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EXPERIMENTAL ARTICLES

Abundance and Diversity of Methanotrophic *Gammaproteobacteria* in Northern Wetlands

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Abstract—Numeric abundance, identity, and pH preferences of methanotrophic *Gammaproteobacteria* (type I methanotrophs) inhabiting the northern acidic wetlands were studied. The rates of methane oxidation by peat samples from six wetlands of European Northern Russia (pH 3.9–4.7) varied from 0.04 to 0.60 μg CH_4 g^{-1} peat h^{-1}. The number of cells revealed by hybridization with fluorochrome labeled probes M84 + M705 specific for type I methanotrophs was 0.05–2.16 × 10^5 cells g^{-1} dry peat, i.e., 0.4–12.5% of the total number of methanotrophs and 0.004–0.39% of the total number of bacteria. Analysis of the fragments of the *pmoA* gene encoding particulate methane monooxygenase revealed predominance of the genus *Methylocystis* (92% of the clones) in the studied sample of acidic peat, while the proportion of the *pmoA* sequences of type I methanotrophs was insignificant (8%). PCR amplification of the 16S rRNA gene fragments of type I methanotrophs with TypeIF–Type IR primers had low specificity, since only three sequences out of 53 analyzed belonged to methanotrophs and exhibited 93–99% similarity to those of *Methylovulum, Methylomonas*, and *Methylobacter* species. Isolates of type I methanotrophs obtained from peat (strains SH10 and 83A5) were identified as members of the species *Methylomonas paludis* and *Methylovulum miyakonense,* respectively. Only *Methylomonas paludis* SH10 was capable of growth in acidic media (pH range for growth 3.8–7.2 with the optimum at pH 5.8–6.2), while *Methylovulum miyakonense* 83A5 exhibited the typical growth characteristics of neutrophilic methanotrophs (pH range for growth 5.5–8.0 with the optimum at pH 6.5–7.5).

Keywords: northern bog ecosystems, methanotrophic bacteria, fluorescent in situ hybridization, *Gammaproteobacteria, Methylomonas, Methylovulum*

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Northern wetlands are among the major natural sources of the greenhouse gas methane [1]. Methane emission from these ecosystems into the atmosphere is controlled by aerobic methanotrophic bacteria inhabiting the upper layers of the wetland profile. The studies of recent decades made it possible to reveal that methanotrophic *Alphaproteobacteria* (type II methanotrophs) belonging to the families *Methylocystaceae* and *Beijerinckiaceae* are the main component of the "methane-oxidizing filter" of northern acidic wetlands [2–4]. Bacteria of the genera *Methylocistis, Methylocapsa, Methylocella*, and *Methyloferula* isolated from the wetlands were characterized as moderately acidophilic organisms capable of methane oxidation in cold acidic environments [5–9].

However, the occurrence of methanotrophic *Gammaproteobacteria* (type I methanotrophs) in acidic wetlands, as well as their possible contribution to methane oxidation, remains an unresolved issue. Analysis of acidic peat using fluorescent in situ hybridization (FISH) demonstrated low abundance of type I methanotrophs (not more than several per cents of the total number of methanotrophs) [2, 10]. Nevertheless, the 16S rRNA and *pmoA* gene sequences exhibiting similarity to those of *Methylobacter* and *Methylomonas* have been repeatedly retrieved from the total DNA extracts obtained from *Sphagnum* peat [3, 11–14]. Since acidophilic representatives have not been found among type I methanotrophs until recently, interpretation of their detection in acidic wetlands by molecular methods remained difficult. The first evidence for the presence of acidophilic type I methanotrophs was obtained by Dutch scientists who isolated two strains of *Methylomonas*- and *Methylovulum*-like bacteria from acidic peat [15]. Unfortunately, these isolates have been only partially characterized. We have recently isolated the first acid-tolerant *Methylomonas* species from *Sphagnum* peat bogs, *M. paludis*. While it is able to grow at pH 3.8–5.0, its optimal growth occurs at higher pH (5.8–6.4) [16], casting doubt upon the role of these organisms in methane oxidation in acidic wetlands.

The goal of the present study was to elucidate the abundance and species diversity of type I methanotrophs in acidic *Sphagnum* peat bogs. We also aimed to obtain the representative isolates of these bacteria and to examine their pH preferences.

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MATERIALS AND METHODS

Objects of the study. The peat samples collected in June–July, 2011–2012, at the oxic–anoxic interface (depth of 5–10 cm) of the peat bog profiles were used in the study. The samples were collected from the Shumnovo and Tarlakovskii Mokh peat bogs (Tver oblast), Obukhovskoye peat bog (Yaroslavl oblast), as well as from three peat bogs in Arkhangelsk oblast: Sholya, Solza, and Tsiglomen (table). The studied ecosystems were typical oligotrophic bogs of the boreal and tundra zones of Russia, with pH of the peat water 3.9–4.2. The Shumnovo peat bog bordered with the burnt forest site, and pH of the peat water increased up to 4.7 because of the introduction of an ash component. The samples were transported to the laboratory in refrigerated packs and were immediately used for determination of the methane-oxidizing activity in the peat, as well as for fixation for the FISH analysis and for DNA extraction.

Determination of the rate of methane oxidation by the peat samples. The samples (10 g of wet peat) were cut into 10–15-mm fragments, placed into 160-mL hermetically sealed sterile glass vials, and then methane was injected up to ~1000 ppm concentration. The vials were incubated at a room temperature. From the headspace the samples (0.5 mL) were taken regularly; methane concentration was determined using a Kristall 5000 chromatograph (Chromateck, Russia) with the flame ionization detector. The measurements were continued until methane in the vials was completely consumed. Using the obtained results, the rate of methane oxidation by the studied peat samples was calculated.

Fluorescent in situ hybridization (FISH). Wet peat (2 g) was resuspended in 10–20 mL of sterile distilled water and treated in a homogenizer for 10 min. An aliquot (0.5 mL) of the resulting suspension was fixed with 4% formaldehyde solution in phosphate buffer (NaCl, 8.0 g; KCl, 0.2 g; Na_2HPO_4, 1.44 g; NaH_2PO_4, 0.2 g; H_2O, 1 L; pH 7.0) for 1.5 h. Then the sample was centrifuged and washed with phosphate buffer. The fixed samples were resuspended in a mixture of 100% of ethanol and phosphate buffer (1 : 1, vol/vol) and stored at –20°C prior to analysis. The fixed suspension (1–2 μL was applied to the wells on the microscope slide and hybridization with the probes was carried out at 46°C, as described previously [17]. The number of cells of type I methanotrophs was determined by hybridization with an equimolar mixture of Cy3-labeled probes M705 + M84, and the number of cells of type II methanotrophs, with the probe M450 [18]. The total bacterial cell number was determined by hybridization with an equimolar mixture of Eub338-mix probes [19]. The Cy3-labeled probes were synthesized by Syntol (Moscow, Russia). The preparations were analyzed using a Zeiss Axioplan 2 epifluorescence microscope (Carl Zeiss, Jena, Germany) with the filters Zeiss 20 for Cy3-labeled probes and Zeiss 02 for detection of cell autofluorescence.

Assessment of methanotroph diversity in the peat by PCR-based analysis of the 16S rRNA and *pmoA* genes. The total DNA was extracted from the peat using the FastDNA SPIN kit for soil (Biol 101, United States), according to the manufacturer's recommendations. Obtained DNA was used as a template for the PCR. General diversity of methanotrophs in the peat was evaluated by the PCR amplification of the *pmoA* gene fragments using the primers A189f and A682r [20]. The *pmoA* gene encodes the polypeptide containing the active center of the particulate methane monooxygenase (pMMO). Diversity of methanotrophic *Gammaproteobacteria* in the samples was determined using the primer systems typeIF–typeIR and MethT1dF–MethT1bR that specifically amplify the 16S rRNA gene fragments (~660 and 900 bp, respectively) of type I methanotrophs [21, 22]. The DNA from *Methylomonas paludis* $MG30^T$ was used as a positive control. The PCR was performed on a PE GeneAmp PCR System 9700 thermocycler (Perkin-Elmer Applied Biosystems, United States). The PCR products were separated in 1.2% agarose gel, stained with ethidium bromide, and visualized using a UV-transilluminator. The amplicons were cloned using the pGem-T Easy Vector System II Kit (Promega), according to the manufacturer's recommendations. Recombinant clones were selected by amplification of the cloned fragments with the vector-specific primers T7 and SP6. Isolation and purification of the plasmid DNA were carried out using the Wizard® Plus Minipreps DNA Purification System Kit (Promega). Sequencing was performed on an ABI 377A sequencer (Perkin-Elmer Applied Biosystems, United States).

Editing of the nucleotide sequences was carried out using the SeqMan software package (Laser Gene 7.0; DNA Star Package). The nucleotide sequences were analyzed using the BLAST network server [http://www.blast.ncbi.nlm.nih.gov]. Phylogenetic trees were constructed using the ARB software package [http://www.arb-home.de]. The significance levels of interior branch points were calculated using the Phylip software package by bootstrap analysis, constructing 1000 alternative trees.

The obtained *pmoA* and 16S rRNA sequences of peat-inhabiting methanotrophs were submitted to GenBank under accession numbers KF543822–KF543864.

Cultivation and analysis of enrichment cultures of methanotrophic bacteria. Enrichment cultures of methanotrophs were obtained using the modified liquid mineral N medium [15] containing the following (g/L): K_2HPO_4, 0.01; KNO_3, 0.02; $MgSO_4 \cdot 7H_2O$, 0.02; $CaCl_2 \cdot 2H_2O$, 0.02; NaCl, 0.03; 1.5 mL of trace elements solution for methanotrophs [23], pH 6.0–6.3. Peat (1 g) was added to the 500-mL vials containing 50–100 mL of the liquid medium described above.

Methane oxidation rates and methanotroph cell numbers in the studied wetlands

Bog, location, sampling time	pH	Bog type	Vegetation composition	Methane oxidation rate, μg/g/h	Number of cells revealed by hybridization with the probes for bacteria (EUB338-mix) and type I (M705 + M84) and type II (M450) methanotrophs, per g of wet peat		
					EUB338-mix ($N \times 10^8$)	M705 + M84 ($N \times 10^5$)	M450 ($N \times 10^6$)
Solza, Arkhangelsk oblast, 64°48′ N, 39°56′ E 07.2012	3.9	Oligotrophic	*Sphagnum fuscum, Eriophorum latifolium, Drosera rotundifolia, Oxycoccus palustris, Vaccinium uliginosum, Rubus chamaemorus, Carex* spp., *Pinus silvestris*	0.25	4.09 ± 1.53	0.96 ± 0.32	2.02 ± 1.54
Tsiglomen, Arkhangelsk oblast, 64°52′ N, 40°32′ E 07.2012	3.9	Oligotrophic	*Sph. fuscum, E. latifolium, D. rotundifolia, Empetrum nigrum, O. palustris, V. myrtillus, V. uliginosum, R. chamaemorus, Carex* spp., *Andromeda polifolia, Betula pendula*	0.47	0.64 ± 0.10	0.96 ± 0.31	2.13 ± 1.24
Sholya, Arkhangelsk oblast, 64°53′ N, 40°16′ E 07.2012	4.1	Oligotrophic	*Sph. fuscum, Sph. angustifolium, V. uliginosum, R. chamaemorus, C. calyculata, A. polifolia, P. silvestris, Equisetum fluviatile*	0.04	1.12 ± 0.16	0.05 ± 0.03	1.18 ± 0.84
Shumnovo, Tver oblast, 56°42′ N, 36°52′ E 06.2012	4.7	Oligotrophic, in the region of a burnt forest	*Sph. angustifolium, Carex* spp., *P. silvestris, B. pendula, Eq. fluviatile, A. polifolia, Vaccinium* sp.	0.23	3.38 ± 0.86	0.57 ± 0.23	2.46 ± 1.51
Tarlakovskii Mokh, Tver oblast, 56°37′ N, 36°52′ E 06.2012	3.9	Oligotrophic	*Sph. angustifolium, Sph. fuscum, Carex* spp., *Oxicoccus* sp., *Vaccinium* sp.	0.20	0.54 ± 0.08	2.16 ± 0.73	1.73 ± 0.92
Obukhovskoye, Yaroslavl oblast, 57° N, 39° E 06.2011	4.2	Oligotrophic	*Sph. angustifolium, Sph. fuscum, Carex* spp., *Oxicoccus* sp., *A. polifolia*	0.60	7.23 ± 1.72	1.01 ± 0.42	21.40 ± 5.60

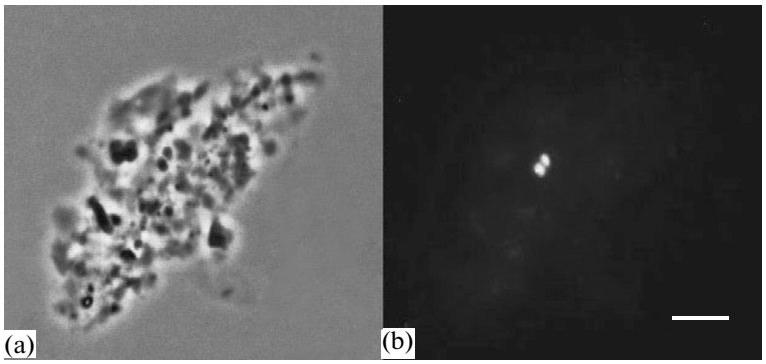

Fig. 1. Detection of the cells of type I methanotrophs in the microbial community of a *Sphagnum* peat sample from Shumnovo bog: the peat conglomerate with absorbed microbial cells, phase contrast microscopy (a) and fluorescent micrograph of hybridization with the mixture of Cy3-labeled probes M705 + M84 which specifically detect the cells of type I methanotrophs (b). Scale bar, 5 μm.

Methane (10–20 vol %) was injected into the gaseous phase, and the vials were incubated with or without shaking (120 rpm) at 20°C for 2–3 weeks.

Type I methanotrophs in these enrichment cultures were revealed using FISH, by hybridization of the cell suspensions with an equimolar mixture of the probes M84 + M705 (see above).

Isolation of methanotrophs and determination of their pH optima. Aliquots of the enrichment cultures in which type I methanotrophs have been detected were spread plated onto the modified N medium with agar-agar (1.5%) or phytagel (0.9%) as the gelling agents. Screening for the presence of the colonies formed by type I methanotrophs was performed by hybridization of the cell material from these colonies with the probes M84 + M705. The colonies selected were subjected to repeated transfers to obtain pure cultures of methanotrophs. Phylogenetic affiliation of the obtained isolates was determined by the 16S rRNA genes PCR amplification using the primers 9f and 1492r [24] with subsequent comparative analysis of the sequences.

Further cultivation of the obtained isolates was carried out in liquid M2 medium (pH 5.0–5.5) containing the following (g/L): K_2HPO_4, 0.1; $MgSO_4 \cdot 7H_2O$, 0.05; KNO_3, 0.15; $CaCl_2 \cdot 2H_2O$, 0.01; NaCl, 0.02, 1.0 mL of trace elements solution for methanotrophs. Methane (up to 25 vol %) was injected into the gaseous phase as the carbon source, and then the vials were incubated with or without shaking (120 rpm). Optimal growth conditions were determined in the range of temperatures from 4 to 37°C. The dependence of growth on pH was investigated in batch cultures grown in liquid M2 medium by measuring a specific growth rate at a given pH value. The pH values varied from 3.5 to 8.5. Acidity of the medium was adjusted by mixing of 0.1 M of H_3PO_4, KH_2PO_4, K_2HPO_4, and K_3PO_4 solutions without changing the ionic strength. Growth was determined by the measurement of optical density using an Eppendorf Biophotometer AG at 600 nm.

RESULTS

Methane oxidation rates in the *Sphagnum* peat samples. The rate of methane oxidation by peat samples from the studied peat bogs varied from 0.04 to 0.6 μg CH_4 per 1 g of wet peat per 1 hour (table). The highest methane-oxidizing activity was registered in the peat from Obukhovskoye peat bog (pH 4.2). No direct correlation was found between methane oxidation rates and pH of peat water. For example, the rate of methane oxidation by the peat from Shumnovo peat bog (pH 4.7) was similar to that of more acidic peat bogs Solza (pH 3.9) and Tarlakovskii Mokh (pH 3.9).

Differential enumeration of type I and type II methanotrophs in the peat. Analysis of the fixed peat samples by in situ hybridization with fluorescently labeled probes M84 + M705 specific for type I methanotrophs revealed only single cells of the target organisms (Fig. 1). The abundance of type I methanotrophs in the studied peat bogs was low and varied within the range of $0.05–2.16 \times 10^5$ cells/g of wet peat. The maximal cell number was registered for the peat from Tarlakovskii Mokh bog (pH 3.9) (table). The abundance of type II methanotrophs detected in the samples using the probe M450 ($1.18–21.4 \times 10^6$ cells/g of wet peat) was several orders of magnitude higher than population density of the methanotrophic *Gammaproteobacteria*. Total number of bacterial cells that were detected by hybridization with the probes EUB338-mix in the peat from the studied bogs was $0.54–7.23 \times 10^8$ cells/g of wet peat. The proportion of type I and II methanotrophs was 0.52–3.60%. These data are in accordance with the previously published results on the abundance of methanotrophs in *Sphagnum* peat bogs [2, 10].

Composition of the methanotrophic community based on the *pmoA* gene analysis. For more detailed analysis of methanotroph diversity in *Sphagnum* peat, the DNA isolated from the Shumnovo bog peat sample was used. The PCR products obtained using the

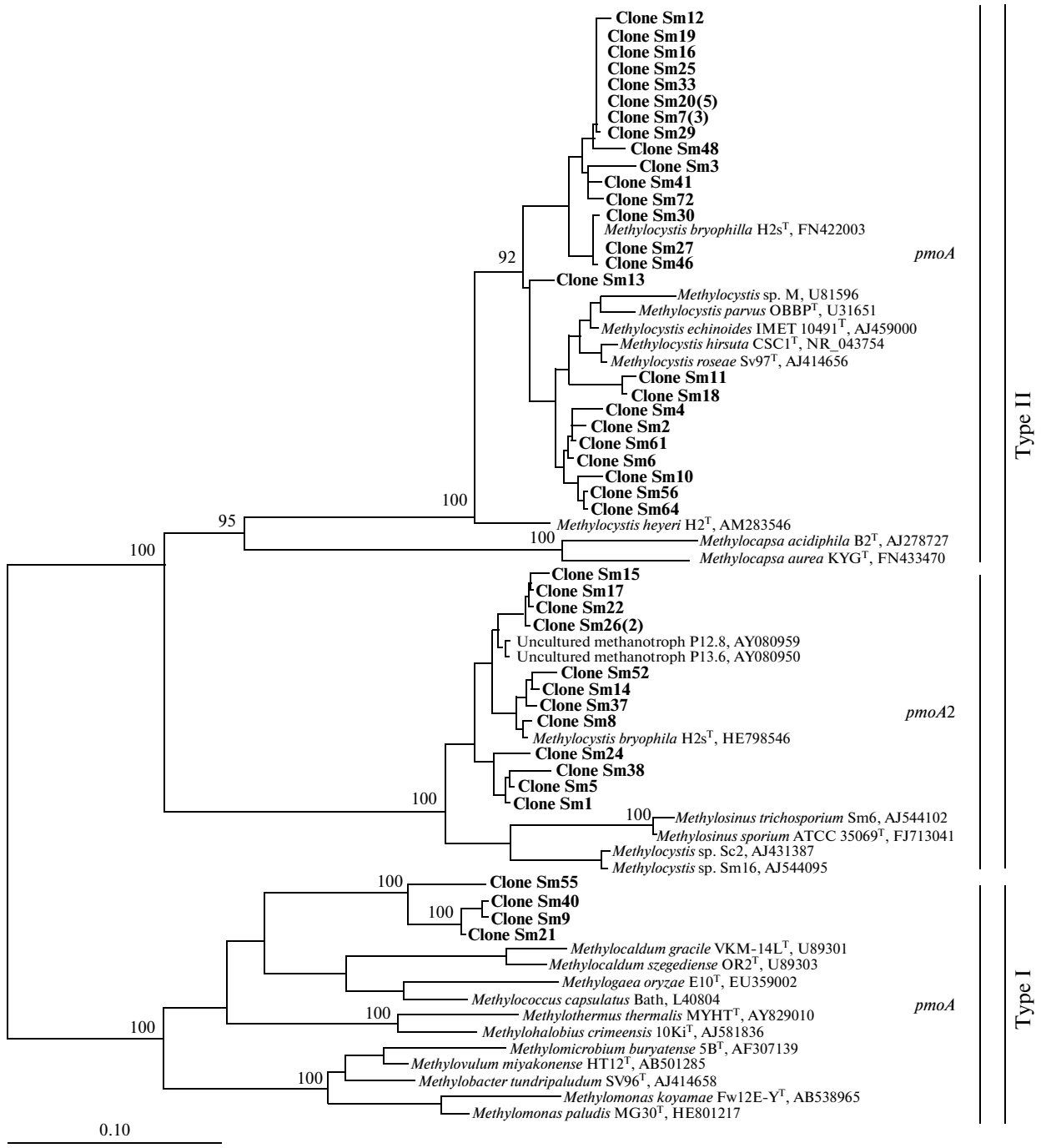

Fig. 2. Phylogenetic tree constructed based on comparative analysis of translated 48 amino acid sequences of the PmoA fragments obtained from the Shumnovo *Sphagnum* peat bog and of some known type I and type II methanotrophs. Scale bar corresponds to 0.1 substitutions per one amino acid position.

primer system A189f–A682r were cloned and sequenced. In total, 52 clones were obtained, of which four were the *amoA* gene (coding for ammonium monooxygenase) sequences and were excluded from further analysis. The remaining 48 sequences represented the particulate methane monooxygenase (pMMO) genes, most of them (44 sequences or ~92% of all clones) belonging to the genus *Methylocystis* (Fig. 2). Of these, 28 clones represented the conventional *pmoA* gene, and 16 other clones, the *pmoA2* gene coding for the pMMO with high affinity to methane [25]. A significant part of these sequences showed high similarity to and formed common clusters with the *pmoA* and *pmoA2* genes from *Methylocystis bryo-*

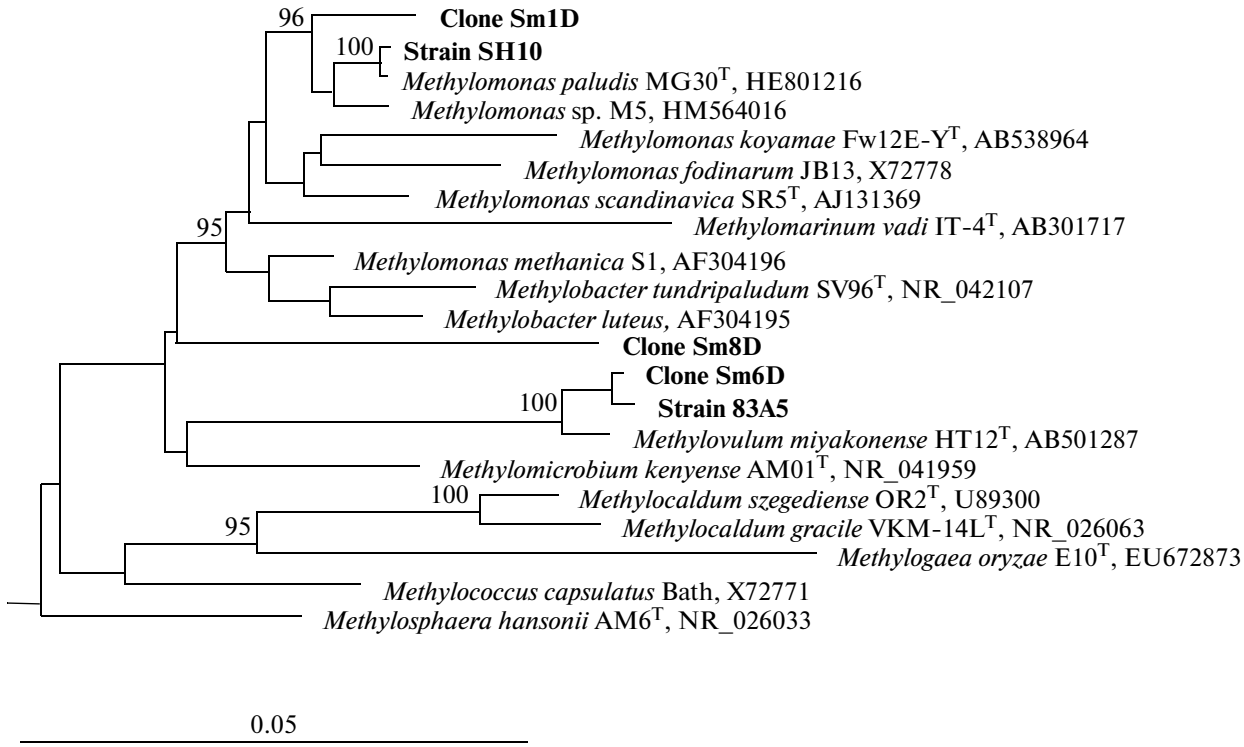

Fig. 3. Phylogenetic tree constructed based on comparative analysis of the cloned nucleotide sequences of the 16S rRNA genes of type I methanotrophs retrieved from the Shumnovo *Sphagnum* peat bog and of some characterized methanotrophic *Gammaproteobacteria*. The 16S rRNA nucleotide sequences from *Methylocapsa acidiphila* B2 (AJ278726), *Methyloferula stellata* AR4^T (FR686343), *Methylocella silvestris* BL2 (NR_075606), *Methylosinus sporium* (Y18946), *Methylocistis parvis* (Y18945), and *Methylosinus trichosporium* OB3b (Y18947) were used as an outgroup. Scale bar corresponds to 0.05 substitutions per one nucleotide position.

phila H2s^T, a typical inhabitant of ombrotrophic peat bogs [9]. Type I methanotrophs were represented by four cloned *pmoA* sequences (8% of all clones) that formed a compact and phylogenetically distinct cluster (Fig. 2). These *pmoA* sequences showed a distant relationship (66–84% of amino acid sequence identity) to those from known methanotrophic *Gammaproteobacteria* and, most likely, represented a new genus of methanotrophs. The *pmoA* sequences from this group exhibited highest similarity (93–95%) to the environmental clone sequences retrieved from wetlands and sediments of freshwater lakes (KC817710, AY488074, AY488072, FN597134).

Assessment of type I methanotroph diversity using the 16S rRNA gene analysis. To confirm the presence of type I methanotrophs forming a defined phylogenetic cluster within the *Methylococcaceae*, we obtained the 16S rRNA gene clone library of these bacteria using the primer system typeIF–typeIR. This library contained 53 cloned 16S rRNA gene fragments, although only three of them (6% of all clones) belonged to type I methanotrophs. Two sequences represented the genera *Methylomonas* and *Methylovulum* (the clones Sm1D and Sm6D, respectively) (Fig. 3). The third cloned sequence, Sm8D, formed a separate, genus-level phylogenetic lineage and displayed low similarity (87–93%) with the 16S rRNA gene sequences from the known type I methanotrophs. Other 50 cloned 16S rRNA genes sequences obtained by PCR with the primers type IF–type IR affiliated with non-methanotrophic *Gammaproteobacteria* from the genera *Legionella, Coxiella, Steroidobacter*, and *Pseudomonas* (24 clones), *Acidobacteria* (16 clones), and some representatives of the *Firmicutes* (10 clones). Apparently, this primer system had low specificity and was not applicable for the analysis of type I methanotroph diversity in wetlands. We made an attempt to use another primer system (MethT1dF and MethT1bR) to detect type I methanotrophs, which also proved inefficient, since the PCR product was not obtained with these primers and the DNA extracts from peat.

Isolation of methanotrophic *Gammaproteobacteria* from *Sphagnum* peat bogs. Although FISH revealed low cell number of type I methanotrophs in the *Sphagnum* peat, we attempted to isolate these microorganisms. Experiments with enrichment cultures using M2 and modified N media showed that the latter was more efficient for the cultivation of type I methanotrophs. The presence of methanotrophic *Gammaproteobacteria* in the enrichment cultures obtained on this

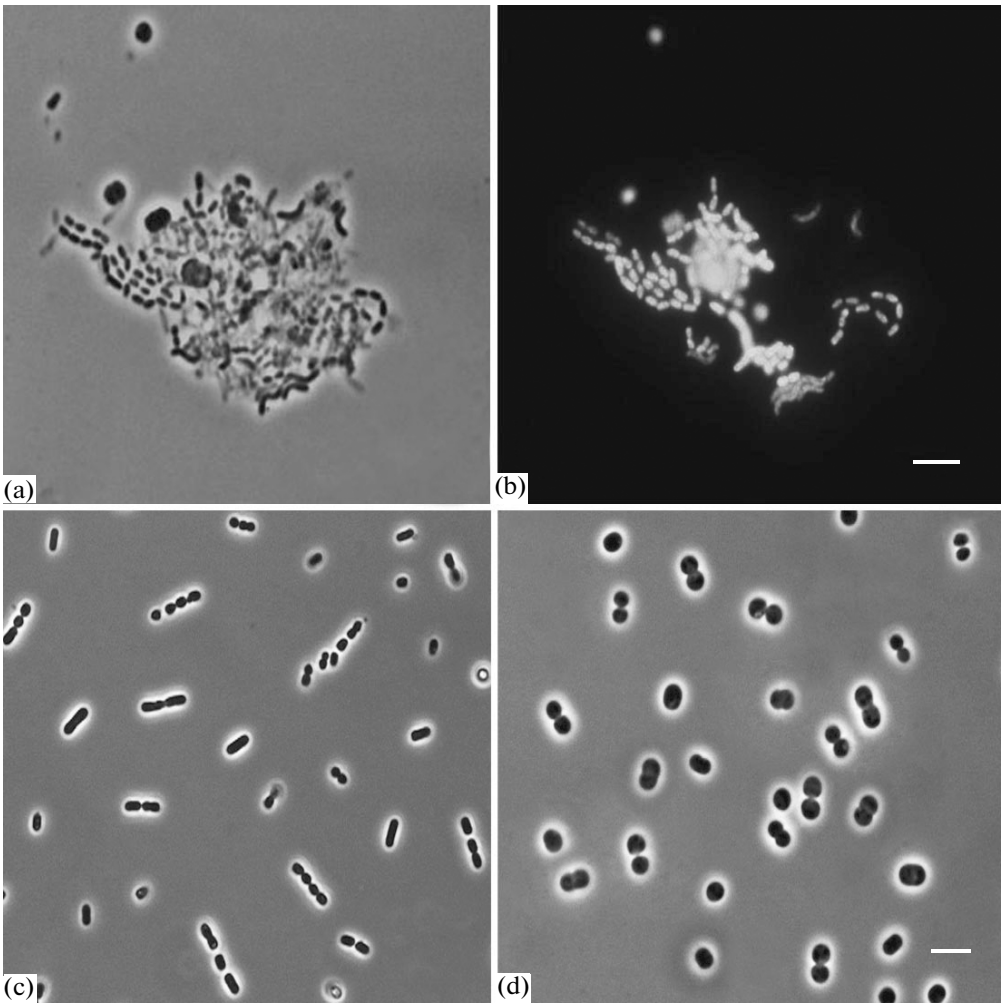

Fig. 4. Identification of type I methanotrophs in an enrichment culture obtained from a peat sample of the Shumnovo bog by FISH and cell morphology of the obtained isolates: phase contrast microscopy of the cells from enrichment culture (a); fluorescent micrograph of hybridization with the probes M84 + M705, specific for type I methanotrophs (b); morphology of the cells of strains SH10 and 83A5, respectively (c and d). Scale bar, 5 μm.

medium was confirmed by in situ hybridization with the probes M84 + M705 (Figs. 4a, 4b). The cells that were targeted with the probes were of two morphotypes: rods in chains and large cocci. Two strains with similar cell morphology, SH10 and 83A5, were subsequently isolated (Figs. 4c, 4d). Analysis of the 16S rRNA gene sequence of strain SH10 showed its identity (100% similarity) with that of the type strain of the peat-inhabiting methanotroph *Methylomonas paludis* MG30^T (HE801216), which was described recently [16]. Identification of the strain 83A5 demonstrated its affiliation with the species *Methylovulum miyakonense* (99% 16S rRNA gene similarity) isolated previously from a forest soil [26].

Characteristics of the isolates of type I methanotrophs from peat bogs. The cells of strain SH10 were short, gram-negative rods of 0.8–1.5 × 1.0–3.5 μm covered with a mucous sheath (Fig. 4c). On solid medium this methanotroph formed spreading mucous pale-pink colonies. The cells of strain 83A5 were large cocci, 1.5–3.5 μm in diameter, with less pronounced capsules (Fig. 4d). On solid medium they formed large compact mucous pink colonies. Both isolates used methane and methanol as the sources of carbon and energy and grew in the range of temperatures from 10 to 30°C. Analysis of the growth rates of these methanotrophs at various pH values revealed a significant difference between strains 83A5 and SH10 with regard to their response to the medium acidity. *Methylomonas paludis* SH10 was able to grow at low pH values (4.2–4.5) with the growth optimum at pH 5.5–6.0. *Methylovulum miyakonense* 83A5, in contrast, could not grow at pH below 5.5 and had the growth optimum at pH 6.5–7.0 (Fig. 5). Thus, only one of the isolated type I methanotrophs had the features of a moderately acidophilic microorganism.

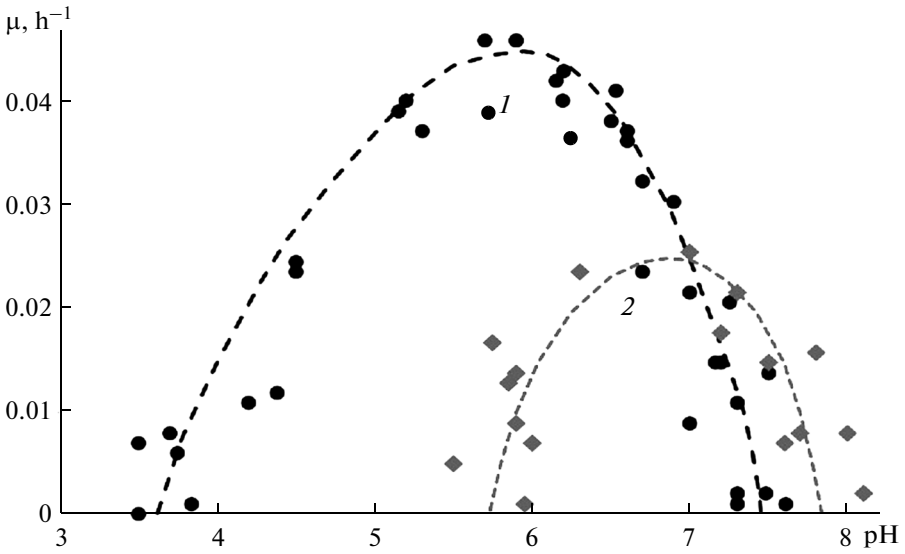

Fig. 5. Influence of medium pH on specific growth rates (μ) of strains SH10 (*1*) and 83A5 (*2*).

DISCUSSION

Our results confirm the previously published data on low abundance of type I methanotrophs in *Sphagnum* peat bogs [2–4, 10, 11]. This fact was confirmed by both FISH results and the relatively small proportion of the *pmoA* sequences from methanotrophic gammaproteobacteria in the clone libraries that were obtained from *Sphagnum*-derived peat and sequenced by Sanger. However, few recent assessments of methanotroph diversity in the *Sphagnum* peat bogs of the Netherlands and Austria, obtained by ultra-deep pyrosequencing of the *pmoA* genes, are in certain contradiction with our results [13, 14, 27]. These authors reported that up to 50% of the *pmoA* sequences identified in the peat belonged to type I methanotrophs, predominantly to *Methylomonas* species. None of these studies, however, employed some alternative method, for example, FISH analysis, to confirm high abundance of methanotrophic *Gammaproteobacteria*. Thus, interpretation of these data may be equivocal. Some *Sphagnum* peat bogs in countries with high population density (such as the Netherlands or Austria) are possibly characterized by higher abundance of type I methanotrophs due to higher concentrations of mineral nutrients in the peat water. On the other hand, pyrosequencing could have introduced some bias in the quantitative assessment of the proportion of different methanotroph groups within the methane-oxidizing community of the studied ecosystems. Both these suggestions, however, need further experimental verification.

Type I methanotrophs capable of growth in acidic peat bogs are scarcely studied. Acidophilic representatives of methanotrophic *Gammaproteobacteria* were unknown until recently. In 2011 Dutch microbiologists isolated several representatives of type I methanotrophs from *Sphagnum* peat [15], and in 2013 we described the first acid-tolerant species of the genus *Methylomonas*, *M. paludis* [16]. In this study we obtained one more isolate of this species, strain SH10. Characteristics of this strain are identical to those given in the description of *M. paludis*. Representatives of this species are able to grow in the media with low pH values (~4.0–4.5), although the growth optimum lies in the range of pH 5.5–6.5; the organism is therefore rather acid-tolerant than acidophilic. Nevertheless, detection of these methanotrophs in the peat by both molecular and cultivation methods suggested their wide occurrence within the acidic wetlands.

The other strain obtained in this work, 83A5, was identified as *Methylovulum miyakonense* [26] and, like the type strain of this species, did not grow at pH values lower than 5.5. Isolation of this methanotroph from the *Sphagnum* peat may be explained only by the possibility of its local growth in some weakly acidic or near-neutral micro-niches within the bog profile. Such organisms probably do not play any important role in methane oxidation in acidic peat bogs.

The *pmoA* gene sequences that were retrieved from the studied peat samples (clones Sm9, Sm21, Sm40, and Sm55) and belong to a phylogenetically separated, genus-level lineage within the *Gammaprotepbacteria* are of special interest. Analysis of the 16S rRNA genes of type I methanotrophs also revealed one sequence (clone Sm8D) divergent from those of the known methanotrophs. The differences were also at the genus level. While available data are insufficient for conclusion whether these *pmoA* and 16S rRNA sequences belong to the same organism, this possibility can not be excluded. The search for potentially novel

methanotrophs and their study are of significant scientific interest.

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