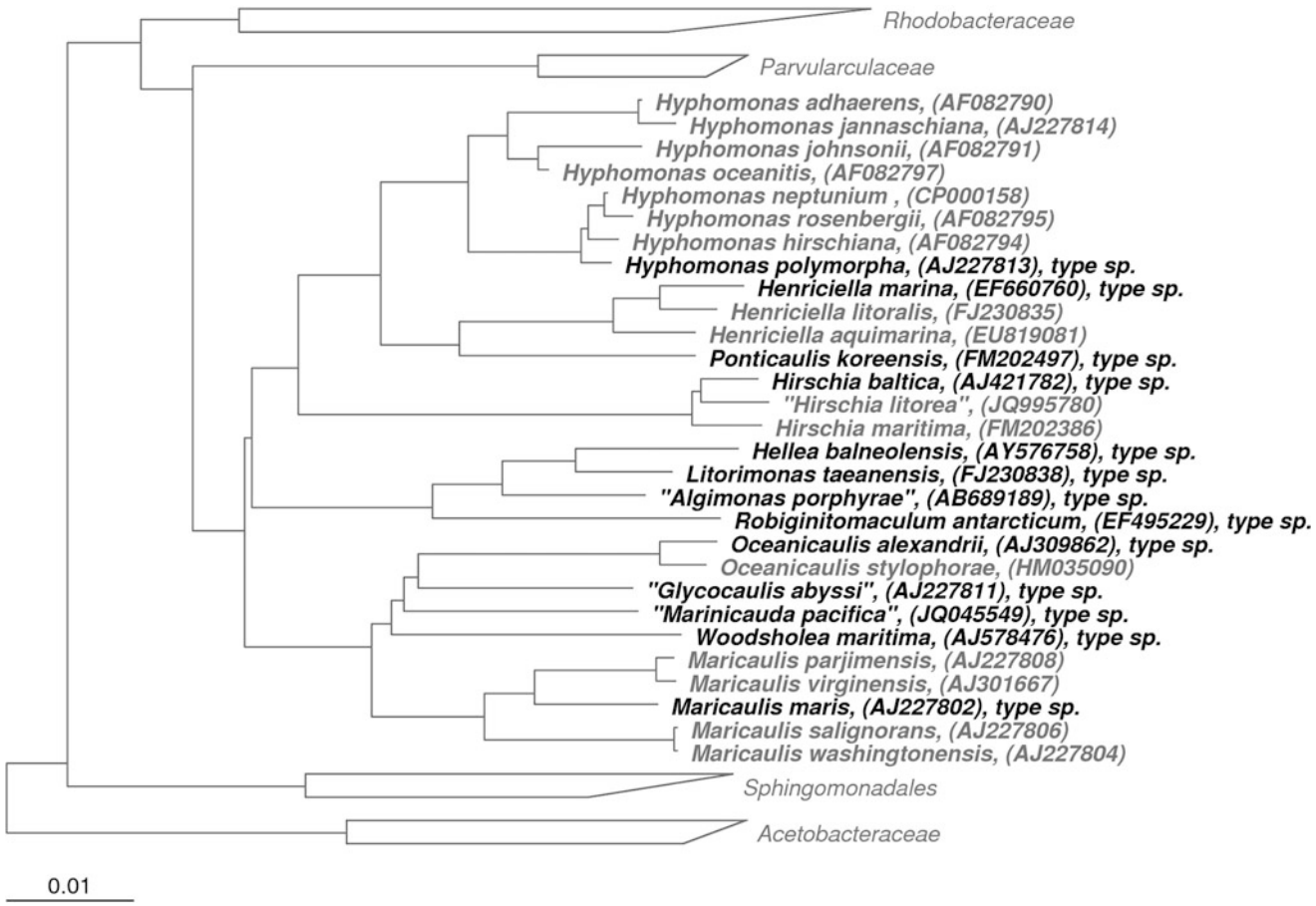

Fig. 12.2

Phylogenetic reconstruction of the family *Hyphomonadaceae* based on 16S rRNA and created using the neighbor-joining algorithm with the Jukes-Cantor correction. Scale bar indicates estimated sequence divergence

Table 12.3

DNA–DNA hybridization between the *Hyphomonas* type strains (Weiner et al. 2000)

	PS-728^T	MHS-3^T	LE-670^T	MHS-2^T	VP-5^T	Sch89^T	VP-1^T	VP-6^T
H. polymorpha PS-728^T	100							
H. adhaerens MHS-3^T	32	100						
H. neptunium LE-670^T	27	66	100					
H. johnsonii MHS-2^T	13	25	25	100				
H. hirschiana VP-5^T	34	19	54	12	100			
H. oceanitis Sch89^T	28	11	12	23	20	100		
H. jannaschiana VP-1^T	8	41	32	18	23	26	100	
H. rosenbergii VP-6^T	5	30	11	21	19	2	4	100

Information on the antibiotic sensitivity of *Hyphomonadaceae* is scarce because only a minority of strains has been tested for their susceptibility against antibiotics. Most strains were susceptible against novobiocin, penicillin, and ampicillin, but *Hyphomonas oceanitis* SCH89^T and *Litorimonas taeanensis* G5^T were resistant against all three. *Litorimonas taeanensis* G5^T and *Robiginitomaculum antarcticum* IMCC3195^T showed also resistance against streptomycin and kanamycin. In the genome of *Oceanicaulis* sp. HTCC 2633, 15 genes for beta-lactamases and a bicyclomycin resistance protein were detected (Oh et al. 2011).

All *Hyphomonadaceae* have high activities in alkaline phosphatase. Esterase (C_4) and esterase/lipase (C_8) activities are

Table 12.4
DNA–DNA hybridization between the *Maricaulis* type strains (Abraham et al. 2002)

	ATCC 15268^T	MCS 25^T	VKM B-1513^T	MCS 6^T	MCS 18^T
M. maris ATCC 15268^T	100				
M. maris ATCC 15269	82				
M. parjimensis MCS 25^T	23	100			
M. virginensis VKM B-1513^T	n.d.	32	100		
M. virginensis VKM B-1514	33	28	83		
M. washingtonensis MCS 6^T	13	21	25	100	
M. salignorans MCS 18^T	32	18	23	59	100

n.d. not determined

present in all species; however, the activity is medium in *Woodsholea*, *Henricella*, and *Ponticaulis* species. Lipase (C_{14}) activity is absent or weak in all species. Leucine arylamidase is also present in all strains and usually high, while the valine and cystine arylamidases activities are always lower and in some species weak. Trypsin activities vary considerably between strains and have not been found in some species, e.g., *Hyphomonas polymorpha* DSM 2665^T, *Algimonas* porphyrae OC-2-2^T, or *Hellea balneolensis* DSM 19091^T. Similarly α-chymotrypsin varies being strong in *Henriciella* spp., *Oceanicaulis* spp., *Maricaulis parjimensis* MCS 25^T, and *M. maris* ATCC 15268^T but weak in *M. salignorans* MCS 18^T and *M. washingtonensis* MCS 6^T and even absent in *Hirschia* spp., *Algominas porphyrae* OC-2-2^T, and *Hyphomonas polymorpha* DSM 2665^T. The activity of acid phosphatase is rather abundant but even more variable being very active in *Maricaulis parjimensis* MCS 25^T, but absent in *M. maris* ATCC 15268^T, *M. washingtonensis* MCS 6^T, *Hellea balneolensis* DSM 19091^T, and *Robiginitomaculum antarcticum* IMCC3195^T. The same behavior was found for naphthol-AS-BI-phosphohydrolase. From the group of sugar-cleaving enzymes, α-glucosidase is the most frequent enzyme. Its activity has been reported from all *Henriciella* species, *Litorimonas taeanensis* G5^T, and *Hirschia baltica* DSM 5838^T, and weak activity was also found in *Maricaulis parjimensis* MCS 25^T and *Hyphomonas polymorpha* DSM 2665^T. All other species are lacking this activity. With the exception of the *Henriciella* species, the same species all showed weak activity of β-glucosidase. Only for *Hirschia maritima GSW*-2^T and *Hellea balneolensis* DSM 19091^T activity of β-galactosidase was reported. *Hirschia maritima* GSW-2^T was the only species showing α-fucosidase activity, while *Woodsholea maritima* CM 243^T was the only species possessing weak *N*-acetyl-β-glucosaminidase activity. None of the species showed any α-mannosidase or α-galactosidase activities.

Hyphomonadaceae display some characteristic fatty acids which can be used for their identification (*Table 12.1*). *Hirschia* species are the only ones containing C14:0. *Woodsholea*, *Oceanicaulis*, *Algimonas*, and *Ponticaulis* species are lacking C15:0; *Maricaulis*, *Robiginitomaculum*, and *Hellea* species have only traces of this fatty acid which is present in higher amounts in the other *Hyphomonadaceae* genera. C16:0 is usually abundant in the cells, but *Woodsholea*, *Oceanicaulis*, *Hellea*, and *Robiginitomaculum* spp. have low amounts. Usually this fatty acid is accompanied by lower amounts of its unsaturated derivative C16:1ω7c. However, *Woodsholea*, *Henriciella*, *Oceanicaulis*, *Ponticaulis*, *Hellea*, and *Litorimonas* are lacking C16:1ω7c completely. The isoforms of C17:0, iC17:0 and iC17:1ω9c, are together with C18:1ω9c abundant only in *Maricaulis* and characteristic within the *Hyphomonadaceae* for this genus. *Oceanicaulis* and *Woodsholea* species contain significantly higher amounts of C18:0 than the species of the other genera. *Henriciella* strains are the only ones showing C20:0. The branched fatty acid, *trans*-11-methyl-12-octadecenoic acid (ECL 18.080) (Abraham et al. 2008), was found in *Maricaulis*, *Hyphomonas*, *Henriciella*, *Oceanicaulis*, *Hellea*, and *Litorimonas* species with unusually high amounts in *Oceanicaulis*. The occurrence of 10-methyl-C18:0 (TSBA) in *Hellea balneolensis* is remarkable. Hydroxy fatty acids are usually esterified with the cell wall and are often used as biomarkers for bacteria. iC11:0 3OH is only present in species of *Maricaulis* and *Hellea*. While *Hyphomonas* and *Hellea* species have more C12:1 3OH than C12:0 3OH, the contrary is the case for *Henriciella* strains. *Oceanicaulis* and *Woodsholea* species possess only C12:0 3OH. C12:0 3OH and C12:1 3OH are absent in species of *Maricaulis*, *Robiginitomaculum*, *Litorimonas*, and *Algimonas*. Only *Algimonas* and *Hellea* strains show high amounts of C18:1 2OH which was also found in much lower amounts in some *Henriciella* strains. *Woodsholea* is the only genus containing considerable amounts of the unknown fatty acid ECL 15.275.

Instead of saponifying the fatty acids for the analysis, the intact polar lipids can be used for chemotaxonomy. A detailed analysis of the polar lipids using tandem-mass spectrometry revealed that they are good biomarkers for the characterization of genera of *Hyphomonadaceae*. The glycolipid fraction of the cells can best be analyzed by chemical ionization (CI) using ammonium as ionization gas. This gives mainly the molecular ions of the individual glycolipids which can be further characterized by collision-induced decay (CID) allowing the identification of the individual fatty acids and their position at the

Table 12.5
Comparison of selected characteristics of *Hyphomonas* species

	1	2	3	4	5	6	7	8
Biofilm formation	+++	Weak	+	−/+	Weak	−	−/+	++
Optimal growth temperature (°C)	25–37	25–31	37	25–37	30–37	20–30	30–37	25–45
Salt range (g l^{-1})	15–120	35–75	35–75	15–60	10–75	10–75	5–50	10–120
Optimal pH	5,7–8.7	7.6	7.6	5.7–8.1	8.0	7.6	7.0–7.4	5.7–8.9
Rosette formation	−	−	Variable	+	−	−	−	+
Nitrate reduction	+	+	+	+	+	+	−	+
Growth in glucose	−	−	−	+	−	−	−	−
G+C %	60	57	60	64	60–62	59	60–61	61

1 H. adhaerens, 2 H. hirschiana, 3 H. jannaschiana, 4 H. johnsonii, 5 H. neptunium, 6 H. oceanitis, 7 H. polymorpha, 8 H. rosenbergii. Data for 1, 4 and 8 from Weiner et al. (2000); for 2, 3, 5, 6, 7 Weiner et al. (1985)

glycerol moiety of the molecules. The pattern of the glycolipids allows a differentiation of isolates often down to the species level. In *Hyphomonadaceae* only the glycolipids α-D-glucopyranosyl diacylglycerol (MGDG) and α-D-glucuronopyranosyl diacylglycerol (GUDG) could be detected. For a similar analysis of phospho- and sulfolipids, ionization by fast atom bombardment (FAB) is more suitable. *Hyphomonadaceae* show here a rich diversity of sulfoquinovosyl diacylglycerols (SQDG), phosphatidyl diacylglycerols (PG), and α-D-glucuronopyranosyl diacylglycerol taurine amide (Tau). The distribution of SQDG, PG, and Tau is not uniform but specific for the individual taxa, and many show reduced amounts of PG which seems to be replaced by SQDG (Abraham et al. 1997). This fits to their habitats and it has been shown that limitation in phosphate leads to a shift from PG to SQDG (Batrakov et al. 1996a; van Mooy et al. 2009).

The main features of members of *Hyphomonadaceae* are listed in ❯ *Tables 12.5*, *12.6*, and *12.7*.

Hyphomonas ex Pongratz (1957), Moore et al. (1984), Weiner et al. (2000)

The description is the one given by Weiner et al. (2000) emended by our own results. *Hyphomonas* species normally generate only a single polar prosthecum and reproduce by budding (❯ *Fig. 12.3*). Members of the genus *Hyphomonas* undergo a rather complex biphasic life cycle. The life cycle consists of a swarm-cell stage that eventually metamorphoses into a benthic, reproductive cell during the biphasic developmental cycle. *Hyphomonas* cells are rod-shaped to oval, mature cells measuring 0.5–1.0 × 1.0–3.0 μm; buds are produced at the tips of polar prosthecae (❯ *Fig. 12.4*), which measure 0.2–0.3 μm in diameter and are 1.5 times the length of the cell body; and swarm cells are motile by means of a single polar to lateral flagellum located on developing buds of younger daughter cells. Gram negative, nonacid fast, aerobic, nonspore forming, and chemoorganotrophic. All strains investigated so far are catalase positive, oxidase positive, urease negative, indole negative, hydrogen sulfide negative, non-saccharolytic, and nonpathogenic for mammals. Peptone is normally required for growth. With one exception, all strains denitrify. The species catabolize amino acids for energy and growth; only *Hyphomonas johnsonii* MHS-2 T can utilize sugars. Members of *Hyphomonas*, especially the vent strains, grow along steep physicochemical gradients involving considerable temperature, pressure, and salinity variations in a broad range of environmental conditions. The optimum growth temperature range is 22–37 °C. *Hyphomonas neptunium*, *Hyphomonas polymorpha*, *Hyphomonas oceanitis*, and *Hyphomonas hirschiana* have a Q-11 ubiquinone type along with a significant amount of Q-10 (approx. 10 % of total ubiquinones) and minor amounts of Q-9 and Q-12. *Hyphomonas jannaschiana* has Q-10 as its major quinone, with trace amounts of Q-9 and Q-11. Polar lipids are 1,2-diacyl-3-α-D-glucopyranosyl-sn-glycerol, 1,2-diacyl-3-α-D-glucuronopyranosyl-*sn*-glycerol, phosphatidyl glycerol, and 1,2-diacyl-3-α-D-glucuronopyranosyl-*sn*-glycerol taurine amide (Batrakov et al. 1996a, b), while sulfoquinovosyl diacylglycerol is absent (Abraham et al. 1997). The dominant fatty acid, common to all *Hyphomonas* spp., is 18:1ω7c. The GC content of the DNA is 57–64 mol%. *Hyphomonas* species were isolated from many ocean niches, including the open ocean (the pelagic zone), mud sloughs, and different hydrothermal vent sites. They are primary colonizers of submerged marine surfaces, some species producing dense biofilms.

Maricaulis Abraham et al. (1999)

L. n. *mare -is*, the sea; L. masc. n. *caulis*, stalk; N.L. masc. n. *Maricaulis*, stalk from the sea.

The description is an emended version of the description given Abraham et al. (1999). Gram-negative cells, rod shaped, fusiform, or vibrioid, 0.4–0.5 by 1–2 μm. Cells possess a prostheca, ca. 0.15 μm in diameter and of varying length depending on the species and environmental conditions, extending from one pole as a continuation of the long axis of the cell. Adhesive material is present at the distal end of the

Table 12.6
Comparison of selected characteristics of *Maricaulis* species

Characteristic	1	2	3	4	5
Enzyme					
Trypsin	+/−	+	+		+
α-Chymotrypsin	+	+/−	+		+
Acid phosphatase	−	+	+/−		−
Naphthol-AS-BI-phosphohydrolase	+/−	+	+/−		+/−
α-Glucosidase	−	+/−	−		−
β-Glucosidase	+	+/−	−		−
Protease	+	+	−		−
β-Galactosidase	−	+	−		−
Nitrate reduction	−	−	+		+
Predominant cellular fatty acids	18:1ω9	18:1ω9	i17:1ω9c	i17:1ω9c	i17:1 ω9c
				18:1ω9	
DNA G+C content		63.0	63.3	65.2	63.0

1 M. maris, 2 M. parjimensis, 3 M. salignorans, 4 M. virginensis, 5 M. washingtonensis

Table 12.7
Comparison of selected characteristics of *Henriciella* species (Lee et al. 2011)

Characteristic	*H. aquamarina*	*H. litoralis*	*H. marina*
Colony color	Grey	Yellow	White
Growth at 40 °C	+	+	−
Esculin hydrolysis	−	+	−
Enzyme activity			
Naphthol-AS-BI-phosphohydrolase	+	Weak	+
α-Glucosidase	+	Weak	+
β-Glucosidase	−	Weak	−
Carbon utilization			
Tween 80	−	−	+
L-Arabinose	−	+	+
D-Fructose	+	+	−
α-D-Glucose	+	−	−
Raffinose	−	+	−
Sucrose	+	−	+
Glycerol	−	+	−
DNA G+C content	61.0	55.2	56.2

prostheca. Occur singly. Multiplication by binary fission. At the time of separation, one cell possesses a prostheca and the other a single polar flagellum. Each new appendage occurs at the cell pole opposite to the one formed during fission. The flagellated cell secretes adhesive material at the base of the flagellum, develops a prostheca at this site, and enters the immotile vegetative phase. Colonies circular, convex, and colorless. Chemoorganotrophic aerobes, but most of the strains tested could grow anaerobically probably using amino acid as fermentable carbon source. Nitrate is reduced to nitrite anaerobically by some strains. All strains are positive for alkaline phosphatase, esterase (C_4), esterase/lipase (C_8), leucine arylamidase, α-chymotrypsin, and naphthol-AS-BI-phosphohydrolase; trypsin activity is always present, although in some species only weakly. All species show weak activities for lipase (C_{14}) and no activities for acid phosphatase, α- and β-galactosidase, β-glucuronidase, α- and β-glucosidase, α-mannosidase, *N*-acetyl-β-glucosaminidase, and α-fucosidase (API ZYM). An exception here is *Maricaulis parjimensis* MCS 25^T which has weak α-chymotrypsin and α- and β-glucosidase activities. Most strains can store carbon as poly-β-hydroxybutyric acid. Mixtures of B vitamins, amino acids, and other organic factors are required for growth. NaCl is required for optimal growth; however, only a few strains tolerate salt concentrations above 100 g NaCl l^{-1}. All strains can grow on peptone yeast extract media with 5 g NaCl l^{-1}; optimal growth between 20 and 60 g NaCl l^{-1}. Growth is inhibited or cells become deformed in media containing 1 % or more organic material. Temperature range of most isolates is 15–40 °C, while 20–30 °C is optimal. The pH range is 6.0–8.0 and optimal pH for growth is around neutral. All strains are characterized by three major fatty acids 16:0, 17:1isoω9c, and 18:1ω7 and the following minor compounds: 17:1ω8, 17:0iso, 17:0, and 18:1ω9. 11:0iso 3-OH is the main hydroxy fatty acid. Polar lipids are α-D-glucopyranosyl diacylglycerol, α-D-glucopyranosyl diacylglycerol, sulfoquinovosyl diacylglycerol, and α-D-glucuronopyranosyl diacylglycerol taurine amide. Most strains contain also phosphatidylglycerol. All isolates were obtained only from seawater. The G+C content is 62.5–65.2 mol%.

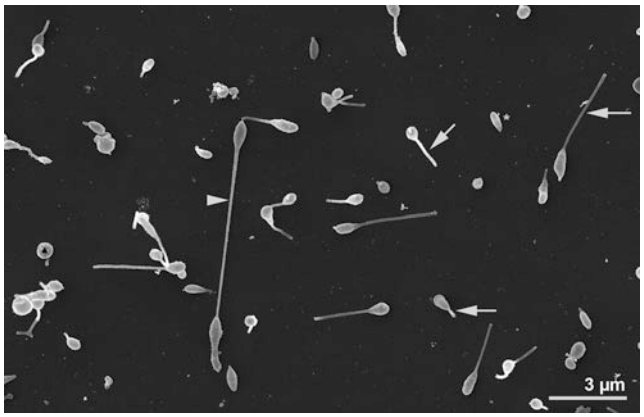

Fig. 12.3
Field emission scanning electron microscopic (FESEM) overview image of logarithmically grown *Hyphomonas jannaschiana* ATCC 33883^T depicting the diverse distinct morphological features of *Hyphomonas* like the formation of prostheca/stalks with different lengths (*arrows*), dividing cell by forming a bud from the distal end of the stalk (*arrowhead*) and swarmer cells (*star*)

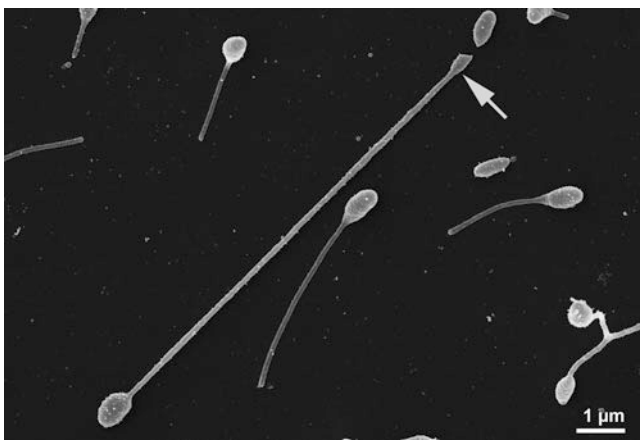

Fig. 12.4
FESEM image showing different lengths of the protheca/stalks of *Hyphomonas jannaschiana* ATCC 33883^T and starting of a budding cell from the distal end of a stalk (*arrow*)

The type species is *Maricaulis maris*. *Caulobacter halobacteroides* Poindexter 1964 was shown to be conspecific to *M. maris* by DNA–DNA hybridization.

Henriciella Quan et al. (2009); emend. Lee et al. (2011)

Henriciella (Hen.ri.ci.el'la. N. L. fem. n. *Henriciella* named after Henrici, A.T., who first described the stalked bacteria genus *Caulobacter*).

Cells are Gram-negative, aerobic, nonspore-forming, motile rods, oxidase, and catalase positive. The major respiratory quinone is Q-10. Cells are usually 0.4–0.7 µm wide and 0.7–2.8 µm long with polar flagellum. Division mode is binary fission. Some cells form mycelium, ranging from 7.8 to 8.0 µm in length. No prostheca were found in the species so far described. Good growth occurs on R2A with 1 % NaCl and MA. Colonies are translucent and shiny. Growth occurs at 10–37 °C (optimum, 20 °C), at pH 5.3–10.5 (optimum, pH 6.9–7.6), and in the presence of 10–150 NaCl g l^{-1} (optimum, 10–20 g l^{-1}). In the API ZYM system, positive for alkaline and acid phosphatase, esterase (C_4), esterase/lipase (C_8), lipase (C_{14}), leucine arylamidase, valine arylamidase, trypsin, α-chymotrypsin, α-glucosidase, and naphthol-AS-BI-phosphohydrolase activities; negative for cystine arylamidase, α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, α-mannosidase, and N-acetyl-β-glucosaminidase activities. *Henriciella marina* differs from this scheme by showing α-fucosidase activity but lack of cystine arylamidase activity. In the API 20NE system, negative for nitrate reduction, indole production, glucose acidification, urease, β-galactosidase, and arginine dihydrolase activities. Some species show β-glucosidase activity. Species do not hydrolyze aesculin and gelatin. The major cellular fatty acids are C18:1ω7c, C16:0, C16:1ω5c, and C17:1ω8c; hydroxyl fatty acids are C12:0 3-OH, C12:1 3-OH, and C18:1 2-OH, but from *Henriciella litoralis* SD10,^{T} no hydroxylated fatty acids were reported. The DNA G+C content of type species is 55.2–62.0 mol %. The type species is *Henriciella marina*. The only species of the genus *Maribaculum*, *Maribaculum marimum*, was transferred to this genus as *Henriciella aquamarina* (Lee et al. 2011).

Hirschia Schlesner et al. (1990); emend. Park and Yoon (2012)

Hirschia (Hirsch'i.a. M.L. fem. n. *Hirschia*, honoring Peter Hirsch, a German microbiologist who is an expert on budding and hyphal bacteria).

The description is an emended version of the description given by Schlesner et al. (1990). Gram-negative, aerobic, motile or nonmotile, oxidase- and catalase-positive, nonspore-forming, prosthecate bacteria with one or occasionally two polarly inserted prostheca (0.06–0.1 µm in diameter). Reproduction occurs by bud formation at the tips of the prostheca. The buds are polarly monotrichously flagellated. Yellow- or orange-pigmented colonies. Mature cells are spherical, oval or rod shaped and measure about 0.6–1.1 × 1.2–1.3 µm. Grow at 10–30 °C (optimum, 30 °C), at pH 6.1–10.1 (optimum, pH 8.1–9.1) with 10 g l^{-1} NaCl. Produces a non-diffusible carotenoid pigment, but bacteriochlorophyll-*a* is absent. Enzyme activities for alkaline phosphatase, trypsin, acid phosphatase, and naphthol-AS-BI-phosphohydrolase were positive, but esterase (C_4), esterase/lipase (C_8), lipase (C_{14}), valine arylamidase, cystine arylamidase, α-chymotrypsin, α-galactosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase were negative (API ZYM). Chemoheterotrophic, C_1 compounds are not utilized as carbon

sources. Polyhydroxybutyrate is not stored. The ubiquinone system is a Q-10 system. Main polar lipids are phosphatidylglycerol and two unidentified lipids. The major cellular fatty acids are C18:1ω7 and C16:0. Hydroxy fatty acids are 3-OH C12:0 for all known species while *Hirschia baltica* had more 3-OH C14:1. The DNA base ratio is 43.7–47 mol% G+C. The type species is *Hirschia baltica*. The genus consists currently of the species *H. baltica*, *H. maritima* Kang and Lee (2009b), and *H. litorea* Park and Yoon (2012). *H. maritima* differs from *H. baltica* by its ability to grow at 4 °C and 37 °C, its tolerance to 20 g l^{-1} NaCl, its ability to hydrolyze gelatin but not DNA, its possession of α- and β-glucosidase, and its lack of β-galactosidase and leucine arylamidase activities (Kang and Lee 2009a).

Oceanicaulis Strömpl et al. (2003)

Oceanicaulis [O.ce.an.i.cau'lis. L. masc. n. oceanus the ocean; L. masc. n. caulis stalk, referring to a prostheca; N.L. masc. n. *Oceanicaulis* stalk (ed organism) from the ocean].

The description of the genus is an emended version given by Strömpl et al. (2003). Gram negative, nonspore forming. Cells are rod shaped or vibrioid. Cultivated cells are 0.5–1 × 0.9–3 μm. In the early stage, cells are non-stalked and motile by means of a single polar flagellum. During binary fission, one cell possesses a stalk (prostheca) and the other a single polar flagellum. During binary fission, at the point of separation, one cell possesses a prostheca and the other a single polar flagellum. Adult cells are nonmotile with prostheca. Adhesive material is present at the distal end of the prostheca. Colonies on MA are round, convex, and non- or lightly pigmented. Aerobic and chemoorganotrophic. The major respiratory quinones are ubiquinone Q-10 and Q-9. Nitrate is reduced to nitrite by most strains. Strains are catalase and oxidase positive. In API ZYM tests, alkaline phosphatase, C4 esterase, C8 esterase/lipase, leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, and naphthol-AS-Bi-phosphohydrolase activities are present, but C14 lipase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase activities are absent. In some strains poly-β-hydroxybutyrate granule accumulation is observed. Cells grow in marine broth at full strength and at 1/10 strength. Strains tested negative for lecithinase, starch, xanthine, indole, o-nitrophenylgalactoside, and Voges–Proskauer, and most tested negative for aesculin, elastinase, gelatinase, and lipase. All strains grow with 20–90 g l^{-1} NaCl and pH 6–10. Mesophilic. Optimum growth occurs at 35–40 °C, 1–2 % NaCl, and pH 7–9. Polar lipids are phosphatidyl glycerol and sulfoquinovosyl diacylglycerol. Major fatty acids are C18:1ω7, C18:0, 7-Me-C18:1ω6, and C17:0 with 3-OH C12:0 as main hydroxy fatty acid. Many strains contain large plasmids. *Oceanicaulis stylophorae* is sensitive to gentamicin, sulfamethoxazole plus trimethoprim, rifampicin, novobiocin, chloramphenicol, nalidixic acid, and tetracycline and resistant to ampicillin, kanamycin, streptomycin, and penicillin G (Chen et al. 2012b). The G+C content of the DNA is 61–62 mol%. The type species is *Oceanicaulis alexandrii*. *Oceanicaulis* comprises two species: *O. alexandrii* and *O. stylophorae* (Chen et al. 2012b). *O. stylophorae* differs from *O. alexandrii* by its temperature range of growth of 15–45 °C (*O. alexandrii* 4–35 °C), lack of C_{14} lipase, the ability to hydrolyze casein but not CM-cellulose, and the utilization of arabinose, adipate, citrate, D-fructose, L-fucose, D-galactose, L-alanine, L-phenylalanine, and L-methionine but not maltose, α-cyclodextrin, α-D-lactose, lactulose, maltose, succinamic acid, hydroxyl-L-proline, or L-threonine.

The following genera *Algimonas*, *Hellea*, *Litorimonas*, *Ponticaulis*, *Robiginitomaculum*, and *Woodsholea* are currently monospecific. Some of their properties have been covered under "short description of genera" and in *Table 12.1*.

Algimonas Fukui et al. (2012)

Algimonas (Al.gi.mo'nas. L. n. alga, seaweed; L. fem. n. *monas*, a monad, unit; N.L. fem. n. *Algimonas*, a unit (bacterium) isolated from seaweed).

The description is the one given by Fukui et al. (2012). The type species is *Algimonas porphyrae* 0C-2-2^T isolated from the red alga *Porphyra yezoensis*. Cells are Gram-negative and straight to slightly curved rods. Many cells are non-stalked and possess a polar flagellum for motility. Some cells possess a prostheca. Multiplication occurs by binary fission. Cells produce orange carotenoid pigments but bacteriochlorophyll-*a* is not found. Aerobic condition and NaCl are required for growth. The predominant isoprenoid quinone is Q-10. Polar lipids are phosphatidylglycerol, glucuronopyranosyldiglyceride, monoglycosyldiglyceride, three unidentified phospholipids, and an unidentified glycolipid. In addition to the description of the genus, the following properties are exhibited by *Algimonas porphyrae*. The cells are 2.13 μm long and 0.37 μm in diameter in MB. Colonies on MA are circular, convex, entire, and 0.22 mm in diameter. Growth of the type strain occurs at 10–30 °C (optimum, 20 °C), pH 6.0–9.0 (optimum, pH 7.0–8.0), and 10–50 g l^{-1} NaCl (optimum, 20–30 g l^{-1}). Oxidase is negative and catalase is positive. Methyl red and Voges–Proskauer reactions are negative. H_2S and indole are not produced. Aesculin and Tween 20, 40, and 80 are hydrolyzed, but agar, alginate, DNA, gelatin, and starch are not. No acid is produced from carbohydrates. Nitrate is reduced. The type strain has alkaline phosphatase, esterase (C_4), esterase/lipase (C_8), leucine arylamidase, valine arylamidase, acid phosphatase, and naphthol-AS-BI-phosphohydrolase activities, but no lipase (C_{14}), cystine arylamidase, trypsin, α-chymotrypsin, α- and β-galactosidase, β-glucuronidase, α- and β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase activities (API ZYM system). The type strain is susceptible to ampicillin, carbenicillin, ciprofloxacin, chloramphenicol, erythromycin, gentamicin, kanamycin, nalidixic acid, neomycin, norfloxacin, novobiocin, penicillin G, rifampicin, streptomycin, and vancomycin but resistant to polymyxin

B and tetracycline. The major cellular fatty acid is C18:1ω7*c*; the major hydroxy fatty acid is 2-OH C18:1. The G+C contents of the DNA are 58.5–60.2 mol%.

Hellea Alain et al. (2008)

Hellea (Hel.le'a. L. fem. n. Helle, a sea goddess in Greek mythology; N.L. fem. n. Hellea named after Helle in reference to the marine origin of the first strain).

The description is an emended version of the description given by Alain et al. (2008). Cells are Gram negative, nonspore forming, rod shaped to vibrioid, and dimorphic. They usually possess one polar stalk (prostheca) and are nonmotile and sessile, or they are nonstalked and motile by means of a polar flagellum. Cells divided by budding and are aerobic, heterotrophic, mesophilic, neutrophilic, and grow best at salt concentrations close to marine salinity. Colonies on MA medium are round, convex, brilliant, and pigmented a brick-red color. Optimal growth occurs at 30 °C, with growth at 15–37 °C. The pH optimum is close to neutral. Cells are catalase positive and oxidase negative and do not reduce nitrate. Hydrolysis of Tween 40 and Tween 80. Grows at NaCl concentrations of 0.2–50 g l^{-1}, with a clear optimum at 30 g l^{-1} NaCl. Growth occurs on acetate, citrate, propionate, pyruvate, succinate, aspartate, glutamate, L-alanine, L-asparagine, L-histidine, L-proline, casamino acids, peptone, tryptone, yeast extract, and D-mannitol. In addition, cis-aconitic acid, D-glucuronic acid, β-hydroxybutyric acid, γ-hydroxybutyric acid, α-ketoglutaric acid, methyl pyruvate, quinic acid, urocanic acid, L-pyroglutamic acid, hydroxy-L-proline, putrescine, *N*-acetylglucosamine, D-arabitol, myoinositol, and xylitol are used. The predominant quinone is Q-10. Polar lipids comprise glucuronopyranosyldiglyceride, monoglycosyldiglyceride, phosphatidylglycerol, an unidentified glycolipid, and two unidentified phospholipids. Main fatty acids are C18:1ω7c, C17:1ω6c, and C16:0; main hydroxy fatty acids are 2-OH C18:1 and 3-OH C10:0. The G+C content of the DNA of the type strain of the type species is 46.8 mol%. The type strain *Hellea balneolensis* 26III/A02/215^T was isolated from the surface microlayer of coastal water in the bay of Banyuls-sur-Mer, in the northwestern Mediterranean Sea on the coast of France.

Litorimonas Jung et al. (2011)

Litorimonas (Li.to.ri.mo'nas. L. n. litus -oris beach; L. fem. n. monasmonad, unit; N.L. fem. n. *Litorimonas* beach bacterium).

The description of the genus is an emended version given by Jung et al. (2011). Cells are Gram-reaction-negative, nonspore-forming, budding, straight to slightly curved rods. Usually nonstalked and motile by means of a polar flagellum; some cells possess one polar stalk (prostheca) and are nonmotile. Cells divide by budding. Oxidase and catalase positive. Neither nitrate nor nitrite is reduced. The only isoprenoid quinone detected is ubiquinone-10 (Q-10). The DNA G+C content of the type strain of the type species is 63.6 mol %. In addition to the characteristics described for the genus, the type species is characterized by the following properties. Cells are 0.4–0.6 × 1.6–2.0 μm, strictly aerobic, chemoheterotrophic, and moderately halophilic. Colonies on MA are yellow orange, convex, and round with entire margins. Growth occurs at 15–40 °C (optimum 25–30 °C), at pH 6–9 (optimum pH 7–8), and in 1–6 % (w/v) NaCl (optimum 2–3 %). Positive for assimilation of D-glucose but negative for assimilation of L-arabinose, D-mannose, maltose, D-mannitol, malic acid, potassium gluconate, *N*-acetylglucosamine, capric acid, adipic acid, trisodium citrate, and phenylacetic acid (API 20 NE). Positive for alkaline phosphatase, esterase/lipase (C_8), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, and a-glucosidase activities but negative for lipase (C_{14}), α- and β-galactosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase activities. Weak enzymic activities are observed for esterase (C_4), β-glucosidase, and naphthol-ASB1-phosphohydrolase (API ZYM). Resistant to polymyxin B, streptomycin, gentamicin, kanamycin, novobiocin, oleandomycin, lincomycin, ampicillin, tetracycline, penicillin G, and carbenicillin but sensitive to chloramphenicol. Phosphatidylglycerol, monoglycosyldiglyceride, glucuronopyranosyldiglyceride, and two unidentified glycolipids are the polar lipids detected. The major cellular fatty acids are C18:1ω7c, C17:0, and C16:0; no hydroxy fatty acids were detected. The DNA G+C content of the type strain is 63.6 mol%; however, this value has recently been corrected drastically to 47.1 % (Fukui et al. 2012). The type strain *Litorimonas taeanensis* G5^T was isolated from beach sand in Taean, South Korea.

Ponticaulis Kang and Lee (2009a)

Ponticaulis (Pon.ti.cau'lis. L. neut. n. pontus the sea; L. masc. n. caulis a stalk, referring to a prostheca; N.L. masc. n. *Ponticaulis* stalk from the sea).

The type strain of *Ponticaulis koreensis* GSW-23^T was isolated from seawater taken from Gimnyeong Beach, Republic of Korea, and expresses the following properties (Kang and Lee 2009a). Colonies are colorless, circular, smooth, convex, and 0.3–0.5 mm in diameter after 5 days of incubation. Cells are 3.1–6.6 μm long and 0.4–0.5 μm wide. Cells are strictly aerobic, Gram-negative, nonspore-forming, non-budding, obligately halophilic rods or vibrioids that are motile by means of a polar flagellum. Multiplication occurs by binary fission. Some cells possess a long prostheca (0.2 μm in diameter) and a holdfast. The major isoprenoid quinone is Q-10. The major cellular fatty acids are summed feature 7 (C18:1ω9c, C18:1ω12t, and/or C18:1ω7c), C16:0, and C18:0. Growth occurs at 10–42 °C (optimum 30–37 °C), at pH 6.1–10.1 (optimum pH 7.1) and with up to 60 g l^{-1} NaCl. Hydrolyses DNA but not casein or starch. Degrades DL-tyrosine but not elastin, chitin, cellulose, hypoxanthine, or xanthine. Positive for gelatin hydrolysis but negative for nitrate reduction, indole production, glucose

fermentation, arginine dihydrolase, urease, aesculin degradation, and β-galactosidase (API 20NE). In API ZYM tests, positive for alkaline phosphatase, esterase (C_4) (weak), esterase/lipase (C_8) (weak), leucine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, and naphthol-AS-BI-phosphohydrolase, but negative for lipase (C_{14}), valine arylamidase, cystine arylamidase, α- and β-galactosidase, β-glucuronidase, α- and β-glucosidase, *N*-acetyl-glucosaminidase, α-mannosidase, and α-fucosidase. The DNA G+C content of the type strain is 53.3 mol%.

Robiginitomaculum Lee et al. (2007)

Robiginitomaculum (Ro.bi.gi.ni.to.ma.cul'um. L. n. robigo -inis rust; L. neut. n. tomaculum, a kind of sausage; N.L. neut. n. Robiginitomaculum, a rust-colored sausage).

The type strain *Robiginitomaculum antarcticum* IMCC3195^T was isolated from surface seawater of Maxwell Bay, King George Island, West Antarctica, and possesses the following properties (Lee et al. 2007). Colonies on MA are circular, smooth, convex, viscous, rusty colored and 0.3–1.0 mm in diameter. Cells are Gram-negative; 1.3–4.5 mm long and 0.4–1.0 mm wide, with a tapered end; nonmotile; non-budding; thin prostheca-producing; and obligately aerobic rods or vibrioids. Multiplication occurs by binary fission. Flagella and holdfast are not present. Some cells possess a thin prostheca that extends along the long cell axis from one pole. Carotenoid pigments are found but bacteriochlorophyll-*a* not. Chemoheterotrophic. The predominant fatty acids are C18:1ω7c, C17:1ω8c, C17:0, and C17:1ω6c and hydroxy fatty acids are 3-OH C9:0, 3-OH C10:0, and 3-OH C11:0. The major respiratory quinone is Q-10. Growth occurs at 3–25 °C, optimally at 20 °C, but not above 30 °C. Growth occurs at pH 5–10 and 5–50 g l^{-1} NaCl, optimally at pH 7 and at 20–25 g l^{-1} NaCl. Oxidase negative and catalase positive. In API 20NE strips, positive for nitrate reduction, aesculin hydrolysis, and β-galactosidase activity. Negative for urea hydrolysis, indole production, acid production from glucose, gelatin liquefaction, and arginine dihydrolase. In the API ZYM system, alkaline phosphatase, esterase/lipase (C_8), leucine arylamidase, valine arylamidase, and cystine arylamidase activities are present. Negative for esterase (C_4), acid phosphatase, β-glucosidase, *N*-acetyl-β-glucosaminidase and α-mannosidase, lipase (C_{14}), trypsin, α-chymotrypsin, naphthol-AS-BI-phosphohydrolase, β-galactosidase, β-glucuronidase, α-galactosidase, α-glucosidase, and α-fucosidase activities. Susceptible to chloramphenicol, erythromycin, rifampicin, and tetracycline, but resistant to ampicillin, gentamicin, kanamycin, penicillin G, streptomycin, and vancomycin. The DNA G+C content is 60.3 mol%.

Woodsholea Abraham et al. (2004)

Woodsholea (Woods.hol'e.a. N.L. fem. n. *Woodsholea* named in honor of the Woods Hole Oceanographic Institution, Massachusetts, USA).

The description of the genus is the one given by Abraham et al. (2004). The type strain of *Woodsholea maritima* CM243^T was isolated from seawater taken from Woods Hole, USA, and expresses the following properties (Abraham et al. 2004): Gram-negative cells, rod shaped and vibriod. Cells possess a stalk, varying in length depending on the strain and environmental conditions, extending from one pole as a continuation of the long axis of the cell. Adhesive material is present at the distal end of the stalk. Occur singly. Multiplication by binary fission. Colonies circular, convex, colorless. Chemoorganotrophic, aerobes, cells can store carbon as poly-β-hydroxybutyric acid. Requirement for organic growth factors is complex and not satisfied by mixtures of B vitamins and amino acids. Grows on peptone/yeast extract media with 40 g NaCl l^{-1}. Growth is inhibited or cells become deformed in media containing 1 % (w/v) or more organic material. Growth temperature range is 20–40 °C and optimal pH for growth is approximately neutral. Do not reduce nitrate, oxidize tryptophan to indole, or hydrolyze arginine, urea, aesculin, gelatin, or p-nitrophenyl-3-D-galactopyranoside. Cells show no catalase activity and are positive for alkaline phosphatase, naphthol-AS-BI-phosphohydrolase, leucine arylamidase, acid phosphatase, esterase (C_4), esterase/lipase (C_8), oxidase, and trypsin but negative for α- and β-galactosidase, β-glucuronidase, α- and β-glucosidase, α-mannosidase, and α-fucosidase. Strains have no to weak α-chymotrypsin and *N*-acetyl-β-glucosaminidase activities; some isolates including the type strain have weak lipase activity. The genus is characterized by two major fatty acids, 18:0 and 18:1ω7c, and minor amounts of 12:0 3-OH, 16:0, 17:0, summed feature 3 (14:0 3-OH, 16:1 iso I, ECL 10.928, and/or 12:0 ALDE), and the unidentified fatty acid ECL 15.275. Polar lipids are α-D-glucopyranosyl diacylglycerol, α-D-glucopyranuronosyl diacylglycerol, sulfoquinovosyl diacylglycerol, and α-D-glucuronopyranosyl diacylglycerol taurine amide. Isolated from seawater. The G+C content is 65 mol%. The G+C content of the type strain is 65.2 mol%. The type species is *Woodsholea maritima*.

Isolation, Enrichment, and Maintenance Procedures

Cell densities of *Hyphomonadaceae* in sea samples are usually low because of the oligotrophic nature of the cells. At the same time the cells are adapted to starvation and this can be used for their isolation. For this reason old marine samples are usually enriched in these bacteria. Many *Hyphomonadaceae* stick with their holdfasts to surfaces, and these surfaces can be other bacteria but also eukaryotic cells. Therefore, filtering the sea samples before plating is not advisable and would lead to a loss of bacteria of interest. Strains of *Hyphomonadaceae* can be isolated from seawater using a standard dilution-plating method on an oligotrophic medium, e.g., R2A agar, diluted 1:10 in seawater (1/10R2A). Because all strains are coming from oligotrophic habitats, full media with more than 1 g l^{-1} soluble organic substrates should be avoided which will cause disfiguring of the cells (pleomorphism). Cell growth is slow

and colonies are visible often only after one or even more weeks. Another isolation method is keeping the seawater sample after addition of 10–30 mg l^{-1} peptone or other carbon source at room temperature for 1–2 months and plating the formed surface film (biofilm) on diluted agar. Instead of using the neuston of the samples, substrata can be submerged in the samples acting as target for the attachment of *Hyphomonadaceae*. The plates can then be removed and the attached bacteria scrapped off and plated. At least *Maricaulis*, *Woodsholea*, *Henriciella*, and *Oceanicaulis* species can also be grown in 0.05 % peptone, 0.05 % casamino acids, and 75–80 % seawater (CPS medium, Poindexter 1964) or marine *Caulobacter* medium SPYEM containing 30 g sea salts (Sigma), 0.5 g NH_4Cl, and 1 l deionized water. After autoclaving and cooling, 20 ml 50 × PYE (50 × PYE: 100 g peptone and 50 g yeast extract in 1 l deionized water (autoclaved)), 2 ml 50 % glucose, and 5 ml riboflavin (0.2 mg ml^{-1}) are added (Abraham et al. 1999). Further purification can be done on marine agar 2216 (MA) after culture at 20 °C for 2 weeks. One should keep in mind that most *Hyphomonadaceae* form clumps and the isolates have to be checked carefully for any mixed cultures. Almost all strains need complex media for growth. One exception is Hyphomonas neptunium which can be grown on GAMS medium: 125 mM of each glutamate, aspartate, serine, methionine, 0.26 µM calcium pantothenate, and 30 % seawater (Havenner et al. 1979). Most strains can be preserved as glycerol suspension (10 %, v/v) at −75 °C. Vegetative stocks should be maintained on 1 % 1/10 R2A agar slants at 4 °C and transferred every 8 or 9 weeks, incubated 2 or 3 days at 20–25 °C then kept at 4 °C. For many strains lyophilization is problematic and often not reliable.

Cell Morphology

All species in the family *Hyphomonadaceae* possess dimorphic cells (◉ *Fig. 12.3*) and reproduce either by binary fission or by budding. Meso-diaminopimelic acid is the diagnostic diamino acid of the cell-wall peptidoglycan. Sessile cells are connected via a stalk to their substrata. For replication in *Algimonas*, *Henriciella*, *Maricaulis*, *Oceanicaulis*, *Ponticaulis*, *Robiginitomaculum*, and *Woodsholea*, a cell is separated by binary fission from the progenitor cell. This sibling carries no stalk but a flagellum which propels it through the medium until a suitable site is found (◉ *Fig. 12.5*). Here the sibling then develops a stalk, becomes sessile, and sheds the flagellum. Long axis of cells can be distinctly curved, but may be straight in some species. Cells are long and slender, approximately 1.5–2.5 µm in length when not dividing, and less than 0.5 µm in diameter or approximately 1 µm in length when not dividing and 0.6 µm in diameter. Stalk length dependent on growth condition and species, one to four times the length of the cell body, diameter 0.11–0.18 µm (◉ *Figs. 12.6* and *12.7*). With the exception of cells of *Robiginitomaculum*, the stalk carries adhesive material at its distal end. *Hellea*, *Hirschia*, *Hyphomonas*, and *Litorimonas* replicate in a different manner. Here the stalk is a reproductive

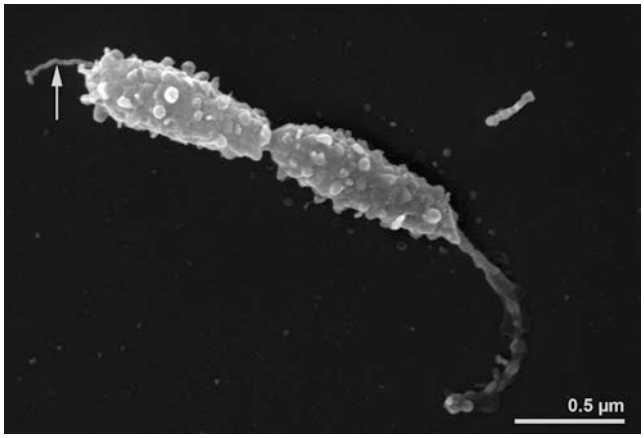

Fig. 12.5
FESEM image of a dividing *Maricaulis salignorans* MSC 18^T cell that depicts the formation of a daughter cell. A flagellum is formed from the distal end of the daughter cell

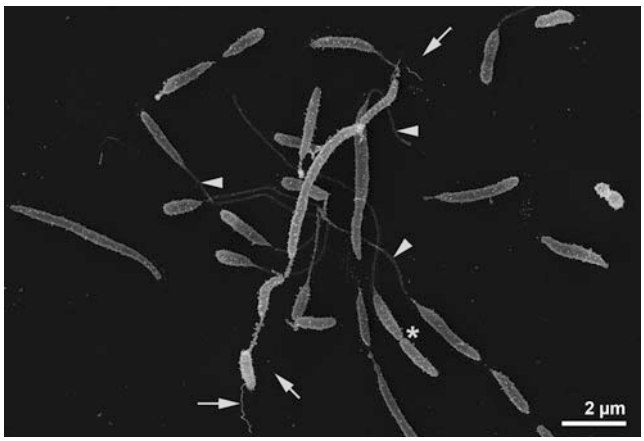

Fig. 12.6
FESEM image of logarithmically grown *Maricaulis salignorans* MSC 18^T showing the diverse distinct morphological features of formation of prostheca/stalks with different lengths (*arrowheads*) and cells with flagella (*arrows*) and a dividing cell (*star*)

structure and DNA and proteins are channeled through it to the new budding cell. Transport within the stalk is achieved in compartments, so-called pseudovesicles (Zerfas et al. 1997) (◉ *Fig. 12.8*). Cells are rod shaped to oval, 0.5–3.0 µm long, and 0.5–1.0 µm in diameter; buds are produced at the tips of polar prosthecae, which measure 0.2–0.3 µm in diameter and are one to five times the length of the cell body. Swarm cells are motile by means of a single polar to lateral flagellum located on developing buds of younger daughter cells (◉ *Fig. 12.9*).

These two groups of cells seem to differ not only in their reproduction but also in their reproductive abilities. While cells of *Maricaulis* or *Caulobacter* produce about 100 offsprings during their lifetime (Poindexter 1964; Ackermann et al. 2003), this number is limited to about 8 in budding genera (Moore 1981).

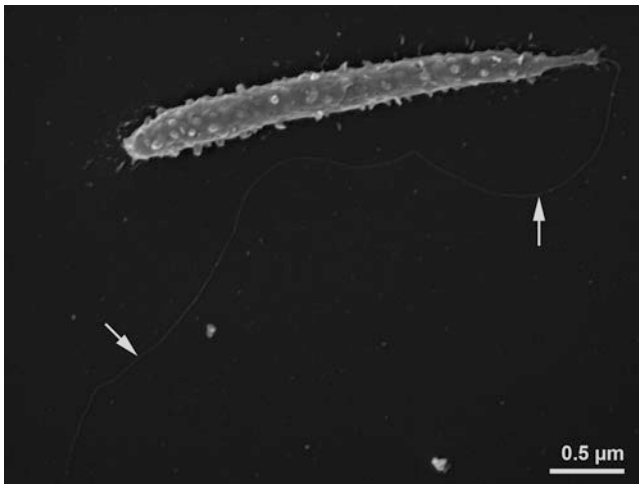

Fig. 12.7
FESEM image of a swarmer cell of *Maricaulis salignorans* MSC 18^T exhibiting a long flagellum

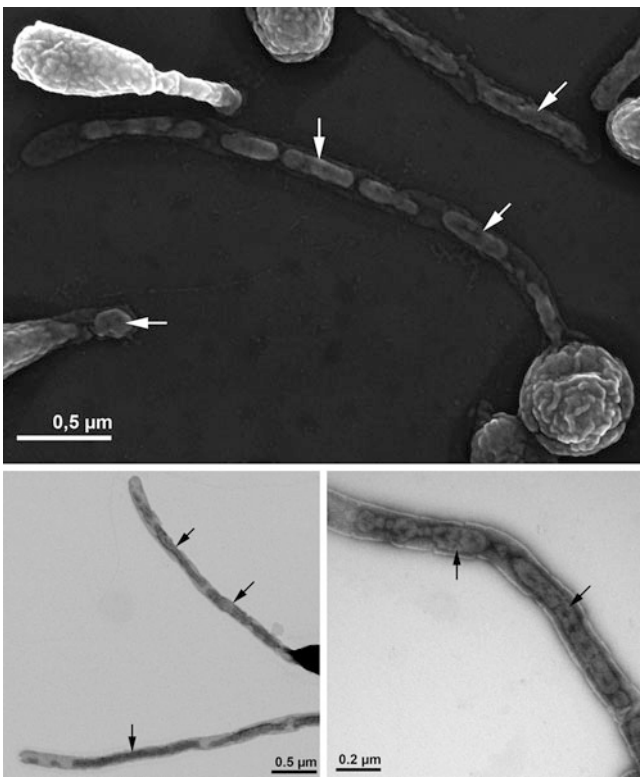

Fig. 12.8
Within the prosthecum/stalk, clearly defined pseudovesicles (*arrows*) can be distinguished by FESEM (*upper image*) or by negatively staining with 2 % uranyl acetate of logarithmically grown *Hyphomonas polymorpha* DSM 2665^T in transmission electron microscopy (*lower two images*). The pseudovesicles are covered by the outer membrane of the bacterium

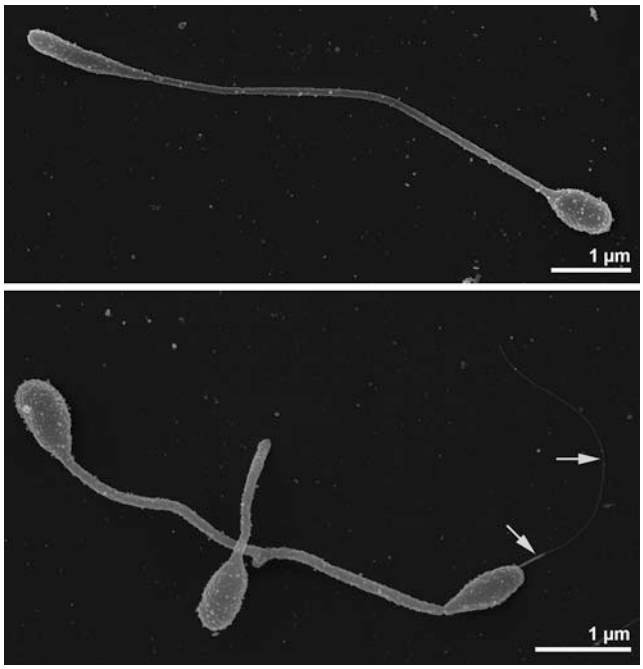

Fig. 12.9
FESEM image depicting a budding cell of *Hyphomonas jannaschiana* ATCC 33883^T from the distal end of a prosthecum/stalk (*upper image*). The formation of a flagellum (*arrows*) after dividing (*lower image*) is visible. The cell with the flagellum will be released as a swarmer cell

One way to protect cells from exoenzyme activities, antibiotics, or other noxious agents produced in biofilms is the formation of S-layers. Such an S-layer was characterized for *Hyphomonas jannaschiana* and two proteins of 29 and 116 kDa were isolated. Both proteins are glycoproteins with the heavier one being the tetramer of the smaller protein. They seemed to be linked by divalent cations because they could be solubilized by EDTA (Shen and Weiner 1998). For the stalk of *Oceanicaulis alexandrii*, it has been shown that it is also covered by an S-layer (Strömpl et al. 2003). S-layers have been found in many *Caulobacteraceae* and it can be assumed that they are also present in a number of *Hyphomonadaceae* species where they have not yet been reported.

Physiology and Metabolism

All species of *Hyphomonadaceae* are strict aerobes although many of them are able to reduce nitrate. None of the isolates have been reported to grow on any mineral medium containing a single substrate as sole source of carbon and energy. Some strains can grow on such a medium if it is supplemented by various growth factors, but most strains need complex (and often ill-defined) mixtures of growth factors or even carbon substrates. *Hyphomonas* are amino-acid-requiring isolates. *Hyphomonas johnsonii* is an outlier species in its genus because it is the only species able to use sugars.

Ecology (Main Habitats)

The main habitat of *Hyphomonadaceae* is the sea with two exceptions: *Hirschia baltica* was isolated from brackish water and *Hyphomonas polymorpha* was isolated from human nasal secretions (but the patient was a deep sea diver). The optimal growth temperature for most species is around 20–30 °C. This is also true for *Robiginitomaculum antarcticum* although it was isolated from surface seawater of Maxwell Bay, King George Island, West Antarctica. This species and species from hydrothermal vents, e.g., *Maricaulis virginensis*, however, can also grow at lower temperature which they probably experience in their special habitats. A true psychrophilic *Hyphomonas* sp. (strain SW47) was isolated from Antarctic sea ice underlining again the ability of some *Hyphomonadaceae* strains to thrive in cold habitats (Bowman et al. 1997).

Although only few reports are available, it can be expected that due to the oligotrophic nature of their habitats, cell numbers of these bacteria will be low. All *Hyphomonadaceae* have swarmer cells which are motile. The dispersal of the progenitor cells is important for species living in oligotrophic habitats because it prevents competition of the siblings for substrates. Genomic and experimental evidences indicate that at least *Hyphomonas neptunium* cells are not chemotactic. The lack of evidence for chemotaxis supports the notion that motility among *Hyphomonas* swarmer cells is a random dispersal method (Badger et al. 2006). However, for most of the *Hyphomonadaceae* species, this has not yet been investigated.

For a number of isolates, associations with eukaryotic cells were reported. *Oceanicaulis alexandrii* was isolated from the marine dinoflagellate *Alexandrium tamarense* (Lebour) Balech. *Maricaulis virginensis* VC-13 was reported to associate in cocultures with several diatoms (Poindexter 2006). The phylogenetic analysis of bacterial communities associated with leaves of the seagrass *Halophila stipulacea* revealed three clones belonging to the Hyphomonadaceae (Weidner et al. 2000). They are phylogenetically positioned between the genera *Ponticaulis* and *Henriciella*. Currently the nature of these associations is not clear, and it has been suggested that the attachment of these strains may simply be caused by the need for a surface to attach. The advantage of an attachment to eukaryotes, however, can easily exceed the mere requirement for a surface to attach. Use of exudates excreted by the eukaryotic host, consumption, and detoxification of oxygen produced by photosynthetic algae or a closed carbon cycle of oxidation of exudates to CO_2 and immediate fixation of the produced CO_2 by the photosynthetic host are only few of the many advantages such epibionts may have in the environment (Poindexter 2006). Still much more research is needed to understand the association of *Hyphomonadaceae* to their hosts.

Biological and Technological Relevance

For none of the isolates, any pathogenicity was shown. This is even the case for *Hyphomonas polymorpha* which comes from a human habitat (Pongratz 1957). Subsequent studies on the diversity of bacteria in the human nasal cavity, however, did not seem to confirm that this is a common habitat for *Hyphomonas* species (Frank et al. 2010).

Only few potential biotechnological applications for strains of the *Hyphomonadaceae* have been reported. A novel architecture has been revealed for the *N*-acetylglutamate synthase of *Maricaulis maris* MCS 10, but it is still too early to predict any biotechnological application from this finding (Shi et al. 2011). *Hyphomonadaceae* have also been shown to be major members in the degradation of municipal solid waste (Trzcinski et al. 2010), but the reported SSU RNA gene sequence is too short for identification of the genus. Strains of *Hyphomonadaceae* seem also to have abilities to degrade complex or even xenophytic organic compounds. Freshwater bacteria degrade the aromatic heterocyclic organic compound carbazole via angular dioxygenation using 1,9a-dioxygenases. A screening for marine isolates possessing this metabolic potential revealed two *Hyphomonadaceae*, strains OC-5 and OC-6 (Maeda et al. 2009). While strain OC-5 falls within the genus *Hyphomonas*, strain OC-6 seems to be a member of the genus *Henriciella*.

To form biofilms many Proteobacteria use *N*-acyl-homoserine lactones (AHLs) for communication for their quorum-sensing systems. It has been first shown for some bacteria that there are enzymes which can split these lactones quenching quorum sensing. There are enzymes cleaving AHL at the lactone site and others cleaving the acyl site chain. While cleavage at the first site is reversible, cleaving the acyl side chains destroys the bioactivity of AHL permanently. These enzymes gained more importance recently as tools to control biofilms (Abraham 2005). Kalia et al. (2011) analyzed the genomes of a number of bacteria for the presence of acyl-homoserine lactone-acylases and lactonases and found both AHL lactonase and acylase in *Hyphomonas neptunium* ATCC 15444. The positive identification of these enzymes has not only possible biotechnological applications, but it tells us also that at least this strain is possible to control biofilm formation of other bacteria by destroying their AHLs.

Concluding Remarks and Perspectives

The last decade has seen descriptions of a number of novel genera belonging to the family *Hyphomonadaceae*, and there is no reason to believe that this will come to an end. We looked at the phylogeny of clones from environmental samples which fall into the space of the *Hyphomonadaceae* to get an impression what may be in store in the future. There are a number of SSU rRNA gene sequences which do not fall into any of the described genera pointing to the existence of genera still novel to science. There are two groups of genera which will probably change if novel species will lead to a sharper definition of these genera. One of these genera is *Henriciella*. Here, only non-stalked species are known but we have isolates in

our laboratory which fall into this genus but clearly show prostheca. Although *Henriciella* is phylogenetically close to *Hyphomonas*, these isolates divide not by budding but by binary fission resembling more *Maricaulis* than *Hyphomonas* species. Another complex of genera for which the genus boundaries seem to be not well defined is formed by the genera *Maricaulis*, *Woodsholea*, and *Oceanicaulis*. Currently criteria can be extracted differentiating these genera; however, only future will tell whether we already know the whole diversity of the species of this complex. This statement is generally valid for any of the single-species genera populating the phylogenetic tree of the *Hyphomonadaceae*. From these considerations it can easily be predicted that we are still facing many exciting new discoveries in this family.

Acknowledgments

The long-standing cooperation with John Smit, Vancouver, and Edward Moore, Gothenburg, is very much acknowledged. We also thank Jennifer Knaak for technical assistance.

References

Abraham W-R (2005) Controlling gram-negative pathogenic bacteria by interfering with their biofilm formation. Drug Design Rev 2:13–33

Abraham W-R, Meyer H, Lindholst S, Vancanneyt M, Smit J (1997) Phospho- and sulfolipids as biomarkers of *Caulobacter sensu lato*, *Brevundimonas* and *Hyphomonas*. Syst Appl Microbiol 20:522–539

Abraham W-R, Strömpl C, Meyer H, Lindholst S, Moore ERB, Christ R, Vancanneyt M, Tindall B, Bennasar A, Smit J, Tesar M (1999) Phylogeny and polyphasic taxonomy of *Caulobacter* species. Proposal of *Maricaulis* gen. nov. with *Maricaulis maris* (Poindexter) comb. nov. as the type species, and emended description of the genera *Brevundimonas* and *Caulobacter*. Int J Syst Bacteriol 49:1053–1073

Abraham W-R, Strömpl C, Bennasar A, Vancanneyt M, Snauwaert C, Swings J, Smit J, Moore ERB (2002) Phylogeny of *Maricaulis* Abraham et al. 1999 and proposal of *Maricaulis virginensis* sp. nov., *M. parjimensis* sp. nov., *M. washingtonensis* sp. nov. and *M. salignorans* sp. nov. Int J Syst Evol Microbiol 52:2191–2201

Abraham W-R, Strömpl C, Vancanneyt M, Bennasar A, Swings J, Lünsdorf H, Smit J, Moore ERB (2004) *Woodsholea maritima* gen. nov., sp. nov., a marine bacterium with a low diversity of polar lipids. Int J Syst Evol Microbiol 54:1227–1234

Abraham W-R, Macedo AJ, Lünsdorf H, Fischer R, Pawelczyk S, Smit J, Vancanneyt M (2008) Phylogeny by a polyphasic approach of the order *Caulobacterales*, proposal of *Caulobacter mirabilis* sp. nov., *Phenylobacterium haematophilum* sp. nov. and *Phenylobacterium conjunctum* sp. nov., and emendation of the genus *Phenylobacterium*. Int J Syst Evol Microbiol 58:1939–1949

Ackermann M, Stearns SC, Jenal U (2003) Senescence in a bacterium with asymmetric division. Science 300:1920

Alain K, Tindall BJ, Intertaglia L, Catala P, Lebaron P (2008) *Hellea balneolensis* gen. nov., sp. nov., a prosthecate alphaproteobacterium from the Mediterranean Sea. Int J Syst Evol Microbiol 58:2511–2519

Badger JH, Eisen JA, Ward NL (2005) Genomic analysis of *Hyphomonas neptunium* contradicts 16S rRNA gene-based phylogenetic analysis: implications for the taxonomy of the orders '*Rhodobacterales*' and *Caulobacterales*. Int J Syst Evol Microbiol 55:1021–1026

Badger JH, Hoover TR, Brun YV, Weiner RM, Laub MT, Alexandre G, Mrazek J, Ren Q, Paulsen IT, Nelson KE, Khouri HM, Radune D, Sosa J, Dodson RJ, Sullivan SA, Rosovitz MJ, Madupu R, Brinkac LM, Durkin AS, Daugherty SC, Kothari SP, Giglio MG, Zhou L, Haft DH, Selengut JD, Davidsen TM, Yang Q, Zafar N, Ward NL (2006) Comparative genomic evidence for a close relationship between the dimorphic prosthecate bacteria *Hyphomonas neptunium* and *Caulobacter crescentus*. J Bacteriol 188:6841–6850

Batrakov SG, Nikitin DI, Pitryuk IA (1996a) Lipid composition of the gram-negative, budding, seawater bacterium *Hyphomonas jannaschiana* lacking in phospholipids. Biochim Biophys Acta 1303:39–46

Batrakov SG, Nikitin DI, Pitryuk IA (1996b) A novel glycolipid, 1,2-diacyl-3-alpha-D-glucuronopyranosyl-sn-glycerol taurineamide, from the budding seawater bacterium *Hyphomonas jannaschiana*. Biochim Biophys Acta 1302:167–176

Bowers LM, Shapland EB, Ryan KR (2008) Who's in charge here? Regulating cell cycle regulators. Curr Opin Microbiol 11:547–552

Bowman JP, McCammon SA, Brown MV, Nichols DS, McMeekin TA (1997) Diversity and association of psychrophilic bacteria in Antarctic sea ice. Appl Environ Microbiol 63:3068–3078

Brilli M, Fondi M, Fani R, Mengoni A, Ferri L, Bazzicalupo M, Biondi EG (2010) The diversity and evolution of cell cycle regulation in alpha-proteobacteria: a comparative genomic analysis. BMC Syst Biol 4:52

Chen H, Jogler M, Rohde M, Klenk HP, Busse HJ, Tindall BJ, Spröer C, Overmann J (2012a) Reclassification and amended description of *Caulobacter leidyi* as *Sphingomonas leidyi* comb. nov., and emendation of the genus *Sphingomonas*. Int J Syst Evol Microbiol 62(Pt 12):2835–2843

Chen MH, Sheu SY, Chen CA, Wang JT, Chen WM (2012b) *Oceanicaulis stylophorae* sp. nov., isolated from the reef-building coral *Stylophora pistillata*. Int J Syst Evol Microbiol 62(Pt 9):2241–2246

Chertkov O, Brown PJB, Kysela DT, Pedro MADE, Lucas S, Copeland A, Lapidus A, Del Rio TG, Tice H, Bruce D, Goodwin L, Pitluck S, Detter JC, Han C, Larimer F, Chang Y-J, Jeffries CD, Land M, Hauser L, Kyrpides NC, Ivanova N, Ovchinnikova G, Tindall BJ, Göker M, Klenk H-P, Brun YV (2011) Complete genome sequence of *Hirschia baltica* type strain (IFAM 1418^T). Stand Genomic Sci 5:287–297

Copeland A, Lucas S, Lapidus A, Barry K, Detter JC, Glavina del Rio T, Hammon N, Israni S, Dalin E, Tice H, Pitluck S, Saunders E, Brettin T, Bruce D, Han C, Tapia R, Gilna P, Schmutz J, Larimer F, Land M, Hauser L, Kyrpides N, Mikhailova N, Viollier P, Stephens C, Richardson P (2011) Complete sequence of *Maricaulis maris* MCS10. GenBank CP000449

Frank DN, Feazel LM, Bessesen MT, Price CS, Janoff EN, Pace NR (2010) The human nasal microbiota and *Staphylococcus aureus* carriage. PLoS One 5:e10598. doi:10.1371/journal.pone.0010598

Fukui Y, Abe M, Kobayashi M, Saito H, Oikawa H, Yano Y, Satomi M (2012) *Algimonas porphyrae* gen. nov., sp. nov., a member of the family *Hyphomonadaceae*, isolated from a red alga *Porphyra yezoensis*. Int J Syst Evol Microbiol 63(Pt 1):314–20. doi:10.1099/ijs.0.040485-0

Garrity GM, Bell JA, Lilburn T (2005) Class I. *Alphaproteobacteria* class. nov. In: Brenner DJ, Krieg NR, Staley JT, Garrity GM (eds) Bergey's manual of systematic bacteriology, 2nd edn. The *Proteobacteria*, vol. 2. The *Alpha-*, *Beta-*, *Delta-*, and *Epsilonproteobacteria*, part C. Springer, New York, p 1

Hardy GGR, Allen C, Toh E, Long M, Brown PJ, Cole-Tobian JL, Brun YV (2010) A localized multimeric anchor attaches the *Caulobacter* holdfast to the cell pole. Mol Microbiol 76:409–427

Havenner JA, McCardell BA, Weiner RM (1979) Development of defined, minimal, and complete media for the growth of *Hyphomicrobium neptunium*. Appl Environ Microbiol 38:18–23

Henrici AT, Johnson D (1935) Stalked bacteria, a new order of *Schizomycetes*. J Bacteriol 29:3–4

Jung JY, Kim JM, Jin HM, Kim SY, Park W, Jeon CO (2011) *Litorimonas taeanensis* gen. nov., sp. nov., isolated from a sandy beach. Int J Syst Evol Microbiol 61:1534–1538

Kalia VC, Raju SC, Purohit HJ (2011) Genomic analysis reveals versatile organisms for quorum quenching enzymes: acyl-homoserine lactone-acylase and -lactonase. Open Microbiol J 5:1–13

Kang HS, Lee SD (2009a) *Ponticaulis koreensis* gen. nov., sp. nov., a new member of the family *Hyphomonadaceae* isolated from seawater. Int J Syst Evol Microbiol 59:2951–2955

Kang HS, Lee SD (2009b) *Hirschia maritima* sp. nov., isolated from seawater. Int J Syst Evol Microbiol 59:2264–2268

Lee K, Lee HK, Choi TH, Cho JC (2007) *Robiginitomaculum antarcticum* gen. nov., sp. nov., a member of the family *Hyphomonadaceae*, from Antarctic seawater. Int J Syst Evol Microbiol 57:2595–2599

Lee KB, Liu CT, Anzai Y, Kim H, Aono T, Oyaizu H (2005) The hierarchical system of the '*Alphaproteobacteria*': description of *Hyphomonadaceae* fam. nov., *Xanthobacteraceae* fam. nov. and *Erythrobacteraceae* fam. nov. Int J Syst Evol Microbiol 55:1907–1919

Lee SH, Shim JK, Kim JM, Choi H-K, Jeon CO (2011) *Henriciella litoralis* sp. nov., isolated from a tidal flat, transfer of *Maribaculum marinum* Lai et al. 2009 to the genus *Henriciella* as *Henriciella aquimarina* nom. nov. and emended description of the genus *Henriciella*. Int J Syst Evol Microbiol 61:722–727

Loeffler F (1890) Weitere Untersuchungen über die Beizung und Färbung der Geisseln bei den Bakterien. Centralbl Bakteriol Parasitenkd 7:625–639

Maeda R, Nagashima H, Widada J, Iwata K, Omori T (2009) Novel marine carbazole-degrading bacteria. FEMS Microbiol Lett 292:203–209

Moore RL (1981) The biology of *Hyphomicrobium* and other prosthecate, budding bacteria. Annu Rev Microbiol 35:567–594

Moore RL, Weiner RM, Gebers R (1984) Genus *Hyphomonas* Pongratz 1957 nom. rev. emend. *Hyphomonas polymorpha* Pongratz 1957 nom. rev. emend. and *Hyphomonas neptunium* (Leifson 1964) comb. nov. emend. (*Hyphomicrobium neptunium*). Int J Syst Bacteriol 34:71–73

Oh HM, Kang I, Vergin KL, Lee K, Giovannoni SJ, Cho JC (2011) Genome sequence of *Oceanicaulis* sp. strain HTCC2633, isolated from the Western Sargasso Sea. J Bacteriol 193:317–318

Park S, Yoon J-H (2012) *Hirschia litorea* sp. nov., isolated from seashore sediment, and emended description of the genus *Hirschia* Schlesner et al. 1990. Int J Syst Evol Microbiol 63(Pt 5):1684–1689. doi:10.1099/ijs.0.044297-0

Poindexter JS (1964) Biological properties and classification of the *Caulobacter* group. Bacteriol Rev 28:231–295

Poindexter JS (1981) The caulobacters: ubiquitous unusual bacteria. Microbiol Rev 45:123–179

Poindexter JS (2006) Dimorphic prosthecate bacteria: the genera *Caulobacter*, *Asticcacaulis*, *Hyphomicrobium*, *Pedomicrobium*, *Hyphomonas* and *Thiodendron*. Prokaryotes 5:72–90

Pongratz E (1957) D'une bactérie pédiculee isolée d'un pus de sinus. Schweiz Z Allg Pathol Bakteriol 20:593–608

Quan ZX, Zeng DN, Xiao YP, Roh SW, Nam YD, Chang HW, Yoon JH, Oh HM, Bae JW (2009) *Henriciella marina* gen. nov., sp. nov., a novel member of the family *Hyphomonadaceae* isolated from the East Sea. J Microbiol 47:156–161

Rosselló-Mora R, Amann R (2001) The species concept for prokaryotes. FEMS Microbiol Rev 25(1):39–67

Schlesner H, Bartels C, Sittig M, Dorsch M, Stackebrandt E (1990) Taxonomic and phylogenetic studies on a new taxon of budding hyphal *Proteobacteria*, *Hirschia baltica* gen. nov., sp. nov. Int J Syst Bacteriol 40:443–451

Shaheen SM, Ouimet M-C, Marczynski GT (2009) Comparative analysis of *Caulobacter* chromosome replication origins. Microbiol 155:1215–1225

Shen N, Weiner RM (1998) Isolation and characterization of S-layer proteins from a vent prosthecate bacterium. Microbios 93:7–16

Shi D, Li Y, Cabrera-Luque J, Jin Z, Yu X, Zhao G, Haskins N, Allewell NM, Tuchman M (2011) A novel *N*-acetylglutamate synthase architecture revealed by the crystal structure of the bifunctional enzyme from *Maricaulis maris*. PLoS One 6:e28825

Smith CS, Hinz A, Bodenmiller D, Larson DE, Brun YV (2003) Identification of genes required for synthesis of the adhesive holdfast in *Caulobacter crescentus*. J Bacteriol 185:1432–1442

Stahl DA, Key R, Flesher B, Smit J (1992) The phylogeny of marine and freshwater caulobacters reflects their habitat. J Bacteriol 174:2193–2198

Staley JT (1968) *Prosthecomicrobium* and *Ancalomicrobium*: new prosthecate freshwater bacteria. J Bacteriol 95:1921–1942

Strömpl C, Hold GL, Lünsdorf H, Graham J, Gallacher S, Abraham W-R, Moore ERB, Timmis KN (2003) *Oceanicaulis alexandrii* gen. nov., sp. nov., a novel stalked bacterium isolated from a culture of the dinoflagellate *Alexandrium tamarense* (Lebour) Balech. Int J Syst Evol Microbiol 53:1901–1906

Trzcinski AP, Ray MJ, Stuckey DC (2010) Performance of a three-stage membrane bioprocess treating the organic fraction of municipal solid waste and evolution of its archaeal and bacterial ecology. Bioresour Technol 101:1652–1661

Van Mooy BAS, Fredricks HF, Pedler BE, Dyhrman ST, Karl DM, Koblizek M, Lomas MW, Mincer TJ, Moore LR, Moutin T, Rappe MS, Webb EA (2009) Phytoplankton in the ocean use non-phosphorus lipids in response to phosphorus scarcity. Nature 458:69–72

Weidner S, Arnold W, Stackebrandt E, Pühler A (2000) Phylogenetic analysis of bacterial communities associated with leaves of the seagrass *Halophila stipulacea* by a culture-independent small-subunit rRNA gene approach. Microb Ecol 39:22–31

Weiner RM, Devine RA, Powell DM, Dagasan L, Moore RL (1985) *Hyphomonas oceanitis* sp. nov., *Hyphomonas hirschiana* sp. nov., and *Hyphomonas jannaschiana* sp. nov. Int J Syst Bacteriol 35:237–243

Weiner RM, Melick M, O'Neill K, Quintero E (2000) *Hyphomonas adhaerens* sp. nov., *Hyphomonas johnsonii* sp. nov. and *Hyphomonas rosenbergii* sp. nov., marine budding and prosthecate bacteria. Int J Syst Evol Microbiol 50:459–469

Zerfas PM, Kessel M, Quintero EJ, Weiner RM (1997) Fine-structure evidence for cell membrane partitioning of the nucleoid and cytoplasm during bud formation in *Hyphomonas* species. J Bacteriol 179:148–156