ISSN 0026-2617, Microbiology, 2011, Vol. 80, No. 4, pp. 549–557. © Pleiades Publishing, Ltd., 2011.
Original Russian Text © I.S. Kulichevskaya, S.E. Belova, V.T. Komov, S.N. Dedysh, G.A. Zavarzin, 2011, published in Mikrobiologiya, 2011, Vol. 80, No. 4, pp. 543–551.

EXPERIMENTAL ARTICLES

Phylogenetic Composition of Bacterial Communities in Small Boreal Lakes and Ombrotrophic Bogs of the Upper Volga Basin

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Received September 23, 2010

Abstract—Fluorescent in situ hybridization (FISH) with rRNA-specific oligonucleotide probes was used to assess the numbers and phylogenetic diversity of prokaryotic microorganisms in the water of small boreal lakes and peatland catchments of the swampy upper Volga basin. The abundance of bacterioplankton in lake water was found to vary from 1.6 to 8.7 × 10^6 cells ml^{-1}, with the highest values detected in neutral eutrophic lakes. The total cell numbers in the peat of ombrotrophic bogs were 3.9-4.3 × 10^8 cells g^{-1} of wet peat. The proportion of bacteria identified by the group-specific probes decreased from 79–85% in neutral (pH 6.6–6.9) mesotrophic and eutrophic lakes to 65–69% in acidic (pH 4.4–5.5) dystrophic lakes and to 51–58% in the peat of acidic (pH 3.6–3.9) ombrotrophic bogs. The diversity of bacterial communities was highest in lakes with neutral water. These communities were dominated by members of the phylum *Actinobacteria* (31–44% of the total bacterial number), while the contribution of *Alphaproteobacteria* (16–19%), *Bacteroidetes* (6–16%), *Betaproteobacteria* (6–7%), *Planctomycetes* (2–8%), and *Gammaproteobacteria* (4–5%) was also significant. In acidic dystrophic lakes, *Actinobacteria* (25–35%) and *Betaproteobacteria* (25–34%) predominated, while peatland catchments were dominated by the *Alphaproteobacteria* (20–23%). The presence of acidobacteria and some planctomycetes common for bogs in the water of acidic dystrophic lakes, as well as the high proportion of bacteria (31–49%) that were not identified by the group-specific probes, suggest the impact of microbial processes in peatland catchments on the microbial composition of the receiving waters.

Keywords: dystrophic lakes, *Sphagnum* peat bogs, prokaryotic microorganisms, FISH, *Actinobacteria*, *Acidobacteria, Planctomycetes.*

DOI: 10.1134/S0026261711040114

Ombrotrophic *Sphagnum* peat bogs of the watersheds, as well as swampy forests, are sources of watercourse formation in the level landscape of the Russian boreal zone. Peatlands of the catchment areas determine the hydrological cycle of the north-flowing rivers and are an important component of the hydrological network. Microbial transformation of organic matter in these peatlands results in the formation of dark, oligotrophic waters enriched with stable humic compounds. These waters, together with the surface flow, feed the numerous lakes and rivers of this region. The microbiological processes occurring in the bogs have a decisive effect on the chemical composition of the water [1–3]. Big lakes, due to their economic importance for the most densely populated part of Russia, have been a subject of thorough investigation, including microbiological research. Small and medium-sized lakes, the feeding of which is mostly atmospheric (ombrotrophic) by rain and by the water inflow from the autonomous ombrotrophic bogs, have received less attention. The communities of ombrophilic microorganisms [4] formed in the low-mineral water of such lakes are still insufficiently studied. In particular, the effect of bog waters feeding small lakes on the formation of the lake microbial community remains unclear.

The goal of the present work was comparative analysis of bacterial communities in small forest lakes and autonomous *Sphagnum* peat bogs of the boggy upper Volga basin.

MATERIALS AND METHODS

Study sites. Research was carried out in the upper Volga basin, where comparative limnological and hydrochemical investigation of small lakes of different trophic status and ombrotrophic bogs of the catchment areas has been carried out in summer 2006–2008. They included:

1. Two humic lakes of different trophic status located in the Darwin State Preserve (Vologda oblast) in the southern part of the peninsula between the Mologa and the Sheksna reaches of the Rybinsk reservoir, namely, the neutral eutrophic Lake Khotavets and the acidic dystrophic Lake Dubrovskoe. Over 40% of the area of this preserve is occupied by *Sphagnum* peat bogs and boggy forests, while the lakes are often

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surrounded by peatlands [5]. The limnological and hydrochemical characteristics of these lakes have been thoroughly investigated by scientists from the Papanin Institute for Biology of Inland Waters, Russian Academy of Sciences [2, 6]. The feeding of these lakes depends mostly on rainfall and on water inflow from boggy catchments. An ombrotrophic *Sphagnum* peat bog typical of northwestern European Russia located 50 m south of Lake Dubrovskoe was used for comparison. The bog is forested by pine (*Pinus silvestris*), while the soil is covered by *Sphagnum* mosses and low shrub (*Ledum palustre, Andromeda polipholia, Oxycoccus quadripetalus*).

2. The ecologically contrasting Lake Bragino and Lake Lesnoe located in the Valdai national park (Novgorod oblast) in the northwestern part of the Valdai Hills. The ombrotrophic bog chosen for investigation was located along the Lake Lesnoe shore and surrounded by birch–pine forest. *Sphagnum angustifolium, Sph. fuscum, Carex* sp., *Oxycoccus* sp., and *Vaccinium* sp. prevailed in the ground cover.

Investigation techniques. The water was sampled from the surface layers of the lakes (0.2–0.5 m depth). The values of pH, temperature, and conductivity were determined at the sampling site. The water color and its hydrochemical characteristics were determined in the analytical laboratory of the Papanin Institute for Biology of Inland Waters, Russian Academy of Sciences, according to the standard procedures [7]. The ionic composition of the water was determined by gas–liquid chromatography on a Staier ion chromatograph equipped with a conductometric detector.

For investigation of the phylogenetic structure of bacterioplankton by fluorescent in situ hybridization (FISH), the water samples were fixed at the sampling site with 100% ethanol (1 : 1 vol/vol) and stored at –20°C prior to analysis. Peat samples collected from the nearby bogs at the water depth (5–10 cm) were used for comparison. Peat samples for FISH analysis were fixed as described earlier [8, 9].

For determination of the cell numbers and composition of bacterioplankton, the fixed samples (1–2 ml) were filtered onto membrane filters (Millipore, 0.22 μm pore diameter). The filters were cut into eight to ten sectors that were used for hybridization with probes specific to different groups of prokaryotes. For the members of the domain *Bacteria*, an equimolar mixture of three probes—EUB338, EUB-338-II, and EUB338-III—was used (EUB338-mix), while the Arch915 probe was used for archaeal cells. More detailed identification of bacteria was achieved using the group-specific probes for the *Alphaproteobacteria* (ALF1b + ALF968), *Betaproteobacteria* (BET42a), *Gammaproteobacteria* (GAM42a), *Bacteroidetes* (CF319a + CFB560), *Actinobacteria* (HGC69a), *Planctomycetes* (PLA46 + PLA886), and *Acidobacteria* (HoAc1402). The nucleotide sequences of these probes and the hybridization conditions used were described in our previous publications [8, 9]. The peat-inhabiting planctomycetes *Schlesneria paludicola* and *Singulisphaera acidiphila* were detected with the probes Shl-129 and Sin-644 + Syn-588 developed in [10]. The Cy3-labeled oligonucleotide probes were synthesized by Syntol (Moscow, Russia).

After hybridization, the preparations were stained for 5 min with 0.5 μM solution of a DNA-specific stain DAPI (4',6'-diamidino-2-phenylindole), washed with distilled water, and air-dried. The preparations were examined under a Zeiss Axioplan 2 (Jena, Germany) epifluorescence microscope with Zeiss 20 and Zeiss 02 filters for Cy3-labeled probes and DAPI, respectively. The target microbial populations were enumerated by counting the hybridized cells in 50–100 fields of view with subsequent calculation of the cell number per 1 ml of water or 1 g of wet peat.

Cultivation and identification of bacteria from the water of dystrophic lakes. The actinobacterial strain NK10 was isolated by plating the water sample from the dystrophic Lake Dubrovskoe on tenfold diluted standard R2A agar medium (Difco). The planctomycete isolate L1 was obtained by plating of a water aliquot from the same lake on the complex M31 agar medium with ampicillin, which has been previously used for isolation of peat-inhabiting planctomycetes [10, 11]. The isolates were identified by partial 16S rRNA gene sequencing (~500 bp), as was described previously [10–12]. The samples for scanning electron microscopy were prepared according to the standard procedure [12].

RESULTS

Limnological and physicochemical characterization of the water. The major limnological and hydrochemical characteristics of the investigated lakes and bogs are listed in Tables 1 and 2. Due to the differences of the respective catchment areas, the lakes differed in pH and the chemical composition of water, as well as in the content of humic compounds. The water of boreal lakes with peatland catchments had low mineralization, pH, and conductivity. Intense coloration of water in these lakes indicated high content of humic compounds, which were exported from the boggy catchment area. The water of neutral eutrophic lakes was collected mostly from fens and swampy forests [2] and was enriched with calcium and magnesium leached from the catchment area.

Total microbial cell numbers. The numbers of microbial cells revealed by DAPI staining in the lakes of different trophic status varied from 1.6 to 8.7 × 10^6 cells ml^{-1}; the highest values were found for the neutral eutrophic lakes (Table 1). The number of cells hybridized with the universal bacterial probes was 1.5–2.0 × 10^6 cells ml^{-1} (35–55% of the total number of DAPI-stained cells (Table 3). The number of archaeal cells was lower by an order of magnitude and varied

Table 1. Characterization of the investigated lakes and *Sphagnum* peat bogs of the Upper Volga basin

Study sites	Lakes and bogs	Trophic status	Area, km^2	Depth, m	pH	Water color, Pt-Co	Conductivity, $\mu S\ cm^{-1}$	C_{org}, $mg\ l^{-1}$	Total N, $mg\ l^{-1}$	Total P, $mg\ l^{-1}$	Chl *a*, $\mu g\ l^{-1}$	Total cell number (DAPI)***
Darwin State Preserve, 58°10′N, 37°33′E	Lake Khotavets	Neutral eutrophic	1.6*	1.9*	6.6	143	30	21.4	1.97	0.20	40.7	5.15 ± 0.31
	Lake Dubrovskoe	Acidic dystrophic	0.2*	1.1*	4.4	177	20	34.1	0.64	0.04	5.37	3.89 ± 0.35
	Ombrotrophic bog 1	Acidic oligotrophic	–	–	3.9	550	50	98.6	1.54**	0.04**	–	3.99 ± 0.54
Valdai national park, 57°45′N, 33°10′E	Lake Bragino	Neutral mesotrophic	1.5	2.4	6.9	170	70	15.2	2.3	0.059	16.3	8.72 ± 0.83
	Lake Lesnoe	Acidic dystrophic	0.8	4.2	5.5	170	10	15.4	2.1	0.160	8.5	3.13 ± 0.25
	Ombrotrophic bog 2	Acidic oligotrophic	–	–	3.6	220	70	142.4	8.7	0.354	–	4.0 ± 0.55

Notes: * From Lazareva and Komov, 1998.;
** V.I. Lazareva, unpublished data.;
*** Cell number $N \times 10^6$ per ml lake water and $N \times 10^8$ per 1 g wet peat.

Table 2. Chemical composition (mg l^{-1}) of the water of investigated lakes and ombrotrophic peat bogs

Lakes and bogs		pH	Cl^-	SO_4^{2-}	NO_3^-	PO_4^{3-}	Total anions	Na^+	K^+	NH_4^+	Mg^{2+}	Ca^{2+}	Total cations
Darwin State Preserve	Lake Khotavets	6.6	0.90	0.80	0.20	0.00	1.90*	0.50	0.10	0.10	11.00	11.67	23.37
	Lake Dubrovskoe	4.4	1.00	1.20	0.70	0.00	2.90	0.84	0	0.00	0.50	1.30	1.90
	Sphagnum peat bog 1	3.9	0.70	1.30	0.03	0.02	2.05	0.30	0.50	0.00	0.40	4.50	5.70
Valdai national park	Lake Bragino	6.9	0.95	1.30	0.14	0.00	2.25	1.46	0.90	0.00	2.09	9.83	14.28
	Lake Lesnoe	5.5	1.23	0.81	0.00	0.00	2.04	0.41	0.79	0.00	0.29	1.67	3.6
	Sphagnum peat bog 2	3.6	2.66	1.38	0.30	0.41	4.04	1.00	1.83	0.44	0.18	0.57	4.02

* HCO_3^- concentration is 22.45 mg l^{-1}.

Table 3. Cell numbers in lake water and peat bog revealed by hybridization with oligonucleotide probes specific for the individual groups of prokaryotes

Area of investigation	Objects	Cell number determined by hybridization with group-specific probes ($N \times 10^6$ cells ml^{-1} water and $N \times 10^8$ cells g^{-1} wet peat)								
		EUB338-mix	Arch915	ALF1b	BET42a	GAM42a	HGC69a	CF319a + CFB560	PLA46 + 886	HoAc1402
Darwin State Preserve	Lake Khotavets	1.96 ± 0.31	0.08 ± 0.02	0.30 ± 0.05	1.14 ± .02	0.09 ± 0.01	0.85 ± 0.01	0.13 ± 0.01	0.15 ± 0.02	0.0004 ± .0002
	Lake Dubrovskoe	1.50 ± 0.11	0.06 ± 0.03	0.03 ± 0.004	0.39 ± 0.08	0.14 ± 0.01	0.54 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.04 ± 0.01
	Sphagnum peat bog 1	1.48 ± 0.33	0.23 ± 0.06	0.36 ± 0.08	0.12 ± 0.03	0.03 ± 0.01	0.08 ± 0.02	0.13 ± 0.03	0.08 ± 0.02	0.07 ± 0.01
Valdai national park	Lake Bragino	2.02 ± 0.11	0.05 ± 0.02	0.36 ± 0.05	0.12 ± 0.02	0.11 ± 0.04	0.63 ± 0.01	0.32 ± 0.02	0.05 ± 0.01	0.002 ± 0.001
	Lake Lesnoe	1.72 ± 0.23	0.04 ± 0.01	0.03 ± 0.007	0.58 ± 0.02	0.01 ± 0.003	0.44 ± 0.01	0.04 ± 0.01	0.1 ± 0.03	0.003 ± 0.001
	Sphagnum peat bog 2	1.5 ± 0.30	0.29 ± 0.04	0.29 ± 0.04	0.08 ± 0.02	0.04 ± 0.01	0.03 ± 0.02	0.04 ± 0.02	0.08 ± 0.03	0.05 ± 0.02

from 0.4 to 0.8 × 10^5 cells ml^{-1} (1–2.5% of the total cell number).

In the peat samples collected at the water level, the number of microorganisms was 3.9–4.3 × 10^8 cells g^{-1} wet peat. About 35% of these cells hybridized with EUB338-mix. The number of archaea in peat was lower by an order of magnitude (2.3–2.9 × 10^7 cells g^{-1} wet peat) (Table 3).

Phylogenetic structure of bacterial communities. The total proportion of bacteria identified with the group-specific probes applied in this study was 79–85% in the water of neutral mesotrophic and eutrophic lakes, 65–69% in the water of acidic dystrophic lakes, and only 51–58% in the peat of acidic ombrotrophic bogs. Thus, the highest proportion of unidentified organisms was detected in acidic and oligotrophic ecosystems (Fig. 1).

The structure of bacterial communities in the neutral eutrophic lakes Khotavets and Bragino exhibited the highest diversity. These communities were dominated by members of the phylum *Actinobacteria* (31–44% of the total bacterial number), while *Alphaproteobacteria* (16–19%), *Bacteroidetes* (6–16%), *Betaproteobacteria* (6–7%), *Planctomycetes* (2–8%), and *Gammaproteobacteria* (4–5%) were also present in significant numbers. Most cells revealed in the water of acidic dystrophic lakes Dubrovskoe and Lesnoe belonged to the *Actinobacteria* (25–35%) and *Betaproteobacteria* (25–34%). Other phylogenetic groups of bacteria were numerically insignificant. The numbers of the *Alphaproteobacteria*, *Gammaproteobacteria, Bacteroidetes*, and *Planctomycetes* in dystrophic lakes were lower than in eutrophic ones. The structure of bacterial populations in the bogs of the catchment areas was different from that of lake water. *Alphaproteobacteria* (20–23% of the total number of the cells hybridizing with EUB338-mix) was the most numerous group of prokaryotes, while *Actinobacteria* constituted only ~5%.

In contrast to lake water, microbial community in the peatlands had a significant proportion of the *Aci-*

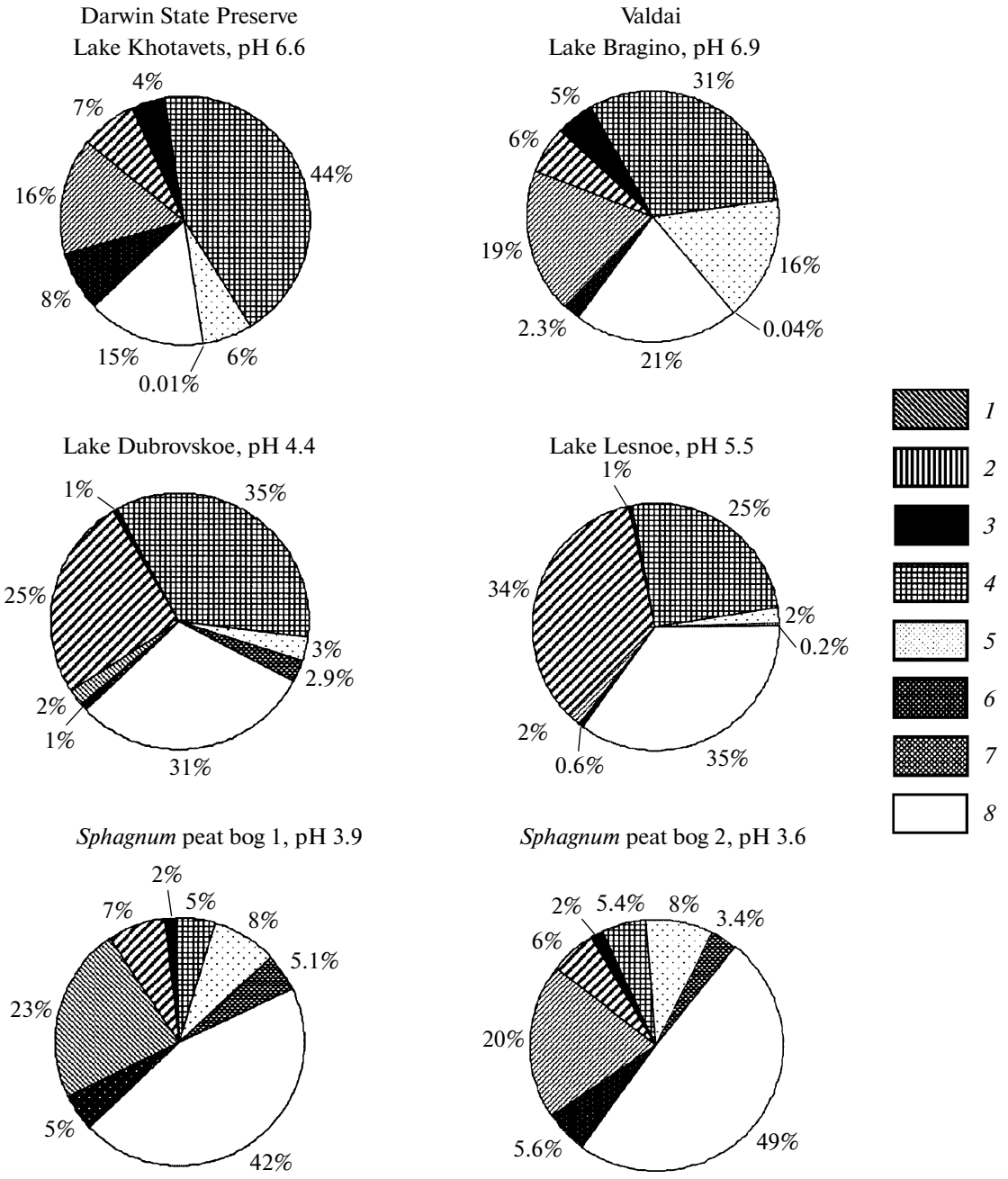

Fig. 1. Composition of bacterial communities in small lakes of different trophic status and *Sphagnum* peat bogs of the catchment areas: *Alphaproteobacteria (1), Betaproteobacteria (2), Gammaproteobacteria (3), Actinobacteria (4), Bacteroidetes (5), Planctomycetes (6), Acidobacteria (7)*, and cells unidentified by the probes (*8*).

dobacteria, which are among the most characteristic peat-inhabiting microorganisms. Practically no acidobacteria were revealed in the water of the lakes Khotavets, Bragino, and Lesnoe, with water pH above 5.0. However, in the more acidic Lake Dubrovskoe (pH 4.4), some cell aggregates of acidobacteria were revealed with the probe HoAc1402 (Figs 2a, 2b). Detection of peat-inhabiting planctomycetes *Schlesneria paludicola* (Figs 2b, 2d) and *Singulisphaera acidiphila* in the water of Lake Dubrovskoe was another evidence for the impact of peatland catchments on the composition of microbial communities in the nearby lakes.

Isolation of some bacterial cultures from the water of dystrophic lakes. Microscopy with the fluorescently labeled probes revealed small C-shaped cells as the predominant actinobacterial morphotype in the water of dystrophic lakes. One bacterium of a similar mor-

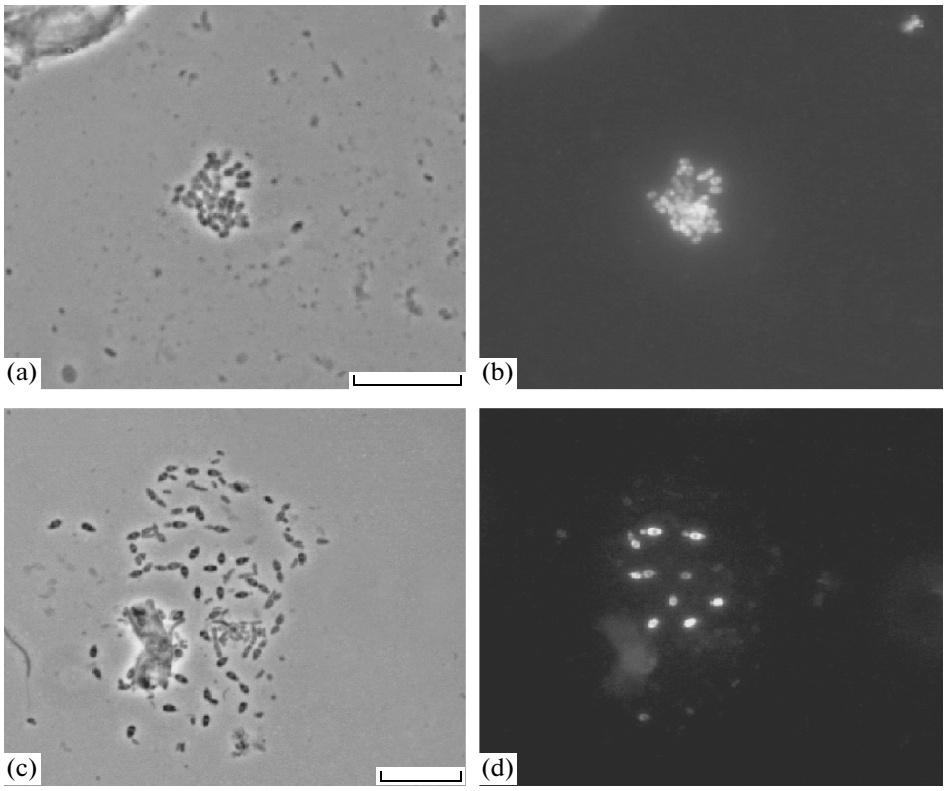

Fig. 2. Specific detection of acidobacteria (a, b) and peat-inhabiting planctomycetes (c, d) in the water of dystrophic Lake Dubrovskoe: phase contrast (a, c), epifluorescence microscopy with the probe HoAc1402 specific for the *Acidobacteria* (b), and epifluorescence microscopy with the probe Shl-129 specific for planctomycetes *Schlesneria paludicola* (c). Scale bars, 10 μm.

phology was isolated from the water of Lake Dubrovskoe and designated isolate NK10 (Fig. 3a). Analysis of 16S rRNA gene sequences of the isolate identified it as one of the chemoorganotrophic nitrate-reducing members of the family *Microbacteriaceae*, *Leifsonia aquatica* (98% similarity).

Detection of peat-inhabiting planctomycetes in the water of dystrophic lakes was confirmed by their isolation in pure culture. For example, a new planctomycete strain L1 was isolated from the water of Lake Dubrovskoe (Fig. 3b). Analysis of the 16S rRNA gene sequence of this isolate revealed 99% similarity to the corresponding gene sequences of the recently described planctomycetes *Singulisphaera acidiphila* [12].

DISCUSSION

Prior to the mid-1990s, the structure of bacterial populations in freshwater habitats was considered taxonomically similar to that of the prokaryotes in adjacent terrestrial ecosystems [13, 14]. This conclusion was drawn from the results of cultivation-based studies, which reveal only a minor fraction of the microbial diversity in natural environments. Since plating of water samples underestimates *Actinobacteria*, they have been traditionally considered typical soil organisms. Accumulation of data on microbial diversity obtained by molecular techniques, which make it possible to identify microorganisms in environmental samples without cultivation, resulted, however, in a drastic change of our understanding of the component structure of microbial populations in various ecosystems, including freshwater ones [15]. Analysis of 16S rRNA gene sequences revealed considerable similarity in the composition of bacterioplankton in geographically remote natural water bodies [16]. Members of *Actinobacteria* and *Betaproteobacteria* were found to predominate in all these ecosystems, while *Alphaproteobacteria*, *Gammaproteobacteria, Bacteroidetes,* and *Verrucomicrobia* were revealed as minor components [16, 17]. Interestingly, 16S rRNA gene sequences of bacteria from freshwater environments form compact clusters, which do not contain any clones from marine of soil ecosystems. Most of these "typically freshwater" clusters have no cultured members.

Predomination of *Actinobacteria* and *Betaproteobacteria* was also found in the eutrophic and dystrophic lakes investigated in the present work. Both in neutral and acidic waters, actinobacteria were among the most numerous groups of bacterioplankton. Detailed analysis of their phylogenetic position was not among the goals of the present work. While up to four phylogenetic clusters (acI–acIV) are presently

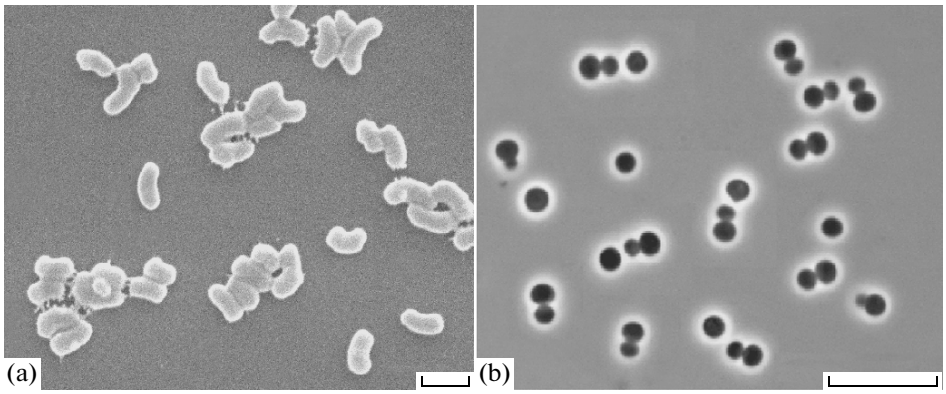

Fig. 3. Cell morphology of actinobacterial isolate NK10 (a) and planctomycete L1 (b) isolated from the water of dystrophic Lake Dubrovsloe: scanning electron microscopy, scale bar, 1 μm (a), phase contrast, scale bar, 10 μm (b).

distinguished among "freshwater" actinobacteria [20], the cultures of the representative organisms have been isolated only for two of them, acI and acII [19–21]. These are characterized by small size (cell volume ≤ 0.1 μm^3) and C-shaped cells. Such bacterial morphotypes were shown to be more resistant to protozoan grazing [19]. These actinobacteria were difficult to cultivate and did not grow in the absence of microbial satellites. They were therefore described as candidates for the new genus- and species-level taxa: *"Candidatus Planktoluna difficilis," "Candidatus Aquiluna rubr*," "*Candidatus Flaviluna lacus*," *"Candidatus Rhodoluna limnophila*," *"Candidatus Rhodoluna planctonica*," *"Candidatus Rhodoluna lacicola*," *"Candidatus Limnoluna rubra*," and *"Candidatus Planctophila limnetica*" [20, 21]. Apart from *"Candidatus Planctophila limnetica*," all of them belong to the family *Microbacteriaceae*. Although the isolate NK10 obtained from the water of acidic Lake Dubrovskoe has similar cell morphology and belongs to the same family, it does not belong to the acII cluster of actinobacteria. Since a group-specific probe was used in the present work, it was not possible to determine the proportion of NK10-like organisms among actinobacteria.

The *Betaproteobacteria*, which were especially numerous in the water of acidic dystrophic lakes (Fig. 1), also had small cells. This correlates well with the results of other studies, which showed predominance of betaproteobacterial sequences in the 16S rRNA gene clone libraries obtained from humic acidic boreal lakes [22–24]. The water of such lakes has a high content of humic substances, which are resistant to microbial degradation, while a small fraction consisting of sugars and organic acids [25] is available to microorganisms. *Betaproteobacteria* were the first to react to the addition of available organic matter to the water of a dystrophic lake, which was evident from a drastic increase in their cell numbers [22, 23].

The present work demonstrated that the phylogenetic structure of prokaryotic microbial communities in the water of boreal lakes varying in their trophic status differs significantly from that found in the peat bogs of the catchment areas. The latter are dominated with the *Alphaproteobacteria* and are characterized by significant cell numbers of the *Acidobacteria* and *Planctomycetes*. This correlates with the results of an earlier molecular analysis of the bacterial diversity in a *Sphagnum* peat bog [8]. Bog water from swampy catchments feeds the boreal lakes located among the peatlands with the surface flow. While *Actinobacteria* and *Betaproteobacteria* predominate in such lakes, the typical peat-inhabiting acidobacteria, as well as planctomycetes *Schlesneria paludicola* and *Singulisphaera acidiphila*, were also found [11, 12]. The latter were revealed by both FISH analysis and by the isolation of the new planctomycete strain L1, which was identified as *S. acidiphila*. Thus, acidobacteria and planctomycetes act as markers of bog waters feeding a lake. The degree of this external influence on formation of bacterioplankton populations in lakes depends, however, significantly on the water exchange rates [26]. A short-term cycle of complete water exchange is known to be typical of the small lakes of the boreal zone [27]. In such lakes, the export of microbial cells, together with allochthonous organic matter from the boggy catchments, is a significant factor affecting the structure of bacterioplankton [26]. On the contrary, in big lakes with low rates of water exchange, the structure of microbial communities depends mainly on the indigenous autochthonous processes in the water column [27].

These results extend the present knowledge of bacterially mediated processes involved in formation of oligotrophic waters in the boggy catchments that are common in northern Russia. They may be used for prediction of the ecological consequences of extreme natural and anthropogenic impacts.

ACKNOWLEDGMENTS

The authors are grateful to V.I. Lazareva, E.S. Gusev, and N.V. Lobus (Papanin Institute for Biology of Inland Waters, Russian Academy of Sciences) for organization and thorough technical assistance during complex field research in the Upper Volga watercatchment areas.

The work was supported by the "Environmental and Climatic Changes: Global Catastrophes" and "Molecular and Cell Biology" programs of the Presidium of the Russian Academy of Sciences, Russian Foundation for Basic Research (project no. 09-04-00004), and the Russian Ministry of Education and Science (contract no. 02.740.11.0023).

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