Microbiology, Vol. 71, No. 6, 2002, pp. 638–650. Translated from Mikrobiologiya, Vol. 71, No. 6, 2002, pp. 741–754.

REVIEW PAPERS

Methanotrophic Bacteria of Acidic *Sphagnum* Peat Bogs

S. N. Dedysh

Institute of Microbiology, Russian Academy of Sciences, pr. 60-letiya Oktyabrya 7, k. 2, Moscow, 117312 Russia

Received April 9, 2002

Abstract—Acidic *Sphagnum* peat bogs cover a considerable part of the territory of Russia and are an important natural source of biogenic methane, which is formed in their anaerobic layers. A considerable portion of this methane is consumed in the aerobic part of the bog profile by acidophilic methanotrophic bacteria, which comprise the methane filter of *Sphagnum* peat bogs and decrease CH_4 emission to the atmosphere. For a long time, these bacteria escaped isolation, which became possible only after the elucidation of the optimal conditions of their functioning in situ: pH 4.5–5.5; temperature, from 15 to 20°C; and low salt concentration in the solution. Imitation of these conditions and rejection of earlier used media with a high content of biogenic elements allowed methanotrophic bacteria of two new genera and species—*Methylocella palustris* and *Methylocapsa acidiphila*—to be isolated from the peat of *Sphagnum* peat bogs of European northern Russia and western Siberia. These bacteria are well adapted to the conditions in cold, acidic, oligotrophic *Sphagnum* peat bogs. They grow in a pH range of 4.2–7.5 with an optimum at 5.0–5.5, prefer moderate temperatures (15–25°C) and media with a low content of mineral salts (200–500 mg/l), and are capable of active dinitrogen fixation. Design of fluorescently labeled 16S rRNA–targeted oligonucleotide probes for the detection of *Methylocella palustris* and *Methylocapsa acidiphila* and their application to the analysis of sphagnum peat samples showed that these bacteria represent dominant populations of methanotrophs with a density of 10^5–10^6 cells/g peat. In addition to *Methylocella* and *Methylocapsa* populations, one more abundant population of methanotrophs was revealed (10^6 cells/g peat), which were phylogenetically close to the genus *Methylocystis.*

Key words: acidophilic methanotrophic bacteria, *Sphagnum* peat bogs, *Methylocella palustris, Methylocapsa acidiphila,* fluorescently labeled 16S rRNA–targeted oligonucleotide probes.

1. SETTING UP THE PROBLEM

Methane is one of the most important greenhouse gases; due to the high rate of its accumulation in the atmosphere (up to 1% per year [1]), it attracts close attention from researchers representing various fields of science. Two methane sources are considered most significant for the global methane budget: natural bogs and waterlogged soils and also rice paddies [1–4]. The significance of each of these sources varies depending on the geographical region. The areas occupied by rice paddies are mainly located in southeastern Asia; fens mainly occur in tropical regions; ombrotrophic and mesotrophic bogs occupy large areas to the north of 60° N. The contribution of these northern bogs to the budget of atmospheric CH_4 is especially considerable for Russia, Scandinavia, Canada, and the north of the United States [2, 4–6]. The largest area, 161 million ha, is occupied by bogs in Russia, and most of these bogs are in western Siberia, where they cover 80% of the territory [7]. Field measurements of methane emission from bogs of western Siberia showed that it is significant, varying from 0.5 to 40 mg CH_4-C/(h m^2) [8–10]. Methane emission from fens is usually insignificant. Maximum emission values have been recorded in ombrotrophic and mesotrophic *Sphagnum* peat bogs, which represent the dominant type of northern wetlands.

One of the key features of *Sphagnum* peat bogs is their high acidity. The pH values of the peat water vary from 3 to 4.5 in ombrotrophic *Sphagnum* peat bogs and from 4 to 5.5 in mesotrophic bogs. In addition, these habitats are ultrafresh or are very poor in mineral salts. As a result of lack of contact with groundwater, the content of mineral salts in ombrotrophic bogs does not exceed 50 mg/l of peat water; this determines its extremely low buffering capacity. This habitat is also characterized by the prevalence of low temperatures. Even in summer, the temperature of surface layers of peat does not exceed 15°C. In addition, the microbial communities of northern bogs are subjected to cyclic processes of freezing and thawing and occur in a frozen state for about half the year. Thus, *Sphagnum* peat bogs are a type of extreme environment, characterized by (i) high acidity (pH 3.0–5.5), (ii) low buffering capacity, (iii) low content of mineral salts, and (iv) prevalence of low temperatures. These are conditions under which the microbial processes of methane production and consumption proceed; their balance determines the value of methane emission.

This review focuses on methanotrophic bacteria forming the methane-oxidizing filter [11], which intercepts the methane produced in the anaerobic layers of *Sphagnum* peat bogs on its way to the atmosphere. As distinct from the methanotrophic communities of neu-

0026-2617/02/7106-0638$27.00

Table 1. Recognized genera of methanotrophic bacteria and their growth pH ranges

Genus	Type	Growth pH range of known representatives	Year of description	Reference
Methylomonas	I	5.5–8.5	1970	[33]
Methylobacter	I	5.5–8.5	1970	[33]
Methylococcus	I	5.5–8.5	1970	[33]
Methylosinus	II	5.5–8.5	1970	[33]
Methylocystis	II	5.5–8.5	1970	[33]
Methylomicrobium	I	5.5–11.0	1995	[34]
Methylosphaera	I	6.0–8.5	1997	[35]
Methylocaldum	I	6.5–8.5	1997	[36]
Methylosarcina	I	5.0–9.0	2001	[37]
Methylocella	II	4.5–7.0	2000	[38]
Methylocapsa	II	4.2–7.2	2002	[39]

tral and weakly alkaline rice paddies [12–15] and fens [16], represented by well-studied type I and type II methanotrophs, the methanotrophic communities of *Sphagnum* peat bogs remained unstudied for a long time. The importance of their investigation is determined by the global distribution of acidic *Sphagnum* peat bogs, which are the dominant terrestrial ecosystems of the boreal zone of the northern hemisphere and a landscape very typical of Russia.

Regularities observed in methane oxidation processes in sphagnum peat. Despite the long list of apparently unfavorable ecotopic factors, methane consumption in sphagnum peat proceeds at an extremely high rate [17–24]. In the presence of acetylene, known to be a specific inhibitor of methanotrophs [25, 26], no methane consumption by peat occurs [24, 27], which confirms the role of microorganisms in this process. The maximum of potential methane-oxidizing activity in the vertical profile of *Sphagnum* peat bogs usually coincides with the water table level or occurs several centimeters below [19–23, 28]. It is here that the boundary between aerobic and anaerobic zones is located and the ratio of the available substrate (CH_4) to oxygen is optimal: below, the concentration of O_2 decreases abruptly, and above the water table level, this tendency is observed for CH_4. All these data suggest the existence in *Sphagnum* peat bogs of an active methane-consuming microbial community. This community is well adapted to existence in an acidic, cold environment. Canadian researchers showed that the pH optimum of methane consumption by sphagnum peat is in the acidic range of pH 5–6, and the incubation of samples at temperatures of 0–10°C results in a methanotroph activity comprising 13–38% of the maximum value [29]. Studies of the kinetics of methane consumption in peat samples taken from the Sosvyatskii Mokh ombrotrophic bog (Tver oblast) revealed maximum methanotroph activity in the pH range of 4.5–5.5 and the temperature range of 15–20°C [30]. A rise in the temperature above 20°C resulted in an abrupt decrease in the methane-oxidizing activity. Contrary to our expectations, methanotrophs of ombrotrophic bogs were not limited by the low content of available bound nitrogen. Introduction of nitrates produced a strong inhibitory effect, which was observed at KNO_3 doses as low as several tens of micrograms per gram peat. Based on these data, a conclusion was made that the methanotrophs of *Sphagnum* peat bogs are psychroactive, moderately acidophilic, and sensitive to increased concentrations of mineral salts. First of all, screening was performed among known methanotrophs for organisms possessing the above characteristics.

Known methanotrophic bacteria and their pH growth ranges. Methanotrophic bacteria are a unique group of microorganisms able to use methane as the sole source of carbon and energy. The key feature of methanotrophs is that they possess methane monooxygenase (MMO), the enzyme catalyzing the first step of methane oxidation (to CH_3OH). Based on morphological, physiological, biochemical, and chemotaxonomic properties, methanotrophic bacteria were subdivided into two groups: type I and type II methanotrophs, which respectively belong to the γ and α subclasses of Proteobacteria [31, 32]. The list of taxa of methanotrophic bacteria validated by year 2002 includes representatives of 11 genera (Table 1). In the mid 1990s, when the search for microorganisms able to oxidize methane in cold acidic *Sphagnum* peat bogs was started, this list included eight genera. Psychrophilic methanotrophs able to grow in a temperature range of 3–20°C had already been isolated by that time [35, 40, 41], but they had a pH optimum at neutral pH values and could not grow at pH values lower than 5.5. It was known that some strains of *Methylococcus capsulatus* can tolerate acidification of the medium to pH 5.0–5.2; however, their growth was suppressed under these conditions [42]. Thus, the analysis of properties of known methanotrophs provided no grounds to relate some of

them to methane oxidation occurring in acidic bogs. The task of detection and isolation of yet unknown methanotrophs able to actively function in cold, acidic, oligotrophic *Sphagnum* peat bogs became pertinent.

2. THE HISTORY OF THE SEARCH FOR ACIDOPHILIC METHANOTROPHS

Use of traditional methods for isolation of methanotrophic bacteria. After Whittenbury and his colleagues managed to isolate more than 100 strains from various natural substrates, his approach and the mineral media NMS and AMS (nitrate mineral salts and ammonium mineral salts) [33] have soundly been regarded classical and have been generally accepted. Later, several other mineral media were suggested for the isolation of methanotrophic bacteria [32, 43, 44]. They varied in the source of available nitrogen, phosphate salt (Na or K), concentrations of individual components, and trace element composition. However, all these media had a high content of mineral salts (1.5–3 g/l) and high buffering capacity and were neutral (pH 6.0–7.5). Selectivity of these media was determined by the presence of a sole carbon source, methane, which allowed initiation of growth of methanotrophic microorganisms only. However, the selectivity of these media had one more aspect: they provided for the development of neutrophilic methanotrophs only. These media worked well and allowed researchers to isolate new species of the genera described by Whittenbury from diverse natural sources: soils, fresh and saline waters, fens, rice paddies, plant rhizosphere, etc. [32, 45, 46]. Acidic soils of coniferous forests and ombrotrophic *Sphagnum* peat bogs were gaps in this list. Traditional media did not allow even enrichment methanotrophic cultures to be obtained from these acidic substrates. The only exception was the work of German researchers who managed to isolate *Methylosinus* spp. strains from sediments of a peat lake (pH 4.4) on a traditional medium with pH 6.5 [47]. The population density of these bacteria was as low as 10^3 cells per gram wet peat, and their ability to grow at acidic pH values was not confirmed. Therefore, it remained unclear whether the cultures isolated represented methanotrophic populations metabolically active in situ or they survived in the acidic habitat in the form of resting cells.

Application of molecular techniques. The failure of the traditional cultivation approaches to reveal the methanotrophs of *Sphagnum* peat bogs impelled several researchers to use methods of molecular ecology, which allow microorganisms to be identified in situ, without the isolation of pure cultures. The molecular methods developed for the detection and identification of methanotrophic bacteria can be subdivided into direct (hybridization of whole cells with 16S rRNA–targeted fluorescent probes) and indirect (which imply isolation of DNA from a natural sample and its analysis with the use of the polymerase chain reaction (PCR)) [48, 49]. The first attempt to reveal acidophilic methanotrophs by molecular methods was undertaken by English researchers who worked with DNA extracted from peat samples taken from *Sphagnum* peat bogs in northern England [24, 50, 51]. By using primer pairs universal for bacteria, fragments of genes encoding 16S rRNA were amplified and cloned, and a voluminous clone library (about 6000 clones) was created. Screening of the clones by their hybridization with oligonucleotide probes specific for *Methylomonas, Methylobacter, Methylococcus*, and *Methylosinus* revealed a group of clones that hybridized with the probe targeting *Methylosinus* [24]. Analysis of the nucleotide sequences of the cloned 16S rDNA fragments showed that they belonged not to *Methylosinus* but to a yet unknown phylogenetic relative of the genera *Methylosinus* and *Methylocystis*. This led to an assumption about the existence of unknown acidophilic methanotrophs within α-Proteobacteria. Subsequent PCR amplification and sequencing of fragments of the main functional genes of methanotrophs, *pmoA* (coding for membrane-bound MMO) and *mxaF* (coding for methanol dehydrogenase) [50, 51], also showed the presence of earlier unknown type II methanotrophs phylogenetically close to the genus *Methylocystis*. Thus, it was established that sphagnum peat is in fact colonized by methanotrophic bacteria and these bacteria are distinct from collection cultures. However, the lack of isolates did not allow researchers to give an answer to the question of whether the methanotrophs revealed by molecular methods are true acidophiles or are acidotolerant forms of known methanotrophs.

Attempts to identify bog-inhabiting methanotrophs by the analysis of fatty acids. The composition of fatty acids is one of the most developed diagnostic criteria in the taxonomy of methanotrophic bacteria [44, 52–54]. The predominance of monounsaturated fatty acids with 16 or 18 carbon atoms allows a methanotrophic isolate to be immediately identified as a representative of type I or type II methanotrophs, respectively. Methanotrophs also contain certain unusual fatty acids that, in other bacteria, are rare or do not occur at all. It was suggested to use these fatty acids, 16 : 1ω8*c* and 18 : 1ω8*c*, as biomarkers (indicative fatty acids) for type I and type II methanotrophs, respectively [53, 55]. Therefore, some researchers attempted to use the analysis of fatty acids extracted from sphagnum peat for investigation of the composition of indigenous methanotrophic communities [23, 56, 57]. The total content of 16 : 1ω8*c* and 18 : 1ω8*c* in peat samples correlated with the value of potential methanotrophic activity, and the content of 18 : 1ω8*c* was at least an order of magnitude higher than the content of 16 : 1ω8*c* [23, 56]. From these results, it was inferred that type II methanotrophs dominate in sphagnum peat. However, the calculations of the population densities of type I and type II methanotrophs, made proceeding from the data on the contents of the indicator acids, used a number of averaged values [56]; the roughest of the approximations was the use of *Methylomonas* spp. and *Methylosinus trichosporium* as

typical type I and type II methanotrophs, whose average cell volume was taken to be equal to 0.7 and 2.0 μm^3, respectively. Calculations that used these assumptions yielded an estimate of methanotroph population density of $(0.3–50) \times 10^6$ cells per gram wet peat; the contributions of type I and type II methanotroph populations were estimated as approximately equal. Later performed experiments with fluorescently labeled probes showed that the actual cell size of bog type II methanotrophs is much smaller than that of *M. trichosporium* and their cell volume does not exceed 0.5 μm^3 (Dedysh *et al.*, unpublished). Thus, from the valuable data on fatty acids, invalid conclusions were made on the population densities of methanotroph groups in peat. Another drawback of the method discussed was the assumption that all methanotrophic bacteria contain highly specific fatty acids, 16 : 1ω8*c* and 18 : 1ω8*c*. At present, it is evident that this assumption was invalid, since, later, two genera of methanotrophs were described, *Methylocella* and *Methylocapsa*, whose cells lack these indicator fatty acids [39]. Although these bacteria belong to type II methanotrophs, they lack 18 : 1ω8*c* and instead contain another isomer, 18 : 1ω7*c*, with another location of the double bond. Unfortunately, this fatty acid cannot be used as an indicator because it is contained by a number of methylotrophic bacteria incapable of methane oxidation [53]. It is noteworthy that the content of 18:1ω7*c* in sphagnum peat was several times higher than that of 16 : 1ω8*c* or 18 : 1ω8*c*, and its peak coincided with the maximum of methanotrophic activity in the bog vertical profile [23].

3. ISOLATION OF ACIDOPHILIC METHANOTROPHS AND THEIR PROPERTIES

Novel strategy for the search for acidophilic methanotrophs. The increasing volume of indirect evidence for the existence of unknown methanotrophs in *Sphagnum* peat bogs, the establishment of their pH and temperature optima, and recognition of the fact that they are inhibited by KNO_3 concentrations appropriate for known methanotrophs [30] gave impetus to the rejection of traditionally used nutrient media and cultivation conditions. The new strategy implied making laboratory media as similar as possible to the conditions of the natural environment and creating conditions that provide for maximum activity of methane consumption by peat. With this in mind, a mineral medium was developed that contained the same components as NMS [33] or P medium [32] (except for the alkaline component of phosphate buffer, Na_2HPO_4), but with the content of salts reduced to 250 mg/l. By means of acidification with H_3PO_4, a spectrum of media was obtained with pH from 3 to 6. For the isolation of dinitrogen-fixing methanotrophs, the same media were prepared without KNO_3. The incubation temperature was chosen to be 20°C. As a result, methanotrophic enrichments were obtained from peat samples taken from four *Sphagnum* peat bogs of different geographic location: the Sosvyatskii Mokh bog in Tver oblast and the Kirgiznoe, Krugloe, and Bakcharskoe bogs in Tomsk oblast [58].

Acidophilic methanotrophic communities. The enrichments obtained utilized methane as the source of carbon and energy and showed stable growth in acidic dilute media in a pH range of 4–6 [58]. Growth occurred in nitrogen-free medium and at a low content (200 mg/l) of a source of available nitrogen (e.g., nitrate). Cells grown on media with nitrate could switch to growth in nitrogen-free medium without any lag phase. On the contrary, cells grown in the regime of dinitrogen fixation and transferred to nitrate-containing medium resumed growth only after a prolonged (40-h) lag phase. Thus, growth in the regime of dinitrogen fixation is more natural for bog communities. Determination of the rates of methane consumption by the enrichments at different pH values showed that maximum rates—up to 0.5 mmol CH_4/(h g biomass)—occurred in the pH range of 4.5–5.5, which was also optimal for methane consumption by native peat samples [30]. The decrease in methane consumption rate by the enrichments observed at lower pH values was much less considerable than that observed at neutral pH values. Testing the enrichments obtained for growth on media with varied content of biogenic mineral elements confirmed that they could not grow on media containing more than 1 g mineral salts per liter. A content of salts of 25–250 mg/l was optimal for growth. On the whole, the properties of these moderately acidophilic communities corresponded to the conditions of their natural habitats.

It remained to be determined what methanotrophic bacteria are components of these communities. To answer this question, DNA was isolated from the enrichments and PCR amplification with two pairs of specific primers [59, 60] and cloning were conducted to obtain fragments of the main functional genes of methanotrophs: *mmoX* (encoding soluble MMO) and *pmoA* (encoding membrane-bound MMO). PCR amplification with primers for *mmoX* yielded products only with DNA from three enrichments, which were obtained on nitrogen-containing medium from the Sosvyatskii Mokh, Kirgiznoe, and Krugloe bogs [58]. Determination of the nucleotide sequences of the cloned *mmoX* fragments showed that all of the three communities contained methanotrophic bacteria whose *mmoX* was considerably different (20–23% divergence of the inferred peptide sequences) from the *mmoX* of the two known groups of these genes: the *Methylococcus capsulatus* (Bath)/*Methylomonas* spp. group and the *Methylosinus/Methylocystis* group [61]. This implied that the *mmoX* gene of acidophilic methanotrophs belongs to an evolutionary group distinct from the two aforementioned recognized groups of *mmoX* genes. In the DNA of the community from the Krugloe bog, a second type of *mmoX* was additionally detected. These *mmoX* fragments were fairly close (96.6% identity of the inferred peptide sequences) to those of *Methylocystis*

representatives, which suggested that they belonged to a new *Methylocystis* species.

The only sample which did not yield *mmoX* fragments in the PCR was the DNA from the enrichment obtained from the Bakcharskoe bog. This enrichment showed rather rapid exponential growth ($\mu = 0.03\ h^{-1}$) in nitrogen-free medium at pH 4.0 [62]. PCR amplification of *pmoA* gene fragments and analysis of their nucleotide sequences showed that this enrichment contained only one methanotrophic organism, whose *pmoA* gene also belonged to an earlier unknown group of *pmoA* genes. The divergence of the amino acid sequence corresponding to this *pmoA* fragment from *pmoA* sequences of type I and type II methanotrophs comprised 32–45 and 28–30%, respectively. Thus, in each of the methanotrophic enrichments obtained, the presence of unknown methanotrophic bacteria was established. The genes coding for MMO of these bacteria did not belong to any of the earlier known groups of *pmoA* and *mmoX* genes and represented new evolutionarily distinct groups of these genes [63].

***Methylocella* and *Methylocapsa*: two new genera of acidophilic methanotrophic bacteria.** The isolation of pure cultures of methanotrophic bacteria from the acidophilic enrichments was performed by two methods, which allowed two different organisms to be isolated. The first, more common plating method allowed the isolation of pure cultures (strains S6, K, and M131) only from three enrichments obtained on nitrogen-containing medium from the Sosvyatskii Mokh, Kirgiznoe, and Krugloe bogs. These isolates were later described as representatives of a new genus and new species of methanotrophic bacteria, *Methylocella palustris* [38, 64]. With the fourth enrichment, obtained on nitrogen-free medium from the Bakcharskoe bog in Siberia, the plating method did not yield positive results. As became clear later, the methanotrophic component of this community can grow only in liquid media. Therefore, another method was used: multiply repeated serial dilutions in nitrogen-free low-mineralization medium. This method allowed one more pure methanotrophic culture to be obtained, which was later described as *Methylocapsa acidiphila* [39].

Both bacteria were moderately acidophilic organisms able to utilize methane as the sole source of carbon and energy. Growth occurred in a rather wide pH range: 4.5–7.0 for *Methylocella palustris* and 4.2–7.2 for *Methylocapsa acidiphila*, with an optimum at 5.0–5.5. As distinct from the majority of known methanotrophs, the bog isolates did not grow at temperatures above 30°C and preferred the temperature range of 15–25°C. An important property of the cultures was their dinitrogen-fixing ability, which plays a decisive role for survival in a habitat extremely poor in available nitrogen. Like the enrichments from which they had been isolated, *Methylocella palustris* and *Methylocapsa acidiphila* could grow only in media with a low content of mineral salts (200–500 mg/l). Moreover, for *Methylocapsa acidiphila* cultures growing in the regime of dinitrogen fixation, growth inhibition by the addition of KNO_3 was observed, which was in agreement with the earlier described inhibitory effect of nitrate on methane oxidation by peat [30].

The two acidophilic methanotrophs are morphologically very different. The cells of *Methylocella palustris* are small (0.6–1.0 by 1.0–2.5 μm), nonmotile, straight or curved, bipolar rods (Fig. 1a). Resting cells—exospores similar to those of *Methylosinus*—occur very rarely. Growth is possible both on agarized and liquid media. Cells of *Methylocapsa acidiphila* are coccobacilli measuring 0.7–1.0 by 0.8–1.2 μm (Fig. 2a); a peculiarity of this culture is the formation of aggregates of cells embedded in a dense polysaccharide matrix (Fig. 2b). At the periphery of these aggregates, resting cells occur, which are similar in their fine structure to the cysts of *Azotobacter* (such cysts are more typical of type I methanotrophs). As distinct from *Methylocella palustris*, *Methylocapsa acidiphila* can grow in liquid media only.

The fine structure of *Methylocella palustris* and *Methylocapsa acidiphila* is also different. A unique peculiarity of *Methylocella palustris* cells is the lack of intracytoplasmic membranes (Fig. 1b) typical of all earlier known methanotrophs. *Methylocella palustris* cells have a wide periplasmic space and a system of spheric vesicles formed via invagination of the plasma membrane. Similar vesicular membrane structures were earlier observed in some representatives of *Hyphomicrobium* and phototrophic bacteria of the genera *Rhodospirillum* and *Rhodopseudomonas* [65, 66]. As distinct from *Methylocella palustris* cells, the cells of *Methylocapsa acidiphila* possess a well-developed system of intracytoplasmic membranes (ICM) (Fig. 2b) whose organization is different from that of ICM of earlier known type I and type II methanotrophs. In *Methylocapsa* cells, membranes are arranged in tightly packed bundles, which is characteristic of type I ICM; however, the membranes in these bundles are oriented parallel to the cell wall, as in type II methanotrophs. An additional peculiarity is that *Methylocapsa* ICM are always localized on only one side of the cell. We classified this type of ICM arrangement as a new type of ICM organization in methanotrophs, type III [39].

An important distinction between the two acidophilic methanotrophs is that they have different MMO forms: *Methylocella* cells possess soluble MMO only, and *Methylocapsa* cells contain only membrane-bound MMO. Whereas the latter situation is rather typical of methanotrophs, the possession of only soluble MMO is a unique feature currently known only for *Methylocella palustris* [38].

The nucleotide sequences of fragments of the *mmoX* gene of *Methylocella palustris* and of the *pmoA* gene of *Methylocapsa acidiphila* proved to be identical to the sequences of the *mmoX* and *pmoA* fragments amplified from the DNA of the initial enrichments. Both *mmoX*

Table 2. Major characteristics of the new genera of acidophilic methanotrophic bacteria *Methylocella* and *Methylocapsa*

Characteristics	Genus *Methylocella*	Genus *Methylocapsa*
Cell morphology	Bipolar straight or curved rods, 0.6–1.0 × 1.0–2.0 μm, single or aggregated	Coccobacilli, 0.7–1.0 0.8–1.2 μm, often arranged in aggregates embedded in polysaccharide matrix
Motility	Nonmotile	Nonmotile
Resting cell type	Exospores	Cysts
Type of ICM organization	Vesicular membrane formations	Bundles of membranes, parallel to PM, on one side of the cell (type III)
pH growth range	4.5–7.0	4.2–7.2
pH growth optimum	5.0–5.5	5.0–5.5
Growth at 30/37°C	–/–	+/–
Growth at 0.5% NaCl	–	–
Methane monooxygenase type	Soluble MMO only	Membrane-bound MMO only
Formaldehyde assimilation pathway	Serine pathway	Serine pathway
N_2-fixing capacity	+	+
Major phospholipids	Phosphatidylmethylethanolamine	Phosphatidylglycerol
Major fatty acids	18:1ω7*c*	18:1ω7*c*
DNA G+C content, mol %	61	63
Phylogenetic affiliation	α-subclass of Proteobacteria	α-subclass of Proteobacteria

and *pmoA* of acidophilic methanotrophs represent evolutionarily distinct groups of genes, differing from corresponding gene groups of type I and type II methanotrophs [63, 64]. It is worth noting that the *pmoA* gene of *Methylocapsa acidiphila* is most similar to *pmoA* genes cloned from acidic soils of various geographical zones [67–69].

The carbon and nitrogen metabolisms of acidophilic methanotrophs were similar to those of neutrophilic type II methanotrophs: they used the serine pathway for formaldehyde assimilation and the glutamate cycle for ammonium assimilation.

The fatty acid compositions of the acidophilic methanotrophs were dominated by monounsaturated fatty acids with 18 carbon atoms (18:1 acids). As distinct from other type II methanotrophs, in acidophiles these fatty acids are represented by a sole isomer, 18:1ω7; the 18:1ω8 isomer, specific for *Methylosinus* and *Methylocystis* representatives, is lacking from the cells of the acidophilic methanotrophs [39]. Earlier, the 18:1ω8 fatty acid was considered to be a signature fatty acid of type II methanotrophs and was widely used in ecological studies for the detection of these organisms. Our studies of acidophilic methanotrophs showed that the scope of application of this marker is restricted to the detection of *Methylosinus* and *Methylocystis* representatives.

According to data of phylogenetic analysis, acidophilic methanotrophs belong to a new branch of methanotrophic bacteria within the α-subclass of Proteobacteria and are only remotely related to *Methylosinus* and *Methylocystis* representatives (93.3–93.8% 16S rRNA homology). A much closer relative of the bog-inhabiting methanotrophs (96.5% 16S rRNA homology) proved to be the acidophilic, organotrophic, dinitrogen-fixing bacterium *Beijerinckia indica* subsp. *indica*, which is a typical inhabitant of acidic soils and bogs. The value of 16S rRNA homology between *Methylocapsa acidiphila* and *Methylocella palustris* is rather high (97.3%). However, considerable differences in several important characteristics suggest affiliation of these methanotrophs to two distinct genera (Table 2); this is also supported by data from DNA–DNA hybridization: the DNA–DNA homology between the two acidophilic methanotrophs is as low as 7% [39].

In spite of the similarity of ecophysiological characteristics of the two acidophilic methanotrophs, more detailed comparative analysis of *Methylocapsa acidiphila* and *Methylocella palustris* provides grounds to believe that these organisms realize different ecological strategies and occupy distinct ecological niches. *Methylocella* is to a larger extent dependent on the presence of a source of available nitrogen in the medium than *Methylocapsa*, which is completely autonomous with respect to nitrogen supply; in addition, the latter organism is more acidotolerant. The affinity to methane in *Methylocapsa acidiphila* (K_s, 1–2 μM) [63] is considerably higher than in *Methylocella palustris* ((K_s, 40–60 μM). The advantage of *Methylocella palustris* may be the possession of the soluble form of MMO. It is known that methanotrophs produce membrane-bound MMO only when grown in the presence of Cu^{2+} [70], which is a component of the active center of this enzyme [71, 72]. When the concentration of Cu^{2+} drops below 0.05 mg/l,

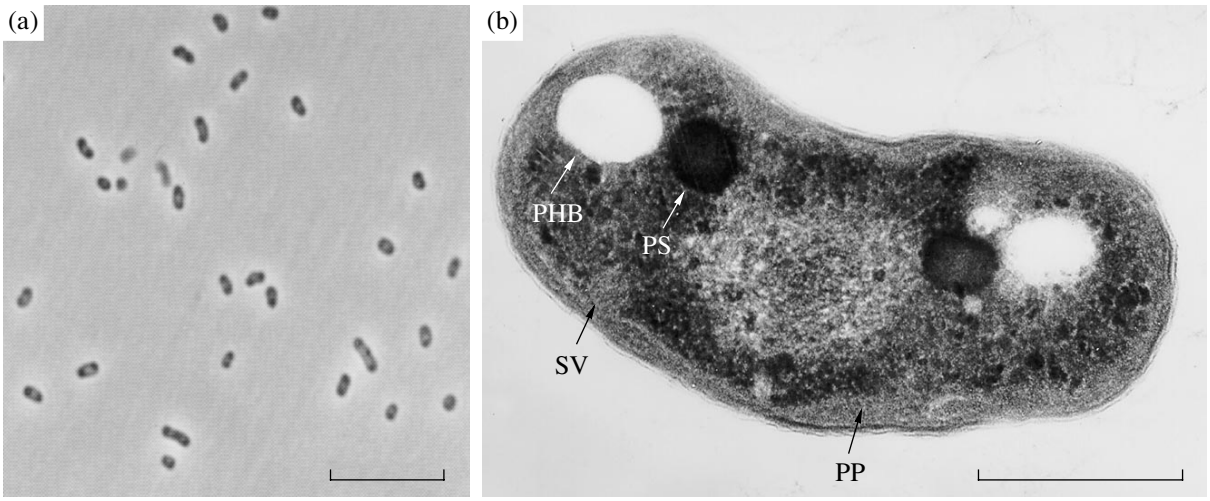

Fig. 1. The acidophilic dinitrogen-fixing methanotrophic bacterium *Methylocella palustris*, strain K, isolated from peat of the Kirgiznoe ombrotrophic *Sphagnum* peat bog (Tomsk oblast, western Siberia) and able to grow in a pH range of 4.5–7.0 with an optimum at 5.0–5.5. (a) Under a phase-contrast microscope, *Methylocella* cells are characterized by pronounced bipolarity: their ends are darker than the middle. Scale bar represents 10 μm. (b) Under an electron microscope, cells exhibit the following peculiarities: a lack of intracytoplasmic membranes and the presence of a broad periplasmic space (PS), spherical membrane vesicles (SV), and granules of poly-β-hydroxybutyrate (PHB) and polyphosphate (PP) at both cell poles. Scale bar represents 0.5 μm. The micrographs were taken by N.E. Suzina.

the soluble form of MMO is expressed [73]. In ombrotrophic bogs, the concentration of Cu^{2+} usually does not exceed 0.04 mg/l [74]. Earlier, a hypothesis was put forward that the possession of soluble MMO may be an ecological advantage of type II methanotrophs in habitats poor in Cu^{2+} [31]. Thus, *Methylocapsa* and *Methylocella* possess different sets of adaptive features that, depending on particular conditions in a microniche, may provide the competitive advantage to this or that acidophilic methanotroph.

4. MOLECULAR METHODS FOR DETECTION OF ACIDOPHILIC METHANOTROPHS

The question of how numerous and widespread acidophilic methanotrophs are in *Sphagnum* peat bogs is of particular interest with regard to the evaluation of the role of these microorganisms in the global processes of methane oxidation. The fact that the first isolates of *Methylocella palustris* were obtained from three acidic bogs of different types and various geographical locations (Tver oblast and the south of western Siberia) [64] suggested wide distribution of these bacteria. This conclusion is confirmed by the data of other researchers, who obtained 16S rDNA clones with similar nucleotide sequences from peat bogs and acidic soils in the north of England [24, 75]. Another evidence for the wide distribution of acidophilic methanotrophs is the cloning of *pmoA* genes close to *pmoA* of *Methylocapsa acidiphila* from acidic (pH 3.4–5.0) forest soils of the United States, Denmark, Brazil, Germany, and Norway [67–69]. The cloned sequences, however, were not identical to corresponding sequences of *Methylocella palustris* and *Methylocapsa acidiphila* and testified rather to the diversity of acidophilic methanotrophs than to the wide distribution of the bacteria of the genera *Methylocella* and *Methylocapsa*.

As it was shown above, neither traditional culture methods nor fatty acid analysis can be used for the detection of acidophilic methanotrophs or for determination of their population density. Much greater opportunities to cope with these tasks are provided by the methods of molecular ecology. Since *Methylocella* and *Methylocapsa* belong to the α-subclass of Proteobacteria, let us consider the main approaches used in investigations on the ecology of type II methanotrophs and the feasibility of their application to the detection of acidophilic representatives (Table 3).

Methods based on the use of PCR. This group of methods implies extraction of DNA from natural samples and PCR amplification and sequencing of the genes encoding 16S rRNA, MMO (the *pmoA* and *mmoX* genes), or methanol dehydrogenase (the *mxaF* gene) (Table 3). These methods are to be considered indirect, since they deal not with microbial cells but with DNA more or less completely extracted from a sample. These methods do not provide quantitative information; nevertheless, they are widely used to study qualitative composition of methanotrophic communities in various habitats. Unfortunately, these methods are of limited value for studies on acidophilic methanotrophs. Thus, group-specific primers designed for PCR detection of type II methanotrophs [76, 77] are inadequate for acidophilic methanotrophs, since they belong to a phylogenetic lineage separate from *Methylosinus* and *Methylocystis.* An attempt to design 16S rDNA–targeted primers specific to acidophilic methanotrophs would be premature because few representatives of this group are currently known and because the

Table 3. Molecular methods for the detection of methanotrophs belonging to the α-subclass of Proteobacteria and their applicability to acidophilic methanotrophs

Method	Target	Target group of organisms	Primer or probe	Nucleotide sequence (5'–3')	Reference	Applicability to	
						Methylocella	*Methylocapsa*
PCR amplification with specific primers	16S rDNA	Type II methanotrophs	Type 2b	CATACCGGRCATGTCAAAAGC	[76]	–	–
The same	16S rDNA	Type II methanotrophs	MethT2R	CATCTCTGRCSAYCATACCGG	[77]	–	–
The same	16S rDNA	*Methylosinus*	Ms1020r	CCCTTGCGGAAGGAAGTC	[78]	–	–
The same	*pmoA*	Type I and type II methanotrophs	A189f	GGNGACTGGGACTTCTGG	[60]	–	+
			A682r	GAASGCNGAGAAGAASGC			
The same	*pmoA*	The same	mb661	CCGGMGCAACGTCYTTACC	[76]	–	+
The same	*mmoX*	Methanotrophs possessing soluble MMO	*mmoX* 882f	GGCTCCAAGTTCAAGGTCGAGC	[59]	+	–
			*mmoX*1403r	TGGCACTCGTAGCGCTCCGGCTCG			
The same	*mmoX*	The same	A166f	ACCAAGGARCARTTCAAG	[79]	+	–
			B1401r	TGGCACTCRTARCGCTC			
The same	*mxaF*	All gram-negative methylotrophs	*mxaF*1003f	GCGGCACCAACTGGGGCTGGT	[50]	+	+
			*mxaF*1561r	GGGCAGCATGAAGGGCTCCC			
Hybridization with 16S rRNA–specific oligonucleotide probes	16S rDNA	Methylotrophs with serine pathway of C assimilation	9α	CCCTGAGTTATTCCGAAC	[80]	–	–
The same	16S rDNA	Type II methanotrophs	MA-221	GGACGCGGGCCGATCTTTCG	[81]	–	–
The same	16S rDNA	Type II methanotrophs	Mα-450	ATCCAGGTACCGTCATTATC	[82]	–	–
The same	16S rDNA	*Methylocella palustris*	Mcell-1026	GTTCTCGCCACCCGAAGT	[83]	+	–
The same	16S rDNA	*Methylocapsa acidiphila*	Mcaps-1032	CACCTGTGTCCCTGGCTC	[84]	–	+
The same	16S rDNA	*M. palustris*, *M. acidiphila*	AcidM-181	TCTTTCTCCTTGCGGACG	[83]	+	+

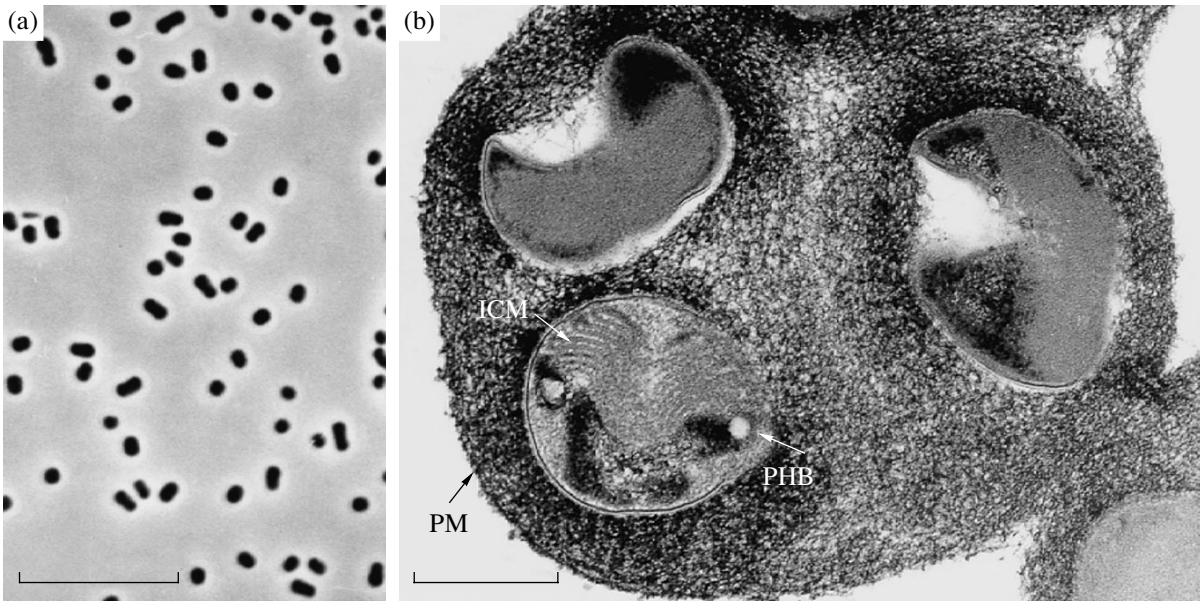

Fig. 2. The acidophilic dinitrogen-fixing methanotrophic bacterium *Methylocapsa acidiphila*, strain B2, isolated from peat of the Bakcharskoe mixed-type sedge–*Sphagnum* peat bog (Tomsk oblast, western Siberia) and able to grow in a pH range of 4.2–7.2 with an optimum at 5.0–5.5. (a) Under a phase-contrast microscope, it is seen that the short cells are surrounded with capsules. Scale bar represents 10 μm. (b) In the electron micrograph of a group of cells embedded in a dense polysaccharide matrix (PM), well-developed intracytoplasmic membranes (ICM) arranged in tightly packed bundles and granules of poly-β-hydroxybutyrate (PHB) can be seen. Scale bar represents 0.5 μm. The micrographs were taken by N.E. Suzina.

phylogenetic boundary with *Beijerinckia* representatives has not yet been drawn. The use of *pmoA* and *mmoX* genes is limited, since the *pmoA* gene is lacking from *Methylocella palustris* and the *mmoX* gene is absent in *Methylocapsa acidiphila*. The last one in this group of methods, implying the use of the *mxaF* gene as the marker, is the most universal, since this gene is present in all gram-negative methylotrophs, including *Methylocapsa* and *Methylocella*. However, this marker does not allow acidophilic methanotrophs to be differentiated from methylotrophic bacteria such as *Methylobacterium*, since the sequences of their *mxaF* genes have proven to be quite similar [63].

In situ hybridization with 16S rRNA-targeted fluorescently labeled oligonucleotide probes. The commonly accepted abbreviation for this method is FISH (fluorescent in situ hybridization). It combines the possibility of direct specific detection and the possibility of the determination of the density of the target microbial population directly in any environment, without need for cultivation [85–87]. A number of oligonucleotide probes have been designed for the detection of type I and type II methanotrophs and their individual genera [78, 81, 82]. Until recently, successful application of these probes was restricted to the analysis of mixed cultures and enrichments [78, 81]; the attempts to determine methanotroph population densities in situ failed because of the low numbers of the target cells in the substrates investigated [82]. As for the detection of acidophilic methanotrophs, neither of the earlier developed probes was suitable for these purposes (Table 3). Therefore, proceeding from the unique regions of the 16S rRNA sequences of *Methylocella palustris* and *Methylocapsa acidiphila*, we designed fluorescently labeled probes for specific detection of these bacteria. One of these probes (Mcell-1026) was specific to *Methylocella* palustris, another one (Mcaps-1032) targeted *Methylocapsa acidiphila*, and the third (AcidM-181) allowed both these bacteria to be detected [83, 84].

To gain insight into the structure of the methanotrophic community in situ, we undertook analysis of peat samples from the acidic (3.6–4.5) Bakcharskoe bog in Tver oblast [83, 84]. The set of probes used in this analysis included probes targeting acidophilic methanotrophs and also earlier known probes specific to type I and type II methanotrophs. This investigation showed that the population densities of *Methylocella palustris* and *Methylocapsa acidiphila* were rather high, comprising 10^6 and 10^5 cells/g wet peat, respectively. The total population density of type I methanotrophs was three orders of magnitude lower: 10^3 cells/g wet peat. An unexpected finding was the detection of one more (in addition to *Methylocella palustris* and *Methylocapsa acidiphila*) numerically significant (10^6 cells/g wet peat) group of methanotrophs inhabiting acidic peat. These were type II methanotrophs phylogenetically close to the genus *Methylocystis*, which, however, did not belong to any of its validated species. It is most probably this *Methylocystis* population that was discovered in the first investigations of DNA extracted from acidic peats [50, 51]. The total number of methanotrophic bacteria revealed in sphagnum peat

by using fluorescent probes was 3.0×10^6 cells/g wet peat, or 0.8% of the total number of eubacteria. In peat samples from different bogs, the latter value varied from 0.4 to 3%. It should be noted that, unlike the probes targeting type I and type II methanotrophs, the probes designed for acidophilic methanotrophs were not group-specific, but targeted two particular species, *Methylocella palustris* and *Methylocapsa acidiphila*, whereas the peat may also be inhabited by other representatives of this acidophilic group. Therefore, the actual population density of acidophilic methanotrophs may be higher than that recorded in our investigation.

5. CONCLUSION

Thus, in the methane-oxidizing filter of *Sphagnum* peat bogs of the boreal zone, three components were identified: the populations of the methanotrophs of the genera *Methylocella* and *Methylocapsa* and the yet unstudied population of methanotrophs of the genus *Methylocystis.* Further studies are needed to find out whether such a composition of the methanotrophic communities is universal for northern acidic bogs. A reliable method for broad-scale investigations is the now available method of in situ detection of acidophilic methanotrophs by hybridization with 16S rRNA–targeted fluorescently labeled oligonucleotide probes. The use of these probes for the analysis of acidophilic methanotrophic enrichments obtained earlier from *Sphagnum* peat bogs of various zones of Russian tundra (Chukotka, Vorkuta, Kolyma, and Kamchatka) [28] allowed us to detect the presence of *Methylocella palustris* cells (Dedysh *et al.*, unpublished data). Evidently, these organisms are representative of the methanotrophic communities of tundra bogs as well. However, the majority of cells in the communities studied could not be recognized by any of the currently existing methanotroph probes, including probes targeting type I and type II methanotrophs. Most probably, the methane-oxidizing filter of acidic tundra bogs includes representatives of yet unknown species or genera of acidophilic methanotrophs.

In summary, the permanently increasing number of studies confirms to the high diversity and wide distribution of acidophilic methanotrophs. Microbiologists currently have at hand only two bacteria, *Methylocella palustris* and *Methylocapsa acidiphila*, and most probably they are only a part of a large group of organisms (within type II methanotrophs) that inhabit not only *Sphagnum* peat bogs but also acidic northern soils. Further studies must fill the gaps in our knowledge on this new bacterial group and its ecological significance.

ACKNOWLEDGMENTS

I am grateful to G.A. Zavarzin (Institute of Microbiology, Russian Academy of Sciences, Moscow) for critical comments and advice on the improvement of this review and to the research group headed by Yu.A. Trotsenko (Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, Pushchino) for fruitful cooperation.

This work was supported by the State Research and Technical Program “Biogenic Sinks, Sources, and Reservoirs of Greenhouse Gases” and by the Russian Foundation for Basic Research, project no. 02-04-04003.

REFERENCES

1. Cicerone, R. and Oremland, R., Biogeochemical Aspects of Atmospheric Methane, *Global Biogeochem. Cycles*, 1988, vol. 2(4), pp. 299–327.
2. Matthews, E. and Fung, I., Methane Emissions from Natural Wetlands: Global Distribution, Area, and Environmental Characteristics of Sources, *Global Biogeochem. Cycles*, 1987, vol. 1, pp. 61–86.
3. Aselmann, I. and Crutzen, P.J., Global Distribution of Natural Freshwater Wetlands and Rice Paddies, Their Net Primary Productivity, Seasonality and Possible Methane Emissions, *J. Atmos. Chemistry*, 1989, vol. 8, pp. 307–358.
4. Hein, R., Crutzen, P.J., and Heinmann, M., An Inverse Modeling Approach to Investigate the Global Atmospheric Methane Cycle, *Global Biogeochem. Cycles*, 1997, vol. 11(1), pp. 43–76.
5. Panikov, N.S., Fluxes of CO_2 and CH_4 in High Latitude Wetlands: Measuring, Modeling and Predicting Response to Climate Change, *Polar Research*, 1999, vol. 18(2), pp. 237–244.
6. Zavarzin, G.A. and Vasil’eva, L.V., Methane Cycle on the Territory of Russia, *Krugovorot ugleroda na territorii Rossii* (Carbon Cycle on the Territory of Russia), Moscow: Izd-vo Mosk. Pravitel’stva, 1999, pp. 202–230.
7. *Land Resources of Russia. CD-ROM Database Created by International Institute for Applied Systems Analysis*, Russ. Acad. Sci., 2002.
8. Panikov, N.S., Titlyanova, A.A., Paleeva, M.V., Semenov, A.M., Mironycheva-Tokareva, N.P., Makarov, V.I., Dubinin, E.V., and Efremov, S.P., Methane Emission from Bogs of West Siberia), *Dokl. Akad. Nauk*, 1993, vol. 330(3), pp. 388–390.
9. Panikov, N.S., Sizova, M.V., Zelenev, V.V., Makhov, G.A., Naumov, A.V., and Gadzhiev, I.M., Emission of CH_4 and CO_2 from Bogs of the South of West Siberia: Spatial and Temporal Variations in the Fluxes, *Zh. Ekol. Chem.*, 1995, vol. 4, pp. 13–24.
10. Panikov, N.S., Glagolev, M.V., Kravchenko, I.K., Mastepanov, M.A., Kosykh, N.P., Mironycheva-Tokareva, N.P., Naumov, A.V., Inoue, G., and Maksyutov, Sh., Methane Emission from Ombrotrophic Bogs of West Siberia as Dependent on the Type of Vegetation Cover, *Zh. Ekol. Chem.*, 1997, vol. 6, no. 1, pp. 59–67.
11. Zavarzin, G.A., Psychrophilic Soengen Cycle, *Ekol. Khim.*, 1995, vol. 4(1), p. 3.
12. Henckel, T., Friedrich, M., and Conrad, R., Molecular Analyses of the Methane-Oxidizing Microbial Community in Rice Field Soil by Targeting the Genes of the 16S rRNA, Particulate Methane Monooxygenase, and Methanol Dehydrogenase, *Appl. Environ. Microbiol.*, 1999, vol. 66, no. 5, pp. 1980–1990.

13. Frenzel, P., Plant-Associated Methane Oxidation in Rice Fields and Wetlands, *Adv. Microb. Ecol.*, 2000, pp. 85–114.
14. Henckel, T., Jackel, U., and Conrad, R., Vertical Distribution of the Methanotrophic Community after Drainage of Rice Field Soil, *FEMS Microbiol. Ecol.*, 2001, vol. 34, pp. 279–291.
15. Eller, G. and Frenzel, P., Changes in Activity and Community Structure of Methane-Oxidizing Bacteria over the Growth Period of Rice, *Appl. Environ. Microbiol.*, 2001, vol. 67, pp. 2395–2403.
16. Namsaraev, B.B., *Microbial Interactions during Methane Oxidation, Cand. Sci. (Biol.) Dissertation*, Moscow, 1973.
17. Yavitt, J.B., Lang, G.E., and Downey, D.M., Potential Methane Production and Methane Oxidation Rates in Peatland Ecosystems of the Appalachian Mountains, United States, *Global Biogeochem. Cycles*, 1988, vol. 2, no. 3, pp. 253–268.
18. Yavitt, J.B., Downey, G.E., Lancaster, E., and Lang, G.E., Methane Consumption in Decomposing *Sphagnum*-Derived Peat, *Soil Biol. Biochem.*, 1990, vol. 22, pp. 441–447.
19. Panikov, N.S., Semenov, A.M., Tarasov, A.A., Belyaev, A.S., Kravchenko, I.K., Smagina, M.V., Palejeva, M.V., Zelenev, V.V., and Skupchenko, K.V., Methane Production and Uptake in Soils of the European Part of the USSR, *J. Ecol. Chem.*, 1993, no. 1, pp. 7–18.
20. Sundh, I., Nilsson, M., Granberg, G., and Svensson, B.H., Depth Distribution of Microbial Production and Oxidation of Methane in Northern Boreal Peatlands, *Microb. Ecol.*, 1994, vol. 27, pp. 253–265.
21. Sundh, I., Mikkela, C., Nilsson, M., and Svensson, B.H., Potential Aerobic Methane Oxidation in a *Sphagnum*-Dominated Peatland—Controlling Factors and Relation to Methane Emission, *Soil Biol. Biochem.*, 1995, vol. 27, pp. 829–837.
22. Nedwell, D.B. and Watson, A., CH_4 Production, Oxidation and Emission in a UK Ombrotrophic Peat Bog: Influence of SO_4^{2-} from Acid Rain, *Soil Biol. Biochem.*, 1995, vol. 27, pp. 893–903.
23. Krumholz, L.R., Hollenback, J.L., Roskes, S.J., and Ringelberg, D.B., Methanogenesis and Methanotrophy within a *Sphagnum* Peatland, *FEMS Microbiol. Ecol.*, 1995, vol. 18, pp. 215–224.
24. McDonald, I.R., Hall, G.H., Pickup, R.W., and Murrell, J.C., Methane Oxidation Potential and Preliminary Analysis of Methanotrophs in Blanket Bog Peat Using Molecular Ecology Techniques, *FEMS Microbiol. Ecol.*, 1996, vol. 21, pp. 197–211.
25. Prior, S.D. and Dalton, H., Acetylene as a Suicide Substrate and Active Site Probe for Methane Monooxygenase from *Methylococcus capsulatus* (Bath), *FEMS Microbiol. Lett.*, 1985, vol. 29, pp. 105–109.
26. Bedard, C. and Knowles, R., Physiology, Biochemistry and Specific Inhibitors of CH_4, NH_4^+ And CO_2 Oxidation by Methylotrophs and Nitrifiers, *Microbiol. Rev.*, 1989, vol. 53, no. 1, pp. 68–84.
27. Dedysh, S.N. and Panikov, N.S., Effect of Methane Concentration on the Rate of Its Oxidation by Bacteria in Sphagnum Peat, *Mikrobiologiya*, 1997, vol. 66, pp. 563–568.
28. Vasil'eva, L.V., Berestovskaya, Yu.Y., and Zavarzin, G.A., Psychrophilic Acidophilic Methanotrophs from Sphagnetta of Permafrost Zone, *Dokl. Akad. Nauk*, 1999, vol. 368, no. 1, pp. 125–128.
29. Dunfield, P., Knowles, R., Dumont, R., and Moore, T.R., Methane Production and Consumption in Temperate and Subarctic Peat Soils: Response to Temperature and pH, *Soil Biol. Biochem.*, 1993, vol. 25, no. 3, pp. 321–326.
30. Dedysh, S.N. and Panikov, N.S., Effect of pH, Temperature, and Concentration of Salts on Methane Oxidation Kinetics in Sphagnum Peat, *Mikrobiologiya*, 1997, vol. 66, pp. 569–573.
31. Hanson, R.S. and Hanson, T.E., Methanotrophic Bacteria, *Microbiol. Rev.*, 1996, vol. 60, no. 2, pp. 439–471.
32. Gal'chenko, V.F., *Metanotrofnye bakterii* (Methanotrophic Bacteria), Moscow: GEOS, 2001.
33. Whittenbury, R., Phillips, K.C., and Wilkinson, T.F., Enrichment, Isolation and Some Properties of Methane-Utilizing Bacteria, *J. Gen. Microbiol.*, 1970, vol. 61, pp. 205–218.
34. Bowman, J.P., Sly, L.I., and Stackebrandt, E., The Phylogenetic Position of the Family *Methylococcaceae*, *Int. J. Syst. Bacteriol.*, 1995, vol. 45, pp. 182–185.
35. Bowman, J.P., McCammon, S.A., and Skerratt, J.H., *Methylosphaera hansonii* gen. nov., sp. nov., a Psychrophilic, Group I Methanotroph from Antarctic Marine-Salinity, Meromictic Lakes, *Microbiology*, 1997, vol. 143, pp. 1451–1459.
36. Bodrossy, L., Holmes, E.M., Holmes, A.J., Kovacs, K.L., and Murrell, J.C., Analysis of 16S rRNA and Methane Monooxygenase Gene Sequences Reveals a Novel Group of Thermotolerant and Thermophilic Methanotrophs, *Methylocaldum* gen. nov., *Arch. Microbiol.*, 1997, vol. 168, pp. 493–503.
37. Wise, M.G., McArthur, J.V., and Shimkets, L.J., *Methylosarcina fibrata* gen. nov., sp. nov. and *Methylosarcina quisquiliarum* sp. nov., Novel Type I Methanotrophs, *Int. J. Syst. Evol. Microbiol.*, 2001, vol. 51, pp. 611–621.
38. Dedysh, S.N., Liesack, W., Khmelenina, V.N., Suzina, N.E., Trotsenko, Y.A., Semrau, J.D., Bares, A.M., Panikov, N.S., and Tiedje, J.M., *Methylocella palustris* gen. nov., sp. nov., a New Methane-Oxidizing Acidophilic Bacterium from Peat Bogs Representing a Novel Sub-Type of Serine Pathway Methanotrophs, *Int. J. Syst. Evol. Microbiol.*, 2000, vol. 50, pp. 955–969.
39. Dedysh, S.N., Khmelenina, V.N., Suzina, N.E., Trotsenko, Y.A., Semrau, J.D., Liesack, W., and Tiedje, J.M., *Methylocapsa acidiphila* gen. nov., sp. nov., a Novel Methane-Oxidizing and Dinitrogen-Fixing Acidophilic Bacterium from *Sphagnum* Bog, *Int. J. Syst. Evol. Microbiol.*, 2002, vol. 52, pp. 251–261.
40. Omel'chenko, M.V., Savel'eva, N.D., Vasil'eva, L.V., and Zavarzin, G.A., Psychrophilic Methanotrophic Community from Tundra Soil, *Mikrobiologiya*, 1992, vol. 61, no. 6, pp. 1072–1077.
41. Omel'chenko, M.V., Vasil'eva, L.V., Zavarzin, G.A., Savel'eva, N.D., Lysenko, A.M., Mityushina, L.L., Khmelenina, V.N., and Trotsenko, Yu.A., A Novel Psychrophilic Methanotroph of the Genus *Methylobacter*, *Mikrobiologiya*, 1996, vol. 65, no. 3, pp. 384–389.

42. Butorova, I.A., Microbial Community on Natural Gas ans Possibilities of Its Regulation, *Cand. Sci. (Biol.) Dissertation*, Leningrad, 1991.

43. Malashenko, Yu.P., Romanovskaya, V.A., and Trotsenko, Yu.A., *Metanokislyayushchie mikroorganizmy* (Methane-Oxidizing Microorganisms), Moscow: Nauka, 1978.

44. Gal'chenko, V.F., Andreev, L.V., and Trotsenko, Yu.A., *Taksonomiya i identifikatsiya obligatnykh metanotrofnykh bakterii* (Taxonomy and Identification of Obligately Methanotrophic Bacteria), Pushchino: Otd. Nauchn. Tekh. Inform. Nauchn. Tsentr Biol. Inf. Akad. Nauk, SSSR, 1986.

45. Topp, E. and Hanson, R.S., Metabolism of Radiatively Important Trace Gases by Methane-Oxidizing Bacteria, *Microbial Production and Consumption of Greenhouse Gases: Methane, Nitrogen Oxides and Halomethanes*, Rogers, J.E. and Whitman, W.B., Eds., Washington, D.C.: Am. Soc. Microbiol., 1991, pp. 71–90.

46. King, G.M., Ecological Aspects of Methane Oxidation, a Key Determinant of Global Methane Dynamics, *Adv. Microb. Ecol.*, 1992, vol. 12, pp. 431–461.

47. Heyer, J. and Suckow, R., Ecological Studies of Methane Oxidation in an Acid Bog Lake, *Limnologica*, 1985, vol. 16, pp. 247–266.

48. Murrell, J.C., McDonald, I.R., and Bourne, D.J., Molecular Methods for the Study of Methanotrophs Ecology, *FEMS Microbiol. Ecol.*, 1998, vol. 27, pp. 103–114.

49. Murrell, J.C. and Radajewski, S., Cultivation-Independent Techniques for Studying Methanotroph Ecology, *Res. Microbiol.*, 2000, vol. 151, pp. 807–814.

50. McDonald, I.R. and Murrell, J.C., The Methanol Dehydrogenase Structural Gene *mxaF* and Its Use as a Functional Gene Probe for Methanotrophs and Methylotrophs, *Appl. Environ. Microbiol.*, 1997, vol. 63, pp. 3218–3224.

51. McDonald, I.R. and Murrell, J.C., The Particulate Methane Monooxygenase Gene *pmoA* and Its Use as a Functional Gene Probe for Methanotrophs, *FEMS Microbiol. Lett.*, 1997, vol. 156, pp. 205–210.

52. Andreev, L.V. and Gal'chenko, V.F., Fatty-Acid Composition and Identification of Methanotrophic Bacteria, *Dokl. Akad. Nauk SSSR*, 1978, vol. 239, pp. 1465–1468.

53. Guckert, J.B., Ringelberg, D.B., White, D.C., Hanson, R.S., and Bratina, B.J., Membrane Fatty Acids as Phenotypic Markers in the Polyphasic Taxonomy of Methylotrophs within the Proteobacteria, *J. Gen. Microbiol.*, 1991, vol. 137, pp. 2631–2641.

54. Bowman, J.P., Sly, L.I., Nichols, P.D., and Hayward, A.C., Revised Taxonomy of the Methanotrophs: Description of the *Methylobacter* gen. nov., Emendation of *Methylococcus*, Validation of *Methylosinus* and *Methylocystis* Species, and a Proposal That the Family *Methylococcaceae* Includes Only the Group I Methanotrophs, *Int. J. Syst. Bacteriol.*, 1993, vol. 43, pp. 735–753.

55. Bowman, J.P., Skerratt, J.H., Nichols, P.D., and Sly, L.I., Phospholipid Fatty Acid and Lipopolysaccharide Fatty Acid Signature Lipids in Methane-Utilizing Bacteria, *FEMS Microbiol. Ecol.*, 1991, vol. 85, pp. 15–22.

56. Sundh, I., Borda, P., Nilsson, M., and Svensson Bo, H., Estimation of Cell Numbers of Methanotrophic Bacteria in Boreal Peatlands Based on Analysis of Specific Phospholipid Fatty Acids, *FEMS Microbiol. Ecol.*, 1995, vol. 18, pp. 103–112.

57. Sundh, I., Nilsson, M., and Borga, P., Variation in Microbial Community Structure in Two Boreal Peatlands as Determined by Analysis of Phospholipid Fatty Acid Profiles, *Appl. Environ. Microbiol.*, 1997, vol. 63, pp. 1476–1482.

58. Dedysh, S.N., Panikov, N.S., and Tiedje, J.M., Acidophilic Methanotrophic Communities from *Sphagnum* Peat Bogs, *Appl. Environ. Microbiol.*, 1998, vol. 64, pp. 922–929.

59. McDonald, I.R., Kenna, E.M., and Murrell, J.C., Detection of Methanotrophic Bacteria in Environmental Samples with the PCR, *Appl. Environ. Microbiol.*, 1995, vol. 61, pp. 116–121.

60. Holmes, A.J., Costello, A., Lidstrom, M.E., and Murrell, J.C., Evidence That Particulate Methane Monooxygenase and Ammonia Monooxygenase May Be Evolutionarily Related, *FEMS Microbiol. Lett.*, 1995, vol. 132, pp. 203–208.

61. Shigematsu, T., Hanada, S., Eguchi, M., Kamagata, Y., Kanagawa, T., and Kurane, R., Soluble Methane Monooxygenase Gene Clusters from Trichloroethylene-Degrading *Methylomonas* sp. Strains and Detection of Methanotrophs during In Situ Bioremediation, *Appl. Environ. Microbiol.*, 1999, vol. 65, pp. 5198–5206.

62. Dedysh, S.N., Liesack, W., Horz, H.-P., and Tiedje, J., A Novel Methane-Oxidizing Strain from Methanotrophic Community Capable of Growth at pH 4, *Abstr. Kongress der VAAM Mikrobiologie 2000*, 2000, Munchen, Germany, 12–16.III, p. 147.

63. Dedysh, S.N., Horz, H.-P., Dunfield, P.F., and Liesack, W., A Novel *pmoA* Lineage Represented by the Acidophilic Methanotrophic Bacterium *Methylocapsa acidiphila* B2, *Arch. Microbiol.*, 2001, vol. 177, pp. 117–121.

64. Dedysh, S.N., Panikov, N.S., Liesack, W., Grokopf, R., Zhou, J., and Tiedje, J.M., Isolation of Acidophilic Methane-Oxidizing Bacteria from Northern Peat Wetlands, *Science*, 1998, vol. 282, pp. 281–284.

65. Conti, S.F. and Hirsch, P., Biology of Budding Bacteria: III. Fine Structure of *Rhodomicrobium* and *Hyphomicrobium* spp., *J. Bacteriol.*, 1965, vol. 89, pp. 503–512.

66. Oelze, J. and Drews, G., Membranes of Photosynthetic Bacteria, *Biochim. Biophys. Acta*, 1971, vol. 265, pp. 209–239.

67. Holmes, A.J., Roslev, P., McDonald, I.R., Iversen, N., Henriksen, K., and Murrell, J.C., Characterization of Methanotrophic Bacterial Populations in Soils Showing Atmospheric Methane Uptake, *Appl. Environ. Microbiol.*, 1999, vol. 65, pp. 3312–3318.

68. Henckel, T., Jackel, U., Schnell, S., and Conrad, R., Molecular Analyses of Novel Methanotrophic Communities in Forest Soil Oxidizing Atmospheric Methane, *Appl. Environ. Microbiol.*, 2000, vol. 66, pp. 1801–1808.

69. Jensen, S., Holmes, A.J., Olsen, R.A., and Murrell, J.C., Detection of Methane Oxidizing Bacteria in Forest Soil by Monooxygenase PCR Amplification, *Microb., Ecol.*, 2000, vol. 39, pp. 282–289.

70. Dalton, H., Methane Oxidation by Methanotrophs: Physiological and Mechanistic Implications, *Methane*

and Methanol Utilizers, Murrell, J.C. and Dalton, H., Eds., New York: Plenum, 1992, pp. 85–114.

71. Semrau, J.D., Zolandz, D., Lidstrom, M.E., and Chan, S.I., The Role of Copper in the pMMO of *Methylococcus capsulatus* (Bath): a Structural Vs Catalytic Function, *J. Inorg. Biochem.*, 1995, vol. 58, pp. 235–244.
72. Murrell, J.C., Gilbert, B., and McDonald, I.R., Molecular Biology and Regulation of Methane Monooxygenase, *Arch. Microbiol.*, 2000, vol. 173, pp. 325–332.
73. Stanley, S.H., Prior, S.D., Leak, D.J., and Dalton, H., Copper Stress Underlies the Fundamental Change in Intracellular Location of Methane Mono-Oxygenase in Methane-Oxidizing Organisms: Studies in Batch and Continuous Cultures, *Biotechnol. Lett.*, 1983, vol. 5, pp. 487–492.
74. Richardson, C.J., Biogeochemical Cycles: Regional, *Wetlands and Shallow Continental Water Bodies*, Patten, B.C., Ed., Hague: SPB Acad., 1990, pp. 259–279.
75. Radajewski, S., Ineson, P., Parekh, N.R., and Murrell, J.C., Stable-Isotope Probing as a Tool in Microbial Ecology, *Nature* (London), 2000, vol. 403, pp. 646–649.
76. Costello, A. and Lidstrom, M.E., Molecular Characterization of Functional and Phylogenetic Genes from Natural Populations of Methanotrophs in Lake Sediments, *Appl. Environ. Microbiol.*, 1999, vol. 65, pp. 5066–5074.
77. Wise, M.G., McArthur, J.V., and Shimkets, L.J., Methanotroph Diversity in Landfill Soil: Isolation of Novel Type I and Novel Type II Methanotrophs Whose Presence Was Suggested by Culture-Independent 16S Ribosomal DNA Analysis, *Appl. Environ. Microbiol.*, 1999, vol. 65, pp. 4887–4897.
78. Holmes, A.J., Owens, N.J.P., and Murrell, J.C., Detection of Novel Marine Methanotrophs Using Phylogenetic and Functional Gene Probes after Methane Enrichment, *Microbiology (UK)*, 1995, vol. 141, pp. 1947–1955.
79. Auman, A.J., Stolyar, S., Costello, A.M., and Lidstrom, M.E., Molecular Characterization of Methanotrophic Isolates from Freshwater Lake Sediment, *Appl. Environ. Microbiol.*, 2000, vol. 66, pp. 5259–5266.
80. Tsien, H.C., Bratina, B.J., Tsuji, K., and Hanson, R.S., Use of Oligodeoxynucleotide Signature Probes for Identification of Physiological Groups of Methylotrophic Bacteria, *Appl. Environ. Microbiol.*, 1990, vol. 56, pp. 2858–2865.
81. Bourne, D.G., Holmes, A.J., Iversen, N., and Murrell, J.C., Fluorescent Oligonucleotide rDNA Probes for Specific Detection of Methane Oxidizing Bacteria *FEMS Microbiol. Ecol.*, 2000, vol. 31, pp. 29–38.
82. Eller, G., Stubner, S., and Frenzel, P., Group Specific 16S rRNA Targeted Probes for the Detection of Type I and Type II Methanotrophs by Fluorescence In Situ Hybridisation, *FEMS Microbiol. Lett.*, 2001, vol. 198, pp. 91–97.
83. Dedysh, S.N., Derakshani, M., and Liesack, W., Detection and Enumeration of Methanotrophs in Acidic *Sphagnum* Peat by 16S rRNA Fluorescence In Situ Hybridization, Including the Use of Newly Developed Oligonucleotide Probes for *Methylocella palustris*, *Appl. Environ. Microbiol.*, 2001, vol. 67, pp. 4850–4857.
84. Dedysh, S.N., Dunfield, P.F., Derakshani, M., Stubner, S., Heyer, J., and Liesack, W., Differential Detection of Type II Methanotrophic Bacteria in Acidic Peatlands Using Newly Developed 16S rRNA-targeted Fluorescent Oligonucleotide probes, *FEMS Microbiol. Ecol.*, 2002 (accepted).
85. Amann, R.I., Krumholz, L., and Stahl, D.A., Fluorescent-Oligonucleotide Probing of Whole Cells for Determinative, Phylogenetic, and Environmental Studies in Microbiology, *J. Bacteriol.*, 1990, vol. 172, pp. 762–770.
86. Amann, R.I., Ludwig, W., and Schleifer, K.-H., Phylogenetic Identification and In Situ Detection of Individual Microbial Cells without Cultivation, *Microbiol. Rev.*, 1995, vol. 59, pp. 143–169.
87. Moter A. and Gobel, U.B., Fluorescence In Situ Hybridization (FISH) for Direct Visualization of Microorganisms, *J. Microbiol. Methods*, 2000, vol. 41, pp. 85–112.