Northern wetlands play a key role in the global carbon budget, particularly in the budgets of the greenhouse gas methane. These ecosystems also determine the hydrology of northern rivers and represent one of the largest reservoirs of fresh water in the Northern Hemisphere. *Sphagnum*-dominated peat bogs and fens are the most extensive types of northern wetlands. In comparison to many other terrestrial ecosystems, the bacterial diversity in *Sphagnum*-dominated wetlands remains largely unexplored. As demonstrated by cultivation-independent studies, a large proportion of the indigenous microbial communities in these acidic, cold, nutrient-poor, and water-saturated environments is composed of as-yet-uncultivated bacteria with unknown physiologies. Most of them are slow-growing, oligotrophic microorganisms that are difficult to isolate and to manipulate in the laboratory. Yet, significant breakthroughs in cultivation of these elusive organisms have been made during the last decade. This article describes the major prerequisites for successful cultivation of peat-inhabiting microbes, gives an overview of the currently captured bacterial diversity from northern wetlands and discusses the unique characteristics of the newly discovered organisms.

**Keywords:** northern wetlands, as-yet-uncultivated bacteria, isolation approaches, methanotrophs, Acidobacteria, Planctomycetes

Apart of their importance in the terrestrial carbon cycle, northern wetlands hold a key role in the global water balance. These wetlands determine the hydrology of northern rivers and represent one of the largest reservoirs of fresh water in the Northern Hemisphere. Despite the carbon sequestering properties of peatlands, they are major sources of dissolved organic carbon (DOC), which is exported to aquatic ecosystems draining peatland catchments (Freeman et al., 2004; Fenner et al., 2007). Currently observed intensified DOC export from peatlands is becoming an increasing cause for concern in the water industry. *Sphagnum*-dominated peatlands represent one of the most extensive types of northern wetlands. *Sphagnum* moss is characteristic of peat bogs and poor fens. Bogs are ombrotrophic ecosystems that are decoupled from the groundwater of the surrounding watershed and receive all water and nutrient inputs from the atmosphere. These peatlands typically have pH values below 4.0 and negligible concentrations of basic cations in surface waters. Poor fens also are acidic, but have somewhat higher surface water pH (4.0–5.5) than bogs, because they receive some minerotrophic drainage in addition to precipitation (Bedford et al., 1999). Both bogs and fens are nutrient-poor by nature. The total concentration of mineral nutrients in these wetlands is usually in the range of 5–50 mg L$^{-1}$. N and S cycling is dominated by organic forms and transformations (Moore et al., 2004). Peat water usually contains very low concentrations of NH$_4^+$ and NO$_3^-$ (3–100 μM or several mg L$^{-1}$; Lamers et al., 2000; Kravchenko, 2002; Moore et al., 2004; Kip et al., 2011). Sulfate concentrations are in the range of 10–300 μM, with the highest values measured in fens or...
in polluted wetlands (Nedwell and Watson, 1995; Blodau et al., 2007; Pester et al., 2010; Kip et al., 2011). Iron concentrations are also very low. Microbial Fe(III) reduction in an upland fen was shown to account for <7% of the anaerobic organic carbon mineralization (Küsel et al., 2008). Therefore, transformations of mineral N, S, and Fe are of minor importance in oligotrophic wetlands.

Degradation of plant litter is the basis of the microbial food chain in these ecosystems. Since *Sphagnum* species are the major primary producers in ombrotrophic peatlands, the litter produced here originates largely from *Sphagnum*, and peat consists primarily of bryophyte remains. *Sphagnum*-derived litter is known to decompose very slowly, with 0.1–25% first-year mass loss (Clymo, 1965; Aerts et al., 2001; Scheffer et al., 2001), which is due to the presence of various highly decay-resistant phenolic compounds and waxes (Verhoeven and Lieveld, 1997) and also due to the low nutrient (particularly, N and P) concentrations in *Sphagnum* litter. Other factors that inhibit decomposition are high acidity, low temperatures and anoxic conditions prevailing within the peat profile. The end-products of anaerobic plant debris degradation are then transformed into methane, which diffuses into the aerobic part of the bog profile.

In summary, the key biogeochemical processes driven by microorganisms in acidic northern wetlands are (i) degradation of plant-derived organic matter, (ii) methanogenesis, (iii) methanotrophy, and (iv) N₂ fixation since available forms of nitrogen are mostly at very low or undetectable levels. Of these, only the microorganisms involved in CH₄ cycle, i.e., methanogenic archaea (Galand et al., 2003; Sizova et al., 2003; Kotsyurbenko et al., 2004, 2007; Juottonen et al., 2005; Bräuer et al., 2006, 2011; Cadillo-Quiroz et al., 2006, 2009, 2010) and methanotrophic bacteria (Dedysh et al., 1998, 2000, 2001, 2002; Dedysh et al., 2004; Dedysh et al., 2007; Morris et al., 2002; Raghoebarsing et al., 2005; Chen et al., 2008a,b; Dedysh, 2009; Kip et al., 2010, 2011) have received considerable research attention. Several representatives of these microbial groups are now available in pure cultures and are described taxonomically. Many of them display unusual characteristics, which are reviewed below. Knowledge about microorganisms responsible for degradation of plant litter in these ecosystems is much more limited. Most of this information is limited to fungi (Thorlmann et al., 2002, 2004; Rice et al., 2006), which are not addressed in this review, while reports on hydrolytic capabilities of peat-inhabiting prokaryotes and their role in decomposition processes are very rare (Pankratov et al., 2011). The same is true for the research on nitrogen-fixing microorganisms (Kravchenko and Doroshenko, 2003; Dedysh et al., 2004b; Doroshenko et al., 2007; Zadorina et al., 2009). Finally, the functional role of many other microbial inhabitants of northern wetlands remains completely unknown.

**“CULTURED VERSUS UNCULTURED” IN SPHAGNUM-DOMINATED WETLANDS**

Despite the importance of northern wetlands in global carbon and water cycles, the microbial community composition in these ecosystems remains insufficiently described. Only a few studies have addressed the overall diversity of bacterial 16S rRNA genes in acidic northern peatlands (Juottonen et al., 2005; Dedysh et al., 2006; Morales et al., 2006; Hartman et al., 2008; Ausec et al., 2009; Pankratov et al., 2011). Peat material used in these studies was collected mostly from the surface layers (0–40 cm depth), around the oxic–anoxic interface, i.e., from the region of highest biological activity.

**Figure 1** gives an overview of the 16S rRNA gene diversity retrieved in six cultivation-independent studies of acidic northern peatlands in different geographic locations. An additional dataset obtained for tropical acidic peatland in Thailand (Kanokratana et al., 2011) was included for comparison (right column in Figure 1). Interestingly, northern and tropical acidic peatlands appear to display similar patterns of bacterial diversity. These habitats are usually dominated by members of the phyla *Acidobacteria* and *Proteobacteria*. Peat-inhabiting acidobacteria belong to subdivisions 1, 3, 4, and 8 of this phylum. Of these, only subdivision 1 is relatively well represented by cultured and characterized strains, many of which were isolated from *Sphagnum*-dominated wetlands. Subdivisions 3 and 8 contain only a few described members, while subdivision 4 does not include taxonomically characterized representatives. Proteobacteria found in peat bogs most commonly belong to the *Alpha*- or *Delta*-classes. Alphaproteobacteria usually prevail in methane-emitting wetlands, and a large proportion of these bacteria is composed of methanotrophs and methylotrophs from the families *Methyllocystaceae* and *Beijerinckiaceae* (Dedysh et al., 2006; Dedysh, 2009). Chemo-heterotrophs from the families *Bradyrhizobiaceae*, *Acetobacteraceae*, *Hyphomicrobiaceae*, and *Caulobacteraceae*, and phototrophs of the genera *Rhodoblastus*, *Rhodomicrobium*, and *Rhodopseudomonas* are also common. Today, much of this diversity is available in culture (Figure 2). Peat-inhabiting *Deltaproteobacteria* belong to phylogenetic lineages represented by the genera *Syntrophobacter*, *Syntrophus*, *Smithella*, *Geobacter*, and *Anaeromyxobacter*. These bacteria seem to prevail in wetlands with somewhat increased sulfate levels (Morales et al., 2006).

Less abundant but numerically significant groups of sequences in clone libraries made from acidic peat affiliate with the phyla *Verrucomicrobia*, *Actinobacteria*, and *Planctomycetes* (Figure 1). With the sole exception of the *Opitutus*-like group, most peat-inhabiting verrucomicrobia belong to taxonomically uncharacterized groups, for which isolates are not available. Surprisingly little is known about *Actinobacteria* that thrive in wetlands. In contrast to well-characterized soil *Actinobacteria*, peat-inhabiting members of this phylum belong to poorly described sub-groups. Significant proportion of *Actinobacteria*-related 16S rRNA gene sequences retrieved from *Sphagnum*-dominated wetlands affiliate with the Acidimicrobiae, a family containing only two characterized members, *Acidimicrobium ferrooxidans* and *Ferrimicrobium acidiphilum*. Another group of commonly detected sequences shows a distant relationship to *Conexibacter woesei*, a deep-rooting member of the phylum *Actinobacteria*.

The *Planctomycetes* is one of the bacterial groups that are strongly underrepresented in clone libraries obtained with the widely used *Bacteria*-specific PCR primer 9-27f. Yet, 16S rRNA gene sequences of planctomycetes are commonly found in these clone libraries made from acidic peat. They are highly diverse and represent nearly all currently known major lineages of this phylum. Many *Planctomycetes*-related 16S rRNA gene sequences...
FIGURE 1 | Taxonomic composition of bacterial communities in northern *Sphagnum*-dominated wetlands of various geographic locations, determined in different cultivation-independent studies (columns 1–6 from left to right). A dataset obtained for a tropical acidic peatland in Thailand (column 7) is included for comparison.

display only a distant relationship (≤90% similarity) to those of taxonomically characterized organisms, although a large proportion of peat-derived sequences can now be classified as belonging to the lineages defined by the recently described acidophilic genera *Schlesneria*, *Singulisphaera*, and *Zavarzinella*.

Finally, minor groups of 16S rRNA gene sequences in the clone libraries affiliate with the *Beta*- and *Gammaproteobacteria*, *Chloroflexi*, *Bacteroidetes*, *Spirochaetes*, *Firmicutes*, and several candidate divisions such as Obsidian Pool 3 (OP3), OD1, and OP8. Of these, sequences related to the *Firmicutes* as well as to the *Beta*- and *Gammaproteobacteria* usually belong to well-characterized microbial groups.

As evidenced by the results of cultivation-independent studies, a large proportion of the indigenous bacteria populations in northern acidic wetlands is represented by as-yet-uncultivated organisms with unknown physiologies and metabolic potentials (Figure 2). Most of these bacteria cannot be cultured using conventional cultivation approaches.

**MAJOR PREREQUISITES FOR SUCCESSFUL CULTIVATION OF PEAT-INHABITING MICROBES**

Direct counts of microbial cells in acidic wetlands are typically about 10^6–10^9 g⁻¹ of wet peat (Williams and Crawford, 1983; Dedysh et al., 2001, 2006; Kotsyurbenko et al., 2004). Twenty to 70% of these cells are detectable by fluorescence in situ hybridization (FISH) with the bacteria-specific probe EUB338-mix, while up to 10% of total cells are targeted with archaeal probes ARCH915 and ARC344. The DAPI-stained objects that are not detected by any of these domain-specific probes are represented by cells of a very small size, i.e., ≤0.5 μm in length (Dedysh et al., 2006). Their nature and metabolic status remain poorly understood.

Only a minor part of peat-inhabiting bacteria can be cultured using a surface plating technique and conventional media such as nutrient agar, R2A and others. The numbers of colony forming units (CFUs) obtained by this conventional approach are low and usually range between 10^5 and 10^6 CFUs g⁻¹ of wet peat (Golovchenko et al., 2005; Dedysh et al., 2006). This corresponds to approximately 0.01–1.0% of total DAPI cell counts.

Nearly one half of the colonies that develop on nutrient agar plates after 1–2 weeks of incubation are formed by fast-growing *Betaproteobacteria*, particularly members of the genus *Burkholderia* (Opelt and Berg, 2004; Belova et al., 2006; Dedysh et al., 2006; Opelt et al., 2007; Vandamme et al., 2007). Other prokaryotes that most commonly develop on these plates affiliate with the *Alphaproteobacteria* (genera *Bradyrhizobium*, *Mesorhizobium*, *Sphingomonas*, *Brevundimonas*, *Caulobacter*, *Hyphomicrobiurn*), *Actinobacteria* (genera *Mycobacterium*, *Rhodococcus*,
Streptomyces, Micromonospora), Gammaproteobacteria (genera Pseudomonas, Serratia, Rahnella), Firmicutes (genera Paenibacillus, Bacillus), and Bacteroidetes (genera Pelobacter, Dyadobacter, Chryseobacterium). These easily cultured isolates are closely related (98–100% 16S rRNA gene sequence similarity) to taxonomically described organisms and, with a very few exceptions, none of them represent the major 16S rRNA gene sequence groups detected in peatlands by means of cultivation-independent techniques. In other words, those who intend exploring the unknown bacterial diversity in northern wetlands cannot rely on a routine approach using standard media and short incubation times. Patience is one of the major virtues required in hunting for novel microbes. Other prerequisites for the successful cultivation of peat-inhabiting microbes are given below.

THE USE OF DILUTE ACIDIC MEDIA
Most commonly used nutrient media have a near-neutral pH and a salt content of 1–3 g L\(^{-1}\). This contrasts dramatically to acidic (pH 3.5–5.5) peat water with a mineral salt content of 5–50 mg L\(^{-1}\) and explains why most peat-inhabiting bacteria do not develop on conventional media. Therefore, a more accurate simulation of the peat bog environment in the laboratory requires dilute (1:10–1:100) and acidic (pH 4.0–5.5) media. High potential of this strategy was demonstrated by isolation of novel acidophilic methane-oxidizing bacteria from northern wetlands (Dedysh et al., 1998). Later, a number of peat-inhabiting methanotrophs were isolated on mildly acidic, low-ionic-strength media (Dedysh, 2009; Kip et al., 2011). Though methanogenic archaea are beyond the scope of this review, they have also been successfully retrieved from acidic wetlands using strongly dilute, acidic media (Sizova et al., 2003; Bräuer et al., 2006; Kotsyurbenko et al., 2007; Cadillo-Quiroz et al., 2009). The same strategy was applied for isolation of heterotrophic bacteria from poorly studied phyla. For example, peat-inhabiting members of the Acidobacteria were cultured using low-ionic-strength, low-nutrient media MM, MM1, or 10-fold diluted R2A (Dedysh et al., 2006; Pankratov et al., 2008). One of these media, MM1, does not contain phosphates...
since they were shown to inhibit growth of some acidobacteria (Pankratov and Dedysh, 2010). In this case, medium pH was adjusted to 4.0–5.0 with alginic acid, which offers a unique possibility of decreasing medium pH without increasing its ionic strength.

**EXTENDED INCUBATION TIME**

Most microbes that thrive in cold and nutrient-poor northern wetlands are slow-growing organisms. Even under optimal growth conditions, isolation of these microbes requires long incubation. Colonies of peat-inhabiting methanotrophs, acidobacteria, planctomycetes, and other fastidious bacteria appear on solid media only after 4–8 weeks of incubation (Dedysh et al., 2000; Pankratov et al., 2008, in press; Kulichevskaya et al., 2009). In the case of the acidotolerant facultative anaerobe *Tehmatospirillum sibiricense*, development of colonies was observed after 5 months of anaerobic incubation (Sizova et al., 2007).

Most of these difficult-to-culture bacteria produce very small (0.1–0.5 mm in diameter) colonies, which can be observed and picked with the use of a dissecting microscope only. The same phenomenon was reported for several rarely cultured groups of soil bacteria, which were most abundant among the mini-colonies that developed after >12 weeks of incubation (Davis et al., 2011). The use of gellan gum (phytagel, gelrite), which produces a very clear medium, allows for easy discrimination of these mini-colonies (Janssen et al., 2002; Stott et al., 2008). This polysaccharide is also free of contaminants, which may inhibit growth of some microbes. Gellan gum was successfully applied for isolation of diverse peat-inhabiting bacteria, including methanotrophs and acidobacteria (Dedysh et al., 2002, in press; Pankratov et al., 2008, in press). Notably, many of these organisms were unable to grow on agar-containing media. It should be kept in mind, however, that polymerization of gellan gum occurs in the presence of bivalent cations, which are normally present in very low concentrations in ombrotrophic wetlands. The possibility that this may negatively effect isolation of some particular bacterial groups cannot be excluded.

**A BIOFILM-MEDIATED APPROACH COMBINED WITH FISH-BASED MONITORING OF THE ENRICHMENT/ISOLATION PROCEDURE**

As discussed above, most colonies that develop rapidly on the surface of various solid media are formed by bacteria which are not predominant in the peat bog environment. Many of these colonies, however, represent not a pure bacterial culture but a co-culture of two or more microorganisms. Examination of such mixed colonies obtained from acidic peat revealed that many of them contained cells of rarely cultured organisms, such as acidobacteria or planctomycetes (Dedysh et al., 2006). This observation suggested that an isolation approach based on enrichment of mixed cultures or microbial biofilms might in some cases be more efficient for isolating these elusive bacteria than a routine “single-colony pick-up” strategy. The two simplest versions of this alternative approach are given below.

Microbial biofilms consisting of cells of peat-inhabiting bacteria can be obtained by using a simple technique invented by Schlesner (1994) for isolation of planctomycetes. Briefly, the bottom of a Petri dish is covered with a layer of sterilized water agar, which contains cycloheximide to inhibit growth of fungi. Up to 20 sterilized cover slides are placed vertically and partially into the agar layer. These plates are then inoculated with 10–15 ml of peat water, which serves as the only source of nutrients for developing microorganisms, and incubated for 1–2 months. Every 2–3 weeks, a few slides are taken out and examined for the presence of target cells. In our experience, the best screening tool to recognize target cells, as opposed to easily culturable cells, is FISH. It allows direct visualization of the target bacteria within the entire population of cells present in the biofilm (Figure 3). This greatly simplifies all further isolation/purification procedures. Various PCR-based techniques can also be used for screening purposes, but FISH offers a clear advantage of seeing the target. The examples of successful application of this biofilm-mediated enrichment approach include isolation of peat-inhabiting subdivision 3 acidobacterium *Bryobacter aggregatus* (Kulichevskaya et al., 2010) as well as cultivation of several mildly acidophilic planctomycetes (Kulichevskaya et al., 2007, 2008, 2009, in press).

![FIGURE 3](https://www.frontiersin.org)
Careful inspection of impure cultures that are commonly discarded in routine cultivation practice is another way of obtaining an unusual organism. A good example is the isolation of a difficult-to-culture acidobacterium, *Telmatobacter bradus* (Pankratov et al., in press). This microorganism is a facultative anaerobe which can grow only under reduced oxygen tension. Despite this fact, *T. bradus* was recovered from aerobically incubated plates where it developed in a co-culture with an unidentified rapidly growing aerobic aliphaproteobacterium. The aliphaproteobacterium created a microhabitat with reduced oxygen concentration through its rapid growth. Application of FISH with the *Acidobacteria*-specific probe HoAc1402 was again the main tool to identify one of the organisms in this co-culture as a target for further isolation work.

**SOME TIPS FOR ISOLATION OF ANAEROBES**

So far, only a very few anaerobic prokaryotes have been cultivated from acidic wetlands. One of the factors that may have hampered isolation of anaerobes is the proper choice of a reducing agent for anaerobic media preparation. As shown by Sizova et al. (2003), many conventional reducing agents such as Na₂S + l-cysteine-HCl and ascorbic acid did not support growth of methanogenic consortia from *Sphagnum*-derived peat, but success was achieved with titanium(III) citrate. Later, a similar approach was successfully applied for isolation of several methanogens from acidic peatlands (Bräuer et al., 2006; Cadillo-Quiroz et al., 2009) as well as for isolation and cultivation of some facultatively anaerobic peat-inhabiting bacteria (Sizova et al., 2007; Pankratov et al., in press).

In summary, the major tools for culturing microbes from acidic northern wetlands are readily available, although they need to be carefully adjusted for each particular target bacterium and site. Nevertheless, the bacterial diversity in acidic northern wetlands remains largely unexplored and should benefit from further development of improved cultivation techniques.

**CURRENTLY CAPTURED BACTERIAL DIVERSITY**

This chapter gives a brief overview of the currently cultured diversity of peat-inhabiting bacteria that have been characterized, named, and deposited in the public culture collections. At present, the list of these prokaryotic organisms does not exceed 30 species (*Table 1*), which compares poorly to the hundreds and thousands of species isolated and described from other ecosystems. Most bacterial taxa characteristic for northern wetlands have been described only during the last decade. Some representatives of the most abundant bacterial groups in acidic wetlands are discussed below.

**MEMBERS OF THE ALPHAPROTEOBACTERIA**

Three different physiological groups of aliphaproteobacteria commonly occur in acidic wetlands: (i) methanotrophs, (ii) chemoorganotrophs, and (iii) phototrophs. Of these, methanotrophic aliphaproteobacteria have received the most research attention due to their important role in reducing methane emission from northern wetlands (reviewed in Dedys, 2009).

**Methanotrophs**

Methanotrophic bacteria that have been cultivated from these ecosystems are members of the families Beijerinckiaceae (the genera *Methylocella*, *Methylocapsa*, and *Methyloferula*) and Methylocystaceae (the genus *Methylocystis*). All of them are cold-tolerant, N₂-fixing organisms that grow between pH 3.5–4.2 and 7.0–7.5. *Methylocella palustris* was the first methanotrophic bacterium isolated from acidic *Sphagnum*-dominated wetlands (Dedys et al., 1998, 2000). Another species of this genus, *Methylocella tundracea*, was later isolated from tundra wetlands (Dedys et al., 2004a). Members of the genus *Methylocella* have many unique features compared to other extant methanotrophs. Notably, they lack an extensive intracellular membrane (ICM) system, use only soluble methane monooxygenase MMO (sMMO) for methane oxidation, and are able to grow on some substrates containing C–C bonds (see below). Another unusual acidophilic methanotroph that possesses only sMMO is *Methyloferula stellata* (Vorobev et al., in press). In contrast to *Methylocella* spp., it is unable to utilize multicarbon compounds. Obligate methanotrophy is also characteristic of the peat-inhabiting methanotroph *Methylocapsa acidiphila* (Dedys et al., 2002). Despite its close phylogenetic relationship to *Methylocella* and *Methyloferula*, *Methylocapsa* possesses a particular MMO (pMMO) enzyme and an extensive ICM system. All methanotrophs from the Beijerinckiaceae are highly sensitive to salt stress and prefer dilute media with a low salt content (0.2–0.5 g L⁻¹). One-carbon substrates are utilized via the serine pathway, although *Methyloferula* also displays activity of ribulose-1,5-bisphosphate carboxylase (RubisCO).

*Methylocystis*-like methanotrophs are one of the numerically dominant and metabolically active methanotroph populations in northern wetlands (Dedys et al., 2001; Dedys et al., 2003; Chen et al., 2008a,b; Belova et al., 2011). Currently, two peat-inhabiting members of this genus are available in cultures – *Methylocystis heyeri* H2 (Dedys et al., 2007) and *Methylocystis* sp. H2s (Belova et al., 2011). These are mesophilic and mildly acidophilic methanotrophs which possess both a particulate and a soluble MMO and a well-developed ICM system. Methane and methanol are utilized via the serine pathway. However, in the absence of C1 substrates, these methanotrophs are able to grow slowly on acetate (Belova et al., 2011). A unique characteristic of *M. heyeri* is the phospholipid fatty acid profile. In addition to the signature fatty acid of alphaproteobacterial methanotrophs (18:1ω8c), cells also contain large amounts of what was previously considered a signature fatty acid of gammaproteobacterial methanotrophs, 16:1ω8c (Dedys et al., 2007).

**Chemo-organotrophs**

Members of the family Rhodospirillaceae are common inhabitants of acidic wetlands. An interesting, non-phototrophic representative of this family, the facultatively anaerobic, acidotolerant bacterium *Telmatospirillum sibirienne*, was isolated by Sizova et al. (2007) from a Siberian fen. These motile spirilla grow chemooorganotrophically on several organic acids and glucose under anoxic and micro-oxic conditions, which span the largest part of the bog profile. At low oxygen partial pressure in the dark, they are also capable of lithotrophic growth on H₂CO₃. Oligotrophy, metabolic versatility, ability to fix N₂ and cell motility make these bacteria perfectly adapted to the peat bog environment.

Two aerobic, acidophilic, peat-inhabiting chemo-organotrophs, *Acidisoma sibiricum* and *Acidisoma tundracea*, were described by...
Table 1 | List of taxonomically characterized bacteria isolated from Sphagnum-dominated wetlands.

| Species name                | Type strain               | Accession no. for 16S rRNA gene | Physiological type                     | pH growth range (optimum) | Carbon sources utilized                  | N₂ fixation | References                      |
|-----------------------------|---------------------------|---------------------------------|----------------------------------------|---------------------------|------------------------------------------|-------------|----------------------------------|
| **ALPHAPROTEOBACTERIA**     |                           |                                 |                                        |                           |                                          |             |                                  |
| Methylocella palustris      | KT (ATCC 700799T)         | Y17144                          | Aerobic methanotroph                  | 4.5–7.0 (5.0–5.5)         | Methane, methanol, acetate, ethanol,      | +           | Dedyshev et al. (2000)           |
| Methylocella tundræ          | T4 (DSM 15673T = NCIMB 13949T) | AJ555244                       | Aerobic methanotroph                  | 4.2–7.5 (5.5–6.0)         | Methane, methanol, acetate, ethanol,      | +           | Dedyshev et al. (2004a)          |
| Methylocapsa acidiphila     | B2 (DSM 13967T = NCIMB 13765T) | AJ278726                       | Aerobic methanotroph                  | 4.2–7.2 (5.0–5.5)         | Methane, methanol                         | +           | Dedyshev et al. (2002)           |
| Methyloxytis heyeri         | B2 (DSM 16984T = VKM B-2426T) | AM283543                       | Aerobic methanotroph                  | 4.4–7.5 (5.8–6.2)         | Methane, methanol, acetate                | +           | Dedyshev et al. (2007)           |
| Methyloferula stellata      | AR4 (DSM 22108T = LMG 25277T = VKM B-2543T) | FR686343                       | Aerobic methanotroph                  | 3.5–7.2 (4.8–5.2)         | Methane, methanol                         | +           | Sizova et al. (2006b)           |
| Rhodoblastus sphagnicola    | RS (DSM 16996T = VKM B-2361T) | AM040096                       | Anaerobic phototroph                  | 4.8–7.0 (5.2–5.5)         | H₂ + CO₂, organic acids, methanol, ethanol | +           | Kulichevskaya et al. (2009)      |
| Telmatospirillum sibiriense | 26-4b1T (ATCC BAA-1305T, KACC 11899T) | AF524863                       | Facultatively anaerobic chemo-organotroph or chemo-lithotroph | 4.0–7.0 (5.7–6.5)         | Several organic acids, glucose, ethanol, H₂ + CO₂ | +           | Sizova et al. (2007)            |
| **BETAPROTEOBACTERIA**      |                           |                                 |                                        |                           |                                          |             |                                  |
| Burkholderia bryophila      | 1S18 ( = LMG 23644T = CCUG 52993T) | AM489501                       | Aerobic chemo-organotroph             | ND                        | Several sugars and organic acids          | +           | Vandamme et al. (2007)          |
| **ACIDOBACTERIA**           |                           |                                 |                                        |                           |                                          |             |                                  |
| Bryobacter aggregatus       | MPL3 (ATCC BAA-1390T = DSM 18758T) | AM162405                       | Aerobic chemo-organotroph             | 4.5–7.2 (5.5–6.2)         | Most sugars, some heteropolysaccharides, galacturonic, and glucuronic acids | –           | Kulichevskaya et al. (2010)      |
| Granulicella paludicola     | OB1010 (IDSM 22464T = LMG 25275T) | AM887758                       | Aerobic chemo-organotroph             | 3.0–7.5 (4.5–5.7)         | Most sugars, heteropolysaccharides        | –           | Pankratov and Dedyshev (2010)   |
| Granulicella rosea          | TP0104 (IDSM 18704T = ATCC BAA-1396T) | AM887759                       | Aerobic chemo-organotroph             | 3.0–7.5 (4.5–5.7)         | Most sugars, heteropolysaccharides, some organic acids | –           | Pankratov and Dedyshev (2010)   |
| Granulicella pectinivorans  | TP6060 (VKM B-2509T = DSM 2487T) | AM947653                       | Aerobic chemo-organotroph             | 3.7–7.6 (5.0–6.5)         | Most sugars, heteropolysaccharides, some organic acids | –           | Pankratov and Dedyshev (2010)   |
| Granulicella aggregans      | TP60628 (LMG 25274T = VKM B-2571T) | AM887756                       | Aerobic chemo-organotroph             | 3.0–7.5 (4.5–5.7)         | Most sugars, heteropolysaccharides, some organic acids | –           | Pankratov and Dedyshev (2010)   |

(Continued)
| Species name                | Type strain | Accession no. for 16S rRNA gene | Physiological type                                | pH growth range (optimum) | Carbon sources utilized                                                                 | N\textsubscript{2} fixation | References                                      |
|-----------------------------|-------------|---------------------------------|--------------------------------------------------|---------------------------|-----------------------------------------------------------------------------------------|-----------------------------|------------------------------------------------|
| Telmatobacter bradus        | TBP601\textsuperscript{T} (= DSM 23630\textsuperscript{T} = VKM B-2570\textsuperscript{T}) | AM887760                                      | Facultatively anaerobic chemo-organotroph         | 3.0–7.5 (4.5–5.0)          | Sugars, several heteropolysaccharides, salicin, galacturonic, and glucuronic acids, cellulose | –                           | Pankratov et al. (in press)                        |
| Bryocella elongata          | SN1\textsuperscript{T} (= LMG 25276\textsuperscript{T} = DSM 22489\textsuperscript{T}) | FR666706                                      | Aerobic chemo-organotroph                         | 3.2–6.6 (4.7–5.2)          | Sugars, ethanol, several heteropolysaccharides                                         | –                           | Dedysh et al. (in press)                          |
| Schlesneriapaludicola       | MPL7\textsuperscript{T} (= ATCC BAA-1393\textsuperscript{T} = VKM B-2452\textsuperscript{T}) | AM162407                                      | Facultatively aerobic chemo-organotroph           | 4.2–7.5 (5.0–6.2)          | Some sugars and heteropolysaccharides, N-acetylglucosamine, salicin                     | –                           | Kulichevskaia et al. (2007)                       |
| Singulisphaera acidiphila   | MOB10\textsuperscript{T} (= ATCC BAA-1392\textsuperscript{T} = VKM B-2454\textsuperscript{T}) | AM850678                                      | Aerobic chemo-organotroph                         | 4.2–7.5 (5.0–6.2)          | Sugars and heteropolysaccharides, N-acetylglucosamine, salicin                         | –                           | Kulichevskaia et al. (2008)                       |
| Singulisphaera rosea        | S26\textsuperscript{T} (= DSM 23044\textsuperscript{T} = VKM B-2599\textsuperscript{T}) | FN391026                                      | Aerobic chemo-organotroph                         | 3.2–7.1 (4.8–5.0)          | Most sugars, several organic acids, and polyalcohols, some heteropolysaccharides N-acetylglucosamine | –                           | Kulichevskaia et al. (in press)                   |
| Zavarzinella formosa        | A10\textsuperscript{T} (= DSM 19928\textsuperscript{T} = VKM B-2478\textsuperscript{T}) | AM162406                                      | Aerobic chemo-organotroph                         | 3.8–7.2 (5.5–6.0)          | Sugars and heteropolysaccharides, N-acetylglucosamine, salicin, pyruvate               | –                           | Kulichevskaia et al. (2009)                       |
| Mucilaginibacter paludis    | TPT56\textsuperscript{T} (= ATCC BAA-1394\textsuperscript{T} = VKM B-2446\textsuperscript{T}) | AM490403                                      | Facultatively aerobic chemo-organotroph           | 4.2–8.2 (6.0–6.5)          | Most sugars, heteropolysaccharides                                                     | –                           | Pankratov et al. (2007)                           |
| Mucilaginibacter gracilis   | TPT18\textsuperscript{T} (= ATCC BAA-1391\textsuperscript{T} = VKM B-2447\textsuperscript{T}) | AM490402                                      | Facultatively aerobic chemo-organotroph           | 4.2–8.2 (5.8–6.2)          | Most sugars, heteropolysaccharides                                                     | –                           | Pankratov et al. (2007)                           |
| Mycobacterium komossense     | Ko 2\textsuperscript{T} (= ATCC 33013\textsuperscript{T} = DSM 44078\textsuperscript{T}) | X55591                                        | Aerobic chemo-organotroph                         | ND                        | Several sugars, polyalcohols, and organic acids                                        | –                           | Kazda and Müller (1979)                          |
| Mycobacterium splagni       | Sph38\textsuperscript{F} (= ATCC 33027\textsuperscript{T} = DSM 44076\textsuperscript{T}) | X55590                                        | Aerobic chemo-organotroph                         | ND                        | Several sugars, polyalcohols, and organic acids                                        | –                           | Kazda (1980)                                     |
| Verrucosispora gilhornersis | HR1-2\textsuperscript{T} (= DSM 44337\textsuperscript{T}) | Y15523                                        | Aerobic chemo-organotroph                         | 6.5–8.2 (7.5)              | Several sugars, salicin                                                                  | –                           | Rheims et al. (1998)                             |
| Bacillus acidicoa           | 105-2\textsuperscript{F} (= DSM 14745\textsuperscript{T} = ATCC BAA-2366 = NRRL B-23453\textsuperscript{T}) | AF547209                                      | Aerobic chemo-organotroph                         | 3.5–70 (ND)               | Sugars                                                                                  | ND                          | Albert et al. (2005)                             |
| Clostridium acidioi         | CK74\textsuperscript{T} (= DSM 12555\textsuperscript{T}) | AJ237756                                      | Anaerobic chemo-organotroph                       | 3.6–6.9 (3.6–6.6)          | Several sugars, polyalcohols, salicin                                                  | +                           | Kuhner et al. (2000)                             |

ND, not determined.
Belova et al. (2009). These psychrotolerant bacteria develop in the pH range of 3.0–7.5 and utilize most sugars, polyalcohols, some organic acids and several polysaccharides, including xylan and starch.

**Photrophs**

Stagnant water bodies with large amounts of organic matter and low oxygen tensions are the preferred habitats for the purple non-sulfur bacteria (PNSB). They also develop in acidic freshwater wetlands. These metabolically flexible microorganisms grow phototrophically under anoxic conditions in the light or chemoorganotrophically under micro-oxic to oxic conditions in the dark. The examples of taxonomically characterized PNSB from acidic wetlands are *Rhodoblastus acidophilus* (Imhoff, 2001), which was originally described as “*Rhodopseudomonas acidiphila*” (Pfenning, 1969), and *Rhodoblastus sphagnicola* (Kulichevskaya et al., 2006b). The latter bacterium was isolated from anaerobic cellulolytic enrichments made with peat suspension incubated under light. Several other phototrophs, such as *Rhodopseudomonas palustris* and *Rhodomicrobium vannielii*, are also often obtained from acidic wetlands. The isolates usually display 99–100% 16S rRNA gene identity to the type strains of these species.

**MEMBERS OF THE BETAPROTEOBACTERIA**

The most typical representatives of this bacterial group in Sphagnum-dominated wetlands are members of the genus *Burkholderia* (Opelt and Berg, 2004; Belova et al., 2006; Opelt et al., 2007). The species *Burkholderia bryophila* was described based on a characterization of a number of strains isolated from Sphagnum mosses (Vandamme et al., 2007). These acidotolerant bacteria utilize various carbon substrates including aromatic compounds, fix N2 and show high antagonistic potential against fungal pathogens.

**THE PHYLUM ACIDOBACTERIA**

The *Acidobacteria* is one of the most diverse groups of prokaryotes in acidic wetlands (Dedysh et al., 2006). Despite this fact, cultured representatives of peat-inhabiting acidobacteria are now available for subdivisions 1 and 3 only. These are the genera *Granulicella* (Pankratov and Dedysh, 2010), *Telmatobacter* (Pankratov et al., in press), and *Bryocella* (Dedysh et al., in press) in subdivision 1 and the genus *Bryobacter* in subdivision 3 (Kulichevskaya et al., 2010). All of these characterized acidobacteria are mesophilic or psychrotolerant, acidophilic chemo-organotrophs that grow at pH values between 3.0 and 6.5–7.5. *Granulicella* spp., *Bryocella elongata*, and *B. aggregatus* are strict aerobes. *T. bradus* is a facultative anaeroobe that grows under reduced oxygen tension and under anoxic conditions. The preferred growth substrates are sugars though several organic acids and polyalcohols can also be utilized by some strains. Most peat-inhabiting acidobacteria can utilize glucuronic and galacturonic acids, which are released during decomposition of Sphagnum moss. The ability to degrade various plant-derived polymers, such as pectin, xylan, laminarin, lichenan, and starch varies between different species, but only *T. bradus* is capable of hydrolyzing cellulose. *B. elongata* possesses an ability to develop in a co-culture with exopolysaccharide-producing acidophilic methanotrophs, presumably by means of feeding on their capsular material. None of the cultured acidobacteria are capable of C1-metabolism or N2 fixation. However, members of this phylum seem to play an important role in degrading plant-derived polymers in acidic oligotrophic wetlands.

**THE PHYLUM PLANCTOMYCETES**

Planctomycetes represent one of the most abundant bacterial groups detectable by FISH in acidic *Sphagnum*-dominated wetlands (Dedysh et al., 2006; Kulichevskaya et al., 2006a). Several peat-inhabiting, acidophilic planctomycetes were recently isolated and described as members of the novel genera *Schlesneria*, *Singulisphaera*, and *Zavarzinella* (Kulichevskaya et al., 2007, 2008, in press). These are mesophilic, moderately acidophilic chemo-organotrophs capable of growth at pH values between 4.2 and 7.5. They grow best in aerobic conditions on media containing carbohydrates or N-acetylglucosamine. However, *Schlesneria paludicola* is also capable of fermentation, while members of the genus *Singulisphaera* grow well in micro-oxic conditions. All planctomycetes isolated from peat are able to degrade various heteropolysaccharides, but not cellulose or chitin. None of them is capable of fixing N2. These cultured representatives, however, do not cover all planctomycete diversity detected in acidic wetlands by means of cultivation-independent approaches (Ivanova and Dedysh, unpublished data). The existence of other, still-unknown physiological types of planctomycetes in wetlands cannot be excluded.

**THE PHYLUM ACTINOBACTERIA**

Currently available cultures of peat-inhabiting actinobacteria include several species of the genus *Mycobacterium* (Kazda and Müller, 1979; Kazda, 1980) and a member of the family Micromonosporaceae, *Verrucomicrobiurn gigihornensis* (Rheims et al., 1998). They utilize sugars, several polyalcohols and organic acids as well as some aromatic compounds, but none of them is capable of cellulose degradation.

**THE PHYLUM BACTERIOIDES**

In contrast to many aquatic and terrestrial habitats, members of the phylum *Bacteroidetes* are not abundant in acidic wetlands. The species cultured from acidic peat lack the ability to degrade cellulose or chitin. Two examples of such bacteria are *Mucilaginibacter* spp. (Pankratov et al., 2007) and *Chitinophaga arvensicola* (Pankratov et al., 2006). They do, however, possess some hydrolytic potential being able to degrade some heteropolysaccharides, such as xylan, laminarin, or pectin in acidic and cold conditions.

**NEW KNOWLEDGE GAINED DUE TO CULTIVATION EFFORTS**

As outlined in Table 1, cultivation efforts of the last decade have resulted in significant success in uncovering the microbial diversity in acidic northern wetlands. Many characteristics of the newly isolated organisms, such as their acid and salt tolerance as well as temperature preferences, distinguish them from bacteria inhabiting neutral mesophilic habitats. This is not surprising since acidic northern wetlands possess unique physico-chemical conditions. More interesting are several unexpected physiological discoveries made after isolation of novel representatives of the bacterial groups presumed to be already well documented. Some of these findings are discussed in this chapter.
EXISTENCE OF METHANOTROPHS CONTAINING ONLY A SOLUBLE METHANE MONOOXYGENASE

Until recently, possession of a particulate methane monooxygenase enzyme and a well-developed intracytoplasmic membrane system in which pMMO is bound was considered a characteristic feature of all extant aerobic methanotrophs. The first methanotrophic bacterium that did not meet these criteria was isolated from acidic Sphagnum-dominated wetlands and named Methylocella palustris (Dedysh et al., 2000). Two other recognized species of this genus, *M. silvestris* (Dunfield et al., 2003) and *M. tundrae* (Dedysh et al., 2004a) were later isolated from an acidic forest soil and tundra wetlands, respectively. All members of the genus *Methylocella* lack extensive intracytoplasmic membrane structures typical of most other methanotrophs. The absence of pMMO in *M. palustris* was initially suggested by the failure to detect a *pmoA* gene, which encodes the 27-kDa polypeptide of pMMO, by PCR or by DNA probing with *pmoA* from *Methylococcus capsulatus* Bath (Dedysh et al., 2000). Analysis of *M. silvestris* BL2T by SDS-PAGE detected no pMMO-specific polypeptides, and DNA probing with *pmoA* gene fragments from two closely related genes also found no evidence for pMMO (Theisen et al., 2005). Finally, the absence of any *pmo* gene homologs in *M. silvestris* BL2T was conclusively verified by analyzing the genome sequence of this bacterium (Chen et al., 2010).

The absence of pMMO in *Methylocella* spp. implies that these bacteria cannot be detected using a *pmoA*-based PCR assay considered universal and specific for all other methanotrophs (Holmes et al., 1995). However, they do possess the *mmoX* gene encoding the α-subunit of sMMO and can be detected via retrieval of these genes from the environment. A real-time quantitative PCR method was recently developed and validated targeting *Methylocella mmoX* that allowed detection and quantification of these unusual methanotrophs in a variety of environmental samples (Rahman et al., 2011). Interestingly, *Methylocella* spp. were detected not only in acidic or neutral habitats, but also in alkaline environments, suggesting that these methanotrophs are not limited to acidic pH in nature.

The diversity of methanotrophs that lack pMMO and use only an sMMO for methane oxidation appears to be not restricted to *Methylocella* species. Recently, three novel isolates were obtained from *Sphagnum*-dominated wetlands and an acidic forest soil and were described as representing a novel genus and species, *Methyloferula stellata* (Vorobev et al., in press). In these bacteria, the *mmoX* gene could not be amplified with any of the previously known *mmoX*-targeted primers, which explains why these methanotrophs escaped detection in all previous cultivation-independent studies. The absence of a universal assay for the specific detection of all pMMO-lacking methanotrophs complicates assessment of their abundance and distribution as well as their contribution to the processes of methane oxidation in northern wetlands and other natural environments. We have only recently begun exploring this new group of pMMO-lacking methanotrophs.

FACULTATIVE METHANOTROPHY

For a long time, all methanotrophs were considered to be obligately methylotrophic, i.e., unable to grow on compounds containing C–C bonds. This notion has recently been revised since the ability to grow on methane as well as on some multicarbon substrates, i.e., facultative methanotrophy, was demonstrated in several methanotrophic bacteria. Interestingly, all currently characterized facultative methanotrophs were isolated from acidic environments, such as *Sphagnum*-dominated wetlands or acidic boreal forest soils. Unusual, pMMO-lacking methanotrophs of the genus *Methylocella* were the first to be conclusively shown to have a facultative capability (Dedysh et al., 2005a). In addition to methane, they are capable of growth on acetate, pyruvate, succinate, malate, and ethanol. Acetate and methane were used as model substrates to conclusively verify facultative growth in *M. silvestris* BL2T. The growth rate and carbon conversion efficiency of *M. silvestris* BL2T was higher on acetate than on methane, and when both substrates were provided in excess acetate was preferably used and methane oxidation shut down. Further detailed experiments demonstrated that transcription of the *mmo* operon in *Methylocella* was repressed by acetate (Theisen et al., 2005).

We now know that a facultative lifestyle may also occur in pMMO-possessing methanotrophs. Several members of the genera *Methylocapsa* and *Methylocystis* were recently shown to be capable of growing on methane as well as on some multicarbon substrates such as acetate or ethanol (Dunfield et al., 2010; Belova et al., 2011; Im et al., 2011). Unlike *Methylocella*, these methanotrophs prefer to utilize methane, but growth can also occur on acetate and/or ethanol in the absence of methane. Two of these organisms, *M. heyeri* H2 and *Methylocystis* sp. strain H2s, were isolated from acidic *Sphagnum* peat. The latter was shown to represent a numerically abundant methanotroph population in geographically distinct northern wetlands (Belova et al., 2011).

METABOLIC VERSATILITY WITHIN THE BEIJERINCKIACEAE

Research on acidic wetlands has changed our perception of the family Beijerinckiaeae. Former knowledge of this bacterial group characterized its representatives as aerobic, acidophilic chemo-heterotrophs with the ability to fix N2. During the last decade, a novel sub-group of acidophilic serine pathway methanotrophs and methylotrophs that are phylogenetically closely related to the genus *Beijerinckia* was discovered in acidic *Sphagnum* peat bogs and forest soils (Dedysh et al., 1998, 2000, 2002; Dedysh et al., 2004a; Dunfield et al., 2003, 2010; Vorob’ev et al., 2009; Vorobev et al., in press). Moreover, methylotrophic autotrophy was found in one earlier described member of the genus *Beijerinckia*, *Beijerinckia mobiles* (Dedysh et al., 2005b). Now this family accommodates bacteria with strikingly different lifestyles, such as chemo-heterotrophs, facultative methylotrophs and also facultative and obligate methanotrophs (*Figure 4*). The 16S rRNA gene sequence similarity values between these phenotypically distinct bacteria range from 96 to 98%, which makes it impossible to predict the phenotype of any novel member in this family based on 16S rRNA gene sequence information alone. Since Beijerinckiaeae is the only bacterial family containing methanotrophic and non-methanotrophic bacteria, it has been chosen for the ongoing comparative genomic study, which should enable elucidating the genetic and metabolic tradeoffs required for a specialized methanotrophic lifestyle compared to more generalist chemoorganotrophic lifestyles.
CELLULOSE DEGRADATION IN MEMBERS OF THE ACIDOBACTERIA

The Acidobacteria is one of the cosmopolitan but poorly characterized groups of the domain Bacteria. The role of these organisms in natural environments remains poorly understood. Recent analysis of the genomes of three bacteria from the phylum Acidobacteria suggested a potential role of these prokaryotes in degradation of plant, fungal, and insect-derived organic matter (Ward et al., 2009). The genomes were shown to contain the genes encoding candidate cellulases and β-glucosidases, suggesting that acidobacteria are able to degrade cellulose substrates, although experimental confirmation for this ability was missing. Recently, however, the first proofs for cellulose degradation by acidobacteria became available (Pankratov et al., in press). This ability was found in a peat-inhabiting facultative anaerobe Telmatobacter bradus. So far, this is the only characterized cellulolytic acidobacterium, although two other uncharacterized subdivision 1 acidobacteria, strains KBS 83 and CCO287, and subdivision 3 acidobacterium KBS 96 were also recently reported to possess cellulytic potential (Eichorst et al., 2011; Pankratov et al., 2011). The actual rates of cellulose decomposition by these bacteria are very low and are not comparable to those in well-characterized cellulose degraders. The latter, however, are either absent or present in only very low numbers in cold and acidic northern wetlands. This makes acidobacteria important members of the indigenous hydrolytic microbial community. This may also be true for other acidic waterlogged habitats such as a primary tropical peat swamp forest in southern Thailand (Kanokratana et al., 2011). Metagenomic analysis of the microbial community in the surface peat layer revealed a variety of putative genes encoding a range of cellulolytic and hemicellulolytic enzymes from the Acidobacteria, suggesting the key role of these microbes in plant debris degradation.

INTRIGUING GROUPS OF AS-YET-UNCULTIVATED MICROBES

Many bacteria that have been detected in acidic northern wetland by means of molecular techniques have so far resisted all cultivation attempts. The reasons behind our failure to culture these microbes are unclear and we also do not have any insights into their phenotypes or ecological functions. Several groups of these elusive bacteria are discussed below.

AN UNCULTURED GROUP OF ALPHAPROTEOBACTERIA ASSOCIATED WITH SPHAGNUM MOSSES

A specific and highly abundant group of alphaproteobacteria was shown to colonize the hyaline cells of the outer stem cortex and the surface of stem leaves of Sphagnum cuspidatum (Raghoebarsing et al., 2005). Molecular identification of these bacteria showed their distant affiliation to acidophilic methanotrophs of the genera Methylocella and Methylocapsa (93% 16S rRNA gene sequence identity) and, therefore, these bacteria were assumed to be "symbiotic" methanotrophs that live in and on Sphagnum mosses. A number of similar 16S rRNA gene sequences were later retrieved from an acidic peat bog in West Siberia (Dedysh et al., 2006) as well as from other boreal ecosystems. These sequences form a common cluster, which is distinct from the Methylocystaceae and the Beijerinckiaeeae, and does not contain cultivated representatives (Figure 2). Interestingly, members of this bacterial group were also detected in cellulolytic peat enrichment cultures, which were incubated without methane (Pankratov et al., 2011). None of the currently available pmoA or mmoX sequence groups retrieved

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from Sphagnum mosses or Sphagnum-derived peat can be linked to these bacteria. Therefore, the biology of organisms within this cluster remains obscure.

UNCHARACTERIZED ACIDOBACTERIA

Despite the recent success in cultivating acidobacteria, most members of this phylum that thrive in northern wetlands resist isolation. This is especially true for subdivision 4 and 8 acidobacteria, which seem to be typical for the peat bog environment. At present, subdivision 4 has no characterized members. Subdivision 8 of the Acidobacteria is currently represented by three neutrophilic organisms that display highly contrasting characteristics. Holophaga foetida is a strictly anaerobic, homoacetogenic bacterium that degrades aromatic compounds (Liesack et al., 1994). Geothrix fermentans is also a strict anaerobe that oxidizes acetate as well as several other simple organic acids and long-chain fatty acids with Fe(III) as the electron acceptor (Coates et al., 1999). By contrast, Acanthopleuribacter pedis is a strictly aerobic chemo-organotroph that utilizes only a very limited number of growth substrates including glucose and several amino-acids (Fukunaga et al., 2008). The 16S rRNA gene sequences commonly retrieved from acidic wetlands affiliate with either Holophaga or Geothrix, but display only a very distant relationship to these characterized bacteria. Further cultivation efforts are needed to elucidate the metabolic potentials of the as-yet-uncultivated acidobacteria and their roles in northern wetlands.

PEAT-INHABITING VERRUCOMICOBIA

Verrucomicrobia-affiliated 16S rRNA gene sequences are abundant in clone libraries obtained from Sphagnum peat (Figure 1). Most of these clones belong to a broad phylogenetic sequence cluster for which cultured representatives have not yet been reported. Therefore, no conclusions can be made concerning the lifestyle of peat-inhabiting verrucomicrobia. Most cultivated members of this phylum are heterotrophs but several recently characterized peat-inhabiting verrucomicrobia. Therefore, the occurrence of methanotrophic verrucomicrobia in acidic northern wetlands remains a highly intriguing question.

CANDIDATE DIVISION OP3

The candidate division OP3 is one of the primary bacterial groups for which no information is currently available. OP3-related 16S rRNA gene sequences have been recovered from many anoxic environments, such as flooded paddy soils, marine sediments, hypersaline deep sea, freshwater lakes, and methanogenic bioreactors (Glöckner et al., 2010). They were also retrieved from Sphagnum-dominated wetlands (Dedysh et al., 2006; Morales et al., 2006; Ivanova and Dedysh, unpublished results). The analysis of metagenomic fosmid libraries constructed from flooded paddy soils revealed that OP3 bacteria share a high proportion of orthologs with members of the Deltaproteobacteria and may possess an anaerobic respiration mode (Glöckner et al., 2010). They may have a facultatively anaerobic lifestyle since OP3-related 16S rRNA gene sequences were found both in oxic and anoxic peat layers.

Designing novel isolation strategies and cultivating uncultured microbes from northern wetlands remain highly challenging tasks given that most microbial inhabitants of these acidic, cold, and nutrient-poor environments are slow-growing, oligotrophic organisms. Hunting for such microbes is perhaps seen as technologically out of step with the increasingly molecular character of modern microbiology. The newly isolated microorganisms, however, are a unique source of novel, unexpected findings, which may revise many old paradigms in our knowledge. They also provide the means to study cell biology, to verify hypotheses emerging from genome sequence data, and to adjust the currently used molecular detection techniques as well as our ideas about the functional role of these microbes in the environment.

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