Chapter 5
Acidophilic Planctomycetes: Expanding the Horizons of New Planctomycete Diversity

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Contents

Members of the bacterial phylum *Planctomycetes* are ubiquitous in a wide range of aquatic and terrestrial environments with diverse conditions (Fuerst 1995). Despite the reported widespread distribution, the known ecophysiological types of planctomycetes are quite limited. Most currently described planctomycetes are both mesophilic and neutrophilic. *Isosphaera pallida* is the only moderately thermophilic planctomycete so far described (Giovannoni et al. 1987). Psychrophilic and alkaliphilic representatives of the *Planctomycetes* have not yet been isolated. Acidophilic members of this phylum also remained unknown for a long time. The first report on the isolation of a planctomycete-like strain from an acidic environment, i.e., peat bog water (pH 4.2) of the Kaltenhofer Moor near Kiel, Germany, was published by Heinz Schlesner in 1994 (Schlesner 1994). This isolate, however, has not been described in detail. More recent research on acidic peatlands identified these ecosystems as a

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unique source of uncharacterized planctomycete diversity and resulted in isolation of several peat-inhabiting, acidophilic planctomycetes in pure cultures. This chapter gives an overview of planctomycete diversity in acidic wetlands, reports phenotypic traits of the newly described acidophilic planctomycetes, and discusses their functional role in natural environments.

5.1 Occurrence of *Planctomycetes* in Acidic Environments

Planctomycetes can be found in environments with pH values ranging from below 3 (Bohorquez et al. 2012) to above 11 (Schlesner 1994). The acidic range of this spectrum is represented mostly by terrestrial environments, such as acidic soils, wetlands, ore deposits, and mining areas. Cultivation-independent molecular recovery of 16S rRNA genes from various acidic (pH 4.2–5.5) soils has repeatedly demonstrated the presence of planctomycetes (Liesack and Stackebrandt 1992; Borneman and Triplett 1997; Jangid et al. 2008; Tsai et al. 2009). These bacteria have been detected among acidophilic microorganisms colonizing waste ore deposits at an acid (pH 3.0) mine drainage site in China (Hao et al. 2007). Analysis of the microbial community composition in an acidic (pH 2.7) hot (29 °C) spring of the Colombian Andes revealed a high relative abundance of 16S rRNA gene sequences from the *Planctomycetes* (Bohorquez et al. 2012). Members of this phylum were identified as one of the dominant bacterial groups in the suspended acidic (pH 4.0–5.1) water within bromeliad tanks in the tropical rainforest canopy (Goffredi et al. 2011). The most extensive acidic habitat colonized by planctomycetes, however, is *Sphagnum*-dominated northern wetlands (Dedysh et al. 2006; Kulichevskaya et al. 2006; Ivanova and Dedysh 2012). The ecology and biology of planctomycetes inhabiting these wetlands are discussed below.

5.2 Acidic Northern Wetlands as a Habitat for Planctomycetes

Wetlands are ecosystems in which the water table is permanently or periodically close to the soil surface. More than half of the global wetland area is located between 50°N and 70°N and is therefore referred to as northern wetland. *Sphagnum*-dominated peatlands represent one of the most extensive types of northern wetlands. *Sphagnum* moss is characteristic of peat bogs and poor fens. These ecosystems typically have pH values between 3.5 and 5.5 and are nutrient poor by nature. The total concentration of mineral nutrients is usually in the range of 5–50 mg L^{-1}. Therefore, transformations of mineral N, S, and Fe are of minor importance in these ecosystems, while degradation of plant litter is the basis of the microbial food chain (Dedysh 2011).

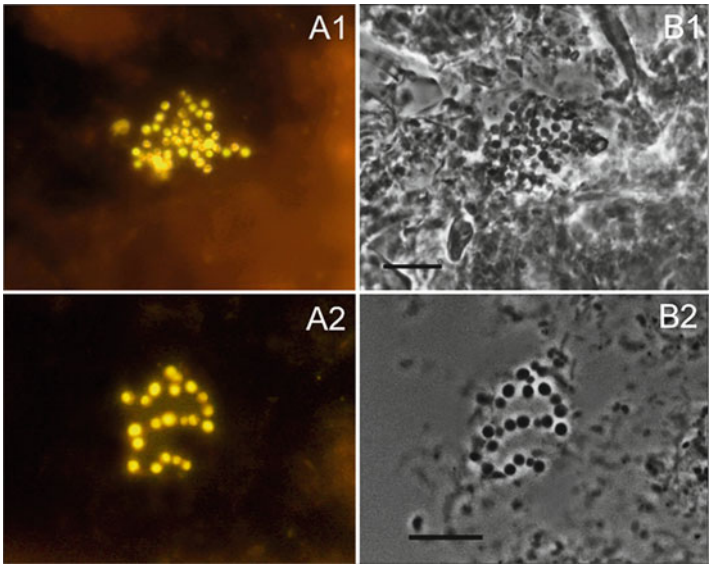

Fig. 5.1 Specific detection of planctomycete cells in acidic *Sphagnum* peat by FISH. Epifluorescent micrographs of in situ hybridizations with Cy3-labelled probes PLA46 and PLA886 (**a**) and the phase-contrast images (**b**) are shown. Bar, 10 **μ**m

As shown in several cultivation-independent diversity studies, nucleotide sequences of planctomycetes commonly comprise 2–5 % of all 16S rRNA gene sequences in the clone libraries made from *Sphagnum* mosses or *Sphagnum*-derived peat (Juottonen et al. 2005; Dedysh et al. 2006; Morales et al. 2006; Ausec et al. 2009). These data, however, have low quantitative value since planctomycetes are strongly underrepresented in clone libraries obtained with the widely used *Bacteria*-specific PCR primer 9-27f (Lane 1991). As shown by Vergin and colleagues (Vergin et al. 1998), this primer contains a mismatch with the corresponding sequence region in full-length 16S rRNA genes in planctomycete genome fragments deposited in fosmid clone libraries. The use of alternative primers may significantly increase the proportion of planctomycete-related sequences. For example, a clone library made from *Sphagnum fallax* with the use of 799f/1492r primers contained 22.5 % of sequences affiliated with the *Planctomycetes* (Bragina et al. 2012).

A combination of two oligonucleotide FISH probes PLA46 and PLA886 with the target specificity for the phylum *Planctomycetes* and the order *Planctomycetales*, respectively (Neef et al. 1998), was applied to determine the in situ abundance of these bacteria in peat sampled from nine *Sphagnum*-dominated wetlands of different geographic locations within European North Russia and West Siberia (Ivanova and Dedysh 2012). The probes hybridized to numerous spherical- or ellipsoid-shaped cells that were arranged in chains or in shapeless cell aggregates and were mostly attached to the particles of nondecomposed organic material (Fig. 5.1). Highest abundances of cells targeted with the probes PLA46 and PLA886 were

observed in the uppermost, oxic layers of the wetland profiles. The cell numbers of planctomycetes in these layers were in the range $1.1–6.7 \times 10^7$ cells per gram of wet peat, comprising 2–14 % of all bacteria. The abundance of planctomycetes in wetlands was positively correlated with pH value of peat water. Highest cell numbers of these bacteria were detected in mildly acidic (pH 5.3–6.0) fens, whereas low planctomycete abundance was more typical for more acidic (pH 3.7–4.5) ombrotrophic bogs, which are nutrient-deficient wetlands that receive water inputs primarily from precipitation. A sharp decline of planctomycete abundance with depth was observed in most peatland sites, suggesting the numerical predominance of aerobic moderately acidophilic planctomycetes.

5.3 Isolation Approaches

A well-established technique for the successful isolation of planctomycetes recommends the use of dilute media with antibiotics affecting the biosynthesis of peptidoglycan in growing cells (Staley 1973; Schlesner 1994; Staley et al. 1992). Dilute media are preferred since many planctomycetes do not grow on nutrient-rich media. In addition, the latter promote development of fast-growing bacteria, which easily overgrow planctomycetes. Therefore, one of the recommended enrichment procedures for samples from aquatic habitats is their long incubation without any addition of nutrients. The use of *N*-acetylglucosamine as a sole source of carbon and nitrogen was also proposed as one of the approaches that gives a selective advantage to planctomycetes (Schlesner 1994).

All of the above-mentioned approaches are clearly applicable for the isolation of planctomycetes from acidic *Sphagnum*-dominated wetlands. A more accurate simulation of the peat bog environment in the laboratory, however, requires the use of acidic (pH 4.0–5.8) media with low concentrations of mineral salts (Dedysh 2011). One example of an appropriate medium is medium M31 (Kulichevskaya et al. 2007a), which is a modification of medium 31 described by Staley et al. (1992). This medium has pH 5.8 and contains *N*-acetylglucosamine as the growth substrate. Several peat-inhabiting planctomycetes, which were further assigned to the novel genera *Schlesneria*, *Singulisphaera*, and *Zavarzinella*, were isolated using medium M31. These bacteria displayed a clear preference for growth in mildly acidic conditions but, in general, their phenotypic traits were quite similar to those in all neutrophilic planctomycetes described earlier. Phenotypically more unusual planctomycetes, *Candidatus* Nostocoida acidiphila and *Telmatocola sphagniphila*, were isolated on the mineral medium M1 containing polysaccharide Phytagel as a solidifying agent (Kulichevskaya et al. 2012b, c). The latter apparently served as the growth substrate for the newly isolated planctomycetes. Interestingly, both *Candidatus* Nostocoida acidiphila and *Telmatocola sphagniphila* were unable to develop on common "planctomycete-specific" media containing relatively high (0.05–0.2 %) concentrations of N-acetylglucosamine or sugars and displayed several other unusual features (see Sect. 8.4). These examples nicely demonstrate the ultimate need of the novel cultivation approaches for discovery of the novel planctomycete phenotypes.

The use of Phytagel (Gellan Gum, Gelrite) instead of agar has one additional advantage: this polysaccharide is free of contaminants which may inhibit growth of some bacteria. Several acidophilic planctomycetes, such as *Telmatocola sphagniphila*, *Candidatus* Nostocoida acidiphila, and some as-yet-undescribed isolates in our collection, were unable to develop on agar media.

Extended incubation time is also one of the important prerequisites for the successful cultivation of peat-inhabiting planctomycetes. Colonies of these fastidious bacteria appear on solid media only after 4–6 weeks or several months of incubation (Kulichevskaya et al. 2009, 2012c).

Finally, one of the most efficient screening tools to monitor the enrichment/isolation procedure and to recognize the presence of planctomycetes is the use of fluorescence in situ hybridization (FISH) with a combination of two *Planctomycete*-specific oligonucleotide probes PLA46 and PLA886 (Neef et al. 1998). FISH-based screening allows direct visualization of the target bacteria within the entire population of cells present in the sample (Fig. 5.1). This screening tool was successfully applied in our work for examination of microbial biofilms consisting of cells of peat-inhabiting bacteria that were obtained by using a Petri dish technique described by Schlesner (1994). Further work on isolation of planctomycetes from these microbial biofilms was also monitored by means of whole-cell hybridization with these probes, which greatly simplified all related identification/purification procedures (Dedysh et al. 2006; Kulichevskaya et al. 2006).

5.4 Cultivated Representatives of Acidophilic Planctomycetes

The use of the above-listed isolation approaches resulted in isolation of several peat-inhabiting, acidophilic planctomycetes in pure cultures. These isolates were further described as representing the novel genera *Schlesneria*, *Singulisphaera*, *Zavarzinella*, and *Telmatocola* (Kulichevskaya et al. 2007a, 2008, 2009, 2012a, c) (Table 5.1). As shown in Fig. 5.2, these newly described planctomycetes do not form a phylogenetically coherent cluster but belong to different subgroups within the family *Planctomycetaceae*. *Schlesneria paludicola* belongs to the phylogenetic lineage defined by the genus *Planctomyces*, *Singulisphaera acidiphila* and *S. rosea* are members of the *Isosphaera* group, while *Zavarzinella formosa* and *Telmatocola sphagniphila* affiliate with the lineage defined by the genus *Gemmata*. These planctomycetes possess different cell morphologies (Fig. 5.3) and differ with regard to some phenotypic traits but are quite uniform with respect to their pH preferences. Members of these genera are moderately acidophilic bacteria growing at pH values between 3.5–4.0 and 7.0–7.5, with an optimum at pH 5–6. In comparison to all previously described planctomycetes, representatives of the genera *Schlesneria*, *Singulisphaera*, *Zavarzinella*, and *Telmatocola* are clearly more acid tolerant (Fig. 5.4a). Some other characteristics of the newly described planctomycetes are given below.

Table 5.1 List of currently characterized acidophilic planctomycetes

Species name	Type strain	GenBank Accession No. for 16S rRNA gene	Relation to oxygen	pH growth range (optimum)	Carbon sources utilized
Schlesneria paludicola	MPL7^T (=ATCC BAA-1393^T = VKM B-2452^T)	AM162407	Facultative aerobe	4.2–7.5 (5.0–6.2)	Some sugars and heteropolysaccharides, *N*-acetylglucosamine, salicin
Singulisphaera acidiphila	MOB10^T (=ATCC BAA-1392^T = VKM B-2454^T)	AM850678	Obligate aerobe	4.2–7.5 (5.0–6.2)	Sugars and heteropolysaccharides, *N*-acetylglucosamine, salicin
Singulisphaera rosea	S26^T (=DSM 23044^T = VKM B-2599^T)	FN391026	Obligate aerobe	3.2–7.1 (4.8–5.0)	Most sugars, several organic acids and polyalcohols, some heteropolysaccharides *N*-acetylglucosamine
Zavarzinella formosa	A10^T (=DSM 19928^T = VKM B-2478^T)	AM162406	Obligate aerobe	3.8–7.2 (5.5–6.0)	Sugars and heteropolysaccharides, *N*-acetylglucosamine, salicin, pyruvate
Telmatocola sphagniphila	SP2^T (=DSM 23888^T = VKM B-2710^T)	JN880417	Obligate aerobe	4.0–7.0 (5.0–5.5)	Sugars and heteropolysaccharides, carboxymethyl cellulose, crystalline cellulose
Candidatus Nostocoida acidiphila	OB1	JQ067914	Obligate aerobe	3.2–5.5 (3.6–4.0)	Some heteropolysaccharides

5.4.1 *Genus* Schlesneria

Schlesneria paludicola is the only currently described species of this genus. It was described based upon a characterization of three strains, MPL7^T, MOB77, and SB2, which were isolated from the *Sphagnum* peat bog Bakchar (pH 4.0), West Siberia, and the peat bog Obukhovskoe (pH 4.2), Yaroslavl region, European North Russia (Kulichevskaya et al. 2007a). Members of this species are represented by budding, ellipsoid-shaped cells that occur singly, in pairs, or are arranged in rosettes (Fig. 5.3a). The cell surface is covered by crateriform pits and numerous fibrillar appendages; stalklike structures are short and rarely observed. These planctomycetes are facultatively aerobic chemoheterotrophs. They grow best in aerobic conditions on media with carbohydrates or *N*-acetylglucosamine. However, they are also capable of fermenting carbohydrates, which might be of special importance for bacteria that inhabit wetlands. *Schlesneria paludicola* possesses weak hydrolytic capabilities and can degrade fucoidan, laminarin, aesculin, pectin, chondroitin sulfate, pullulan, gelatin, and xylan. Growth occurs at pH values between 4.2 and 7.5 (optimum at 5.0–6.2) and at temperatures between 4 °C and 32 °C (optimum at 15–26°C). The major fatty acids are C16:0 and C16:1ω7c. The closest taxonomically described phylogenetic relative of *Schlesneria paludicola* is *Planctomyces limnophilus* (87 % sequence similarity). Several 16S rRNA gene cloned sequences that were retrieved from acidic peat and acidic soil display 98–99 % similarity to the corresponding gene sequence from *Schlesneria paludicola* (Fig. 5.2), suggesting that these planctomycetes are typical for acidic environments.

5.4.2 *Genus* Singulisphaera

At present, this genus includes two species, *S. acidiphila* and *S. rosea* (Kulichevskaya et al. 2008, 2012a). Both species are represented by spherical, nonmotile cells that occur singly, in pairs, or shapeless aggregates (Fig. 5.3b). These cells attach to surfaces by means of amorphous holdfast material; stalklike structures are absent. Members of this genus are chemoheterotrophic aerobes. They are not capable of fermenting carbohydrates but they grow very well in microaerobic conditions. The preferred growth substrates of these planctomycetes are sugars and *N*-acetylglucosamine. *S. rosea* utilizes also some organic acids including citrate, fumarate, lactate, malate, pyruvate, and succinate. Members of both species hydrolyze various heteropolysaccharides such as laminarin, aesculin, or pullulan. In addition, *S. acidiphila* degrades pectin, lichenan, and xylan, while *S. rosea* grows on starch. None of them utilize cellulose or chitin. The major fatty acids are C16:0, C18:1ω9*c*, and C18:2ω6*c*,12*c*; the latter is genus characteristic. *S. rosea* is more acidophilic (growth at pH 3.2–7.1, optimum at 4.8–5.0) (Fig. 5.4b) than *S. acidiphila* (growth at pH 4.2–7.5, optimum at 5.0–6.2). Members of the genus *Singulisphaera* are only distantly related to the thermophilic filamentous neutrophile from hot springs *Isosphaera pallida* (Giovannoni et al. 1987) and to the

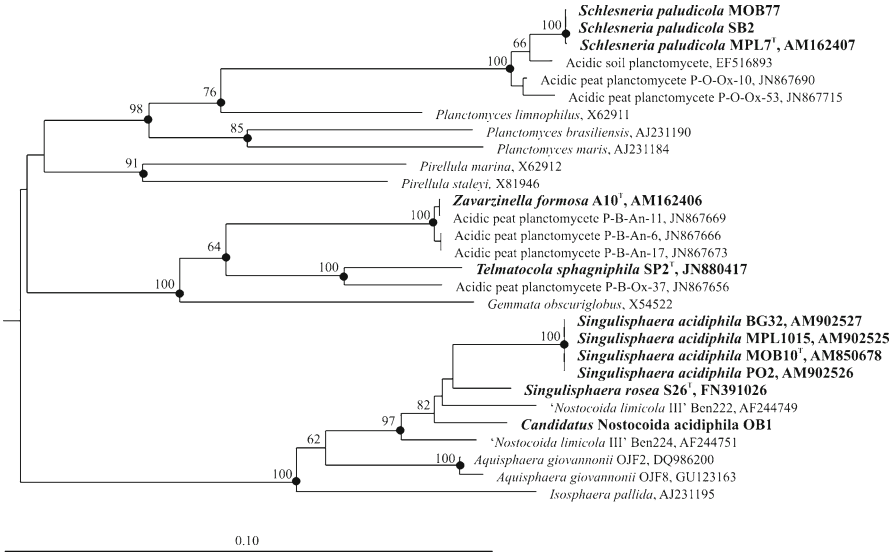

Fig. 5.2 16S rRNA gene-based neighbor-joining tree showing the phylogenetic position of acidophilic planctomycetes (in *bold*) in relation to other taxonomically characterized members of the family *Planctomycetaceae*. Bootstrap values (1,000 data resamplings) of >60 % are shown. *Black circles* indicate that the corresponding nodes were also recovered in the maximum-likelihood and maximum-parsimony trees. The root (not shown) was composed of five 16S rRNA gene sequences from anammox planctomycetes (AF375994, AF375995, AY254883, AY257181, AY254882). The scale bar represents 0.1 substitutions per nucleotide position

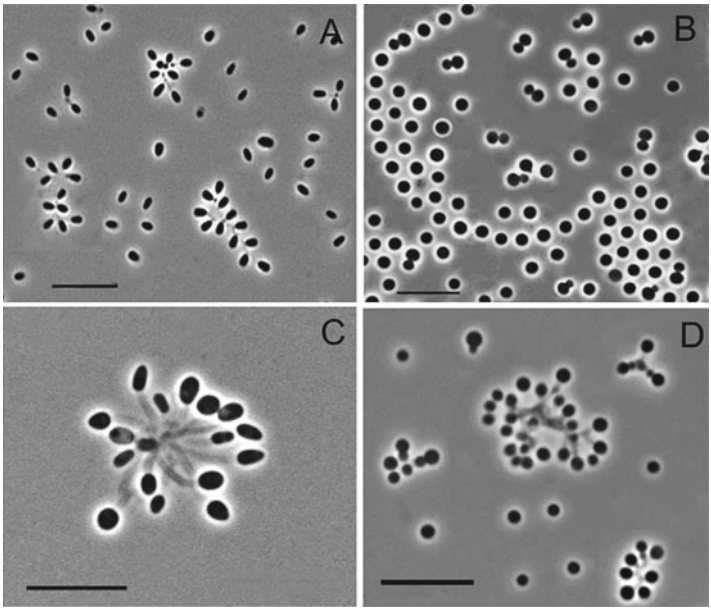

Fig. 5.3 Cell morphology of acidophilic planctomycetes: *Schlesneria paludicola* (**a**), *Singulisphaera acidiphila* (**b**), *Zavarzinella formosa* (**c**), *Telmatocola sphagniphila* (**d**). Bar, 10 μm

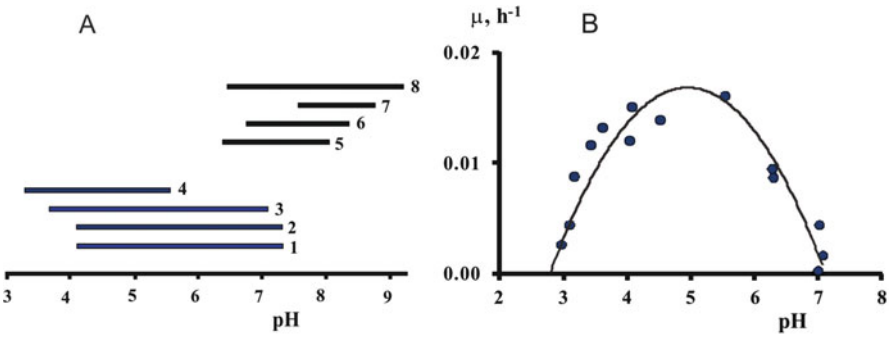

Fig. 5.4 (**a**) pH growth ranges of neutrophilic (*black*) and acidophilic (*blue*) planctomycetes: 1—*Schlesneria paludicola*, 2—*Singulisphaera acidiphila*, 3—*Zavarzinella formosa*, 4—*Candidatus* Nostocoida acidiphila, 5—*Planctomyces limnophilus*, 6—*Isosphaera pallida*, 7—*Gemmata obscuriglobus*, 8—*Aquisphaera giovannonii*. (**b**) Influence of medium pH on the growth of *Singulisphaera rosea* S26^T

mesophilic nonfilamentous neutrophile from the sediments of a freshwater aquarium *Aquisphaera giovannonii* (Bondoso et al. 2011) (89–90% and 92–93 % 16S rRNA gene sequence similarity) (Fig. 5.2). More close relationship (94.5–95.5 % sequence similarity) is observed with a group of filamentous planctomycete strains from activated sludge, "*Nostocoida limicola*" III (Liu et al. 2001), which also belongs to a wide phylogenetic clade defined by the genus *Isosphaera*.

5.4.3 Genus Zavarzinella

Zavarzinella formosa was described based upon a characterization of a single unique isolate, strain A10^T, obtained from the Siberian peat bog Bakchar (Kulichevskaya et al. 2009). Ellipsoid-shaped cells of this isolate are uniformly covered with crateriform pits and possess long (up to 10–15 μm) and unusually thick stalks, which assemble cells in large rosette-like clusters (Fig. 5.3c). Adult cells are immobile, while daughter cells are motile by means of one-two flagella. This planctomycete is an obligately aerobic chemoheterotroph. In contrast to *Schlesneria* and *Singulisphaera*, it is not capable of growth in microaerobic conditions. The growth substrates are sugars, N-acetylglucosamine, and pyruvate, but organic acids other than pyruvate are not utilized. The protein gelatin and the heteropolysaccharides laminarin, pectin, chondroitin sulfate, aesculin, starch, lichenan, and xylan are hydrolyzed, but chitin or cellulose are not. The major fatty acids are C18:0, C18:1ω5c, and C16:1ω5c. Members of this species grow at pH values between 3.8 and 7.2 (optimum at 5.5–6.0) and at temperatures between 10 and 30 °C (optimum at 20–25 °C). The closest described relatives are the neutrophile *Gemmata obscuriglobus* (90 % 16S rRNA gene sequence similarity) and the acidophile *Telmatocola sphagniphila* (86 % sequence similarity) (Fig. 5.2). A number of 16S rRNA gene clones displaying high similarity (99 %) to *Z. formosa* were retrieved from acidic *Sphagnum*-dominated wetlands (Ivanova and Dedysh 2012) and from an acidic hot spring of the Colombian Andes (Bohorquez et al. 2012).

5.4.4 *Genus* Telmatocola

Telmatocola sphagniphila is a recently proposed species, which accommodates morphologically and phenotypically unusual planctomycetes. Two characterized members of this species, strains SP2^T and OB3, were isolated from two North European *Sphagnum* peat bogs, Staroselsky moss and Obukhovskoye (Kulichevskaya et al. 2012c). When grown on solid media, spherical cells of these bacteria are arranged in unusual, dendriform-like structures (Fig. 5.3d). *Telmatocola sphagniphila* is an obligately aerobic chemoheterotroph, which is unable to grow in micro-oxic or anoxic conditions. The preferred growth substrates are various heteropolysaccharides and sugars, the latter being utilized only if provided in low concentrations (below 0.025 %). In contrast to other described planctomycetes, *Telmatocola sphagniphila* possesses cellulolytic potential and is capable of slow growth on carboxymethyl cellulose, microcrystalline cellulose, and fibrous cellulose prepared from Whatman filter paper. Growth occurs at pH 4.0–7.0 (optimum pH 5.0–5.5) and at 6–30 °C (optimum 20–26 °C). The major fatty acids are C16:1ω5c, C18:1ω5c, C16:0, and C18:0. *Telmatocola sphagniphila* is only distantly related to *Zavarzinella formosa* and *Gemmata obscuriglobus* (86% and 87 % 16S rRNA gene sequence similarity, respectively) (Fig. 5.2). Representatives of this species appear to be numerically abundant in acidic peatlands (see Sect. 8.5).

5.4.5 Candidatus *Nostocoida acidiphila*

One of the commonly observed bacterial morphotypes detected in acidic peat by means of hybridization with planctomycete-specific FISH probes is chains composed of spherical cells (Fig. 5.5a, b). For a long time, our attempts to cultivate these filamentous planctomycetes remained unsuccessful. Recently, however, one of these organisms was isolated in a co-culture with several chemoheterotrophic bacteria, which appear to feed on a polysaccharide sheath produced by the planctomycete (Fig. 5.5c, d) (Kulichevskaya et al. 2012b). The consortium consisting of the filamentous planctomycete and several satellite bacteria developed on a surface of a mineral medium solidified with Phytagel (1 %, w/v); the latter apparently was used as the source of carbon and energy. The phylogenetic position of this filamentous bacterium was determined by means of total DNA extraction from the consortium followed by PCR-mediated amplification of the 16S rRNA gene fragment using the combination of the *Planctomycete*-specific forward primer Pla46 (Neef et al. 1998) and the universal bacterial reverse primer Univ1390R. The retrieved 16S rRNA gene fragment displayed 95 % sequence similarity to the corresponding gene fragment in *Singulisphaera acidiphila* and 94.8–96.3 % similarity to 16S rRNA gene sequences of filamentous, taxonomically uncharacterized planctomycetes from activated sludge; those which have been termed by wastewater microbiologists "*Nostocoida limicola*" III (Liu et al. 2001) (Fig. 5.2). In contrast to moderately acidophilic

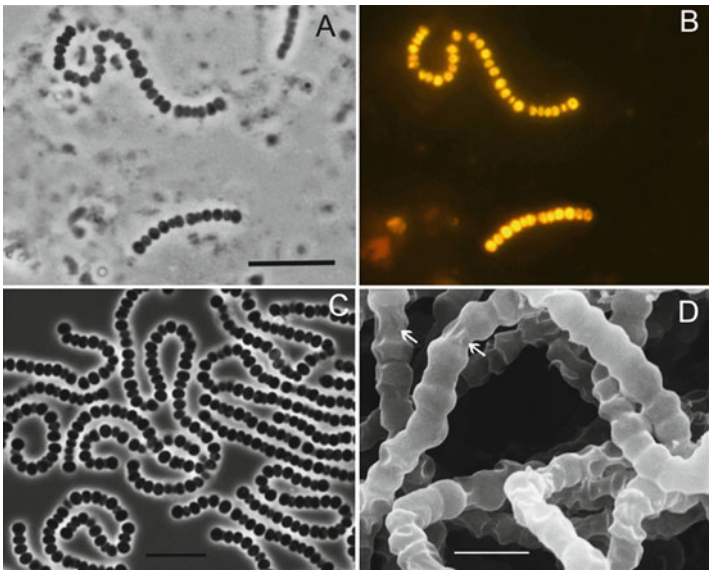

Fig. 5.5 (**a**, **b**) Specific detection of filamentous planctomycetes in acidic peat by FISH: epifluorescent micrograph of in situ hybridization with Cy3-labelled probes PLA46 and PLA886 (**a**) and the phase-contrast image (**b**). (**c**) Phase-contrast image of the acidophilic planctomycete *Candidatus* Nostocoida acidiphila OB1. (**d**) Electron micrograph of cell filaments of *Candidatus* Nostocoida acidiphila OB1 covered with a sheath (shown by *arrows*). Bars, 10 μm (**a**, **c**) and 3 μm (**d**)

members of the genera *Schlesneria*, *Singulisphaera*, *Zavarzinella*, and *Telmatocola*, this filamentous peat-inhabiting planctomycete showed a clear preference for growth in acidic conditions, with an optimum at pH 3.6–4.0. The only growth substrates utilized by this planctomycete were polysaccharides of microbial origin, such as Phytagel, Gellan Gum, or xanthan, which are produced by bacteria of the genera *Sphingomonas* and *Xanthomonas*. Since all attempts to obtain a pure culture of this slowly growing filamentous bacterium were unsuccessful, a tentative name *Candidatus* Nostocoida acidiphila was proposed for this planctomycete (Kulichevskaya et al. 2012b).

5.5 Phylogenetic Diversity and Functional Role of *Planctomycetes* in Wetlands

Phylogenetic diversity of the *Planctomycetes* in acidic *Sphagnum*-dominated wetlands was assessed in two recently published studies (Ivanova and Dedysh 2012; Kulichevskaya et al. 2012c). In the first study, PCR-mediated amplification of the 16S rRNA gene fragments (~1,350 bp) of peat-inhabiting planctomycetes was performed using the combination of the planctomycete-specific forward primer Pla46 (Neef et al. 1998) and the universal bacterial reverse primer Univ1390R. The resulting sequence

pool was highly diverse and included nearly all currently known major lineages of this phylum with the only exception of anammox planctomycetes (Jetten et al. 2005, 2010). The clone libraries constructed for two oxic peat samples from wetlands in West Siberia (peat bog Bakchar) and European North Russia (peat bog Obukhovskoye) had highly similar composition. More than half of all clones (53–61 %) in both clone libraries affiliated with the phylogenetic lineage defined by the genera *Isosphaera* and *Singulisphaera*. The second abundant group of sequences (25–35 % of all clones) obtained from oxic peat belonged to the phylogenetic lineage defined by the genera *Gemmata* and *Zavarzinella*. Finally, two minor groups of clones affiliated with *Planctomyces*- and *Pirellula*-like planctomycetes. By contrast, most 16S rRNA gene sequences (45 % of all clones) retrieved from the anoxic peat sample clustered within the group defined by the genus *Pirellula*. These sequences displayed only a distant relationship (85.7–89.7 % sequence similarity) to those from taxonomically described representatives of this planctomycete group, but were highly similar (95.2–98.7 %) to the environmental clone sequences Molly75 (AY775524), Molly19 (AY775494), and B86 (AM162476) obtained from *Sphagnum*-dominated wetlands in the USA and Russia (Morales et al. 2006; Dedysh et al. 2006).

The study of Kulichevskaya and coauthors (Kulichevskaya et al. 2012c) applied pyrosequencing-based *Bacteria* diversity analysis to get a deeper insight into diversity of peat-inhabiting planctomycetes. PCR in this study was carried out using the *Bacteria*-specific primers 907F and 1392R. A total of 1081 partial (average length ~490 bp) planctomycete 16S rRNA gene sequences were obtained from the *Sphagnum* peat sample collected from the peat bog Obukhovskoye. Taxonomy-based analysis, which was performed at a confidence threshold of 80 %, revealed that only 39 % of these sequences affiliate with phylogenetic lineages defined by taxonomically described organisms, including members of the genera *Isosphaera*, *Pirellula*, *Planctomyces*, *Schlesneria*, *Singulisphaera*, and *Telmatocola* (Fig. 5.6). The most frequently detected organisms were *Isosphaera*- and *Telmatocola*-like bacteria (16% and 10 % of all planctomycete-related sequences, respectively). The majority (61 %) of all planctomycete-related 16S rRNA gene sequences retrieved

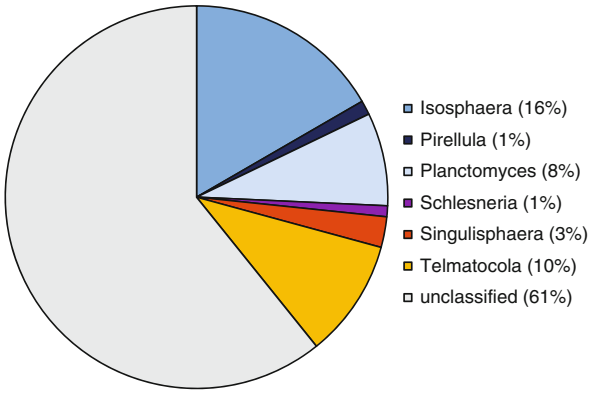

Fig. 5.6 The community composition of the *Planctomycetes* in an acidic (pH 4.0) peat sampled from the *Sphagnum*-dominated, ombrotrophic peat bog Obukhovskoye, European North Russia (58° 14′N, 38° 12′E), as assessed by pyrosequencing-based analysis (cited from Kulichevskaya et al. 2012c)

from acidic peat could not be assigned to taxonomically characterized already isolated or described organisms, thus highlighting the need for further cultivation efforts in uncovering the *Planctomycete* diversity in acidic northern wetlands.

The occurrence of anammox planctomycetes in acidic wetlands remains to be verified. As discussed above, these bacteria were absent in samples taken from the two *Sphagnum* peat bogs where available forms of nitrogen were at undetectable levels. However, anammox planctomycetes were detected in a mildly acidic swampy peat soil fed by nitrate-enriched local groundwater (Hu et al. 2011). The enrichment culture obtained from this peat sample displayed the highest specific activity at pH 7.1, suggesting the acid-tolerant, if not acidophilic, nature of these bacteria.

The functional role of planctomycetes in wetlands remains poorly understood. Based on our current knowledge, these bacteria are slow-acting decomposers of plant-derived organic matter. In our experiments, members of the *Planctomycetes* were identified as a numerically abundant component of a bacterial community participating in *Sphagnum* moss decomposition, which developed at the final stage of decomposition process (Kulichevskaya et al. 2007b). This is not surprising since all currently characterized peat-inhabiting planctomycetes are capable of degrading various heteropolysaccharides of plant and microbial origin. A weak ability to degrade cellulose, the major component of *Sphagnum*-derived litter, was recently demonstrated for *Telmatocola sphagniphila* (Kulichevskaya et al. 2012c). The existence of other, as-yet-uncultivated cellulolytic planctomycetes, therefore, cannot be excluded. Due to slow growth rates, the role of primary degraders is unlikely to be attributed to this group of bacteria. Yet, the biogeochemical role of planctomycetes in peatlands remains to be clarified since characterized representatives make up only a minor part of the whole planctomycete diversity in acidic wetlands.

5.6 Final Remarks

Despite the recent success in cultivation of the first acidophilic planctomycetes, most *Planctomycete*-related 16S rRNA gene sequences retrieved by molecular approaches from various acidic habitats display low similarity to those of characterized organisms. This also reasonably applies to planctomycetes in acidic wetlands, which were addressed in our studies. These bacteria inhabit both oxic and anoxic peat layers, while only aerobic representatives of peat-inhabiting planctomycetes have been characterized so far. Further work is needed, therefore, to characterize the unknown planctomycetes found in this habitat and to explore their physiology, genomic capabilities, and functional role in wetlands.

The existence of extremely acidophilic planctomycetes with growth optima at pH below 3.0 remains an open question. According to the currently available reports on molecular microbial diversity in extremely acidic habitats, members of the *Planctomycetes* are either absent or present in a relatively low abundance in these environments. More research is needed to extend our knowledge of the metabolic types and specific adaptations of planctomycetes that colonize extremely acidic habitats.

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