ISSN 0026-2617, Microbiology, 2013, Vol. 82, No. 1, pp. 91–101. © Pleiades Publishing, Ltd., 2013.
Original Russian Text © V.V. Parfenova, A.S. Gladkikh, O.I. Belykh, 2013, published in Mikrobiologiya, 2013, Vol. 82, No. 1, pp. 94–105.

EXPERIMENTAL ARTICLES

Comparative Analysis of Biodiversity in the Planktonic and Biofilm Bacterial Communities in Lake Baikal

V. V. Parfenova, A. S. Gladkikh[1], and O. I. Belykh

Limnological Institute, Siberian Branch, Russian Academy of Sciences, ul. Ulan-Batorskaya 3, Irkutsk, 664033 Russia

Received February 20, 2012

Abstract—Bacterial communities of the water and the biofilm formed during five years on an artificial substrate in Lake Baikal were studied by the pyrosequencing of 16S rRNA gene fragments; taxonomic diversity of bacterial communities and differences in their structure were revealed. The biofilm community contained mainly representatives of three phyla: *Cyanobacteria, Bacteroidetes,* and *Proteobacteria*; the amounts of other groups were within 1%. Bacterial community of the plankton was more heterogeneous; along with the dominant phyla (*Bacteroidetes, Actinobacteria,* and *Proteobacteria*) 15% of the members were of the other phyla. The use of pyrosequencing allowed to reveal 35 bacterial phyla in Lake Baikal, some of which were identified for the first time; moreover, minor groups of microorganisms (including only several sequences), which were not earlier determined by other molecular methods were found.

Keywords: Lake Baikal, pyrosequencing, 16S rRNA, plankton, biofilm, biodiversity, *Cyanobacteria, Actinobacteria, Bacteroidetes*

DOI: 10.1134/S0026261713010128

Aquatic microorganisms, in spite of their key role in biogeochemical processes and in the functioning of freshwater reservoirs, remain insufficiently studied. Until the 1990s, research on the biodiversity of freshwater bacteria was based on the study of cultured strains [1]. It was known, however, that up to 99% of microorganisms were uncultured and, therefore, remained unknown and could not be used for biotechnological and other purposes [2]. The application of molecular methods, in particular, the polymerase chain reaction (PCR), made it possible to gain insight into the composition of microbial communities in natural systems. The use of the 16S rRNA gene as a marker resulted in the isolation of numerous DNA sequences and revealed a great diversity of planktonic bacteria in various lakes and rivers [1]. In recent years, progress in the study of the composition and metabolic potential of microorganisms is associated with investigating metagenomes of microbiota in freshwater ecosystems. This approach is based on the analysis of thousands (tens of thousands) of sequences obtained from one sample and allows profound characterization of microbial communities, revealing not only the dominant microorganisms but also the minor components which may play an important ecological role [3]. Metagenomic analysis determines not only the qualitative composition of a microbial community, but also a share of individual taxa in it. The development of high-performance parallel sequencing method (pyrosequencing) made it possible to perform in-depth metagenomic studies, which are necessary for the system approach to the investigation of bacterial communities.

Lake Baikal, the most ancient and deep lake on the Earth, is characterized by the highest hydrobiont diversity among freshwater reservoirs [4]. The lake microbial community was formed as a result of long-time development under peculiar hydrophysical and hydrochemical conditions of a unique freshwater ecosystem.

The first studies on bacterial plankton of Lake Baikal based on PCR amplification and sequencing of the 16S rRNA gene were concerned with identification of cultured saprotrophic bacteria. The analysis of uncultured bacterial community revealed a great diversity of bacterial groups. Further molecular genetic studies made it possible to gain insight into the diversity of taxa of both isolated strains and uncultured community of the bacterial plankton [5].

The studies on the biofilm bacterial communities in Lake Baikal had started only recently: the first 16S rRNA gene sequences of biofilm bacteria from the littoral zone of Lake Baikal were analyzed in 2010 [6]. Investigations of biofilms, especially of bacteria forming biofilms in the organisms of humans and animals, as well as in the water supply systems, attract increasing interest worldwide. In recent years, a considerable number of biomedicine-important metagenomic studies was carried out: microbiomes of freshwater biofilms were obtained [7, 8].

[1] Corresponding author; e-mail: gladkikh@lin.irk.ru

At present, metagenomic investigations of freshwater lakes are scarce: only a few studies were concerned with diversity and functional capability of bacterial plankton in meso- and eutrophic lakes [9–13]. There is no information obtained by pyrosequencing method on microbial diversity of plankton and biofilms in oligotrophic lakes, of which Baikal is the largest.

The aim of this work was to apply pyrosequencing of the 16S rRNA gene fragments to investigation of the composition and structure of bacterial communities in the water and in the biofilm formed on an artificial substrate in Lake Baikal.

MATERIALS AND METHODS

Sampling was performed in the littoral zone of Lake Baikal in the region of the Bol'shie Koty settlement in June 2010; the stainless steel plates (2 × 17 cm) were retrieved by divers from a 5-m depth where they have been incubated for five years. Biofilms were removed from the plates and put into sterile Eppendorf tubes. The water (2 L) for plankton sampling was collected close to the plates and passed through a 0.22-μm Millipore polycarbonate filter (United States).

Pyrosequencing. The total DNA was isolated from the samples using a DNKSorb kit (Russia) after preliminary enzymatic lysis. The amplification was performed using the eubacterial primers 9F and 541R flanking the V1–V3 regions of the 16S rRNA gene. The pair of oligonucleotide primers used in the work is specific for conservative sites of the 16S rRNA gene and is usually applied in metagenomic studies to determine bacterial diversity of various communities [14]. Metagenomic sequencing of the 16S rRNA gene fragments was carried out on a Roche/454 Genome Sequencer FLX Titanium (ChemLab Inc., Seoul National University, Korea).

The maximum length of obtained sequences was 558 nucleotides; sequences shorter than 300 nucleotides and chimeric sequences were excluded from analysis. Thus, each sequence contained at least two out of three (V1, V2, and V3) hypervariable 16S rRNA gene sites. On the whole, 11846 sequences with the total length of 5279749 nucleotides were analyzed.

Determination of diversity and taxonomic composition of the communities. Each of the sequences derived was taxonomically identified by comparing with those available in the EzTaxon database by using the BLASTN program and pairwise comparison [15]. The thresholds of similarity (x) were used to identify species ($x \geq 97\%$), genera ($97 > x \geq 94\%$), families ($94 > x \geq 90\%$), orders ($90 > x \geq 85\%$), classes ($85 > x \geq 80\%$), and phyla ($80 > x \geq 75\%$). The sequences were considered as unidentified if the similarity level was below the threshold.

Primary analysis of the pyrosequencing data, removing of short and chimeric sequences, clusterization in operational taxonomic units (OTU), and the biodiversity evaluation by calculating the ACE, Chao1, and Shannon indices were carried out with the use of the Mothur v.1.22.0 program (http://www.mothur.org). Determination of the species diversity, taxonomic composition, and comparison of communities were performed with the aid of the Pyrosequencing pipeline program (http://pyro.cme.msu.edu). The obtained sequences were aligned and cluster analysis was undertaken with the use of the Complete Linkage Clustering program (a component of the Pyrosequencing pipeline). The clustering was carried out at various levels; the distance between the clusters was varied from 0 to 0.25 with a step of 0.01. The phylotypes (OTU) were recognized at the cluster distance of 0.03; evaluation of the taxonomic complexity of the communities was carried out at different distance levels corresponding to the following taxa: species (0.03), genus (0.05), and family (0.1) using the Rarefaction program (a component of the Pyrosequencing pipeline). To characterize the taxonomic composition of the community, cluster analysis was performed at the cluster distance of 0.25 that corresponded to the phylum level. For each cluster, a representative nucleotide sequence corresponding to the cluster center (i.e., the sequence having a minimal sum of squares of the distances to other sequences included in the cluster) was found using the Dereplicate Request program (Pyrosequencing pipeline). The representative cluster sequences were taxonomically classified on the basis of genotypic approach in accordance with the International Code of Nomenclature of Bacteria (ICNB). If the analyzed sequence shared more than 97% similarity with that of a validly described microorganism, the cluster was attributed to the corresponding species.

RESULTS AND DISCUSSION

Bacterial community of the plankton in the littoral zone of Lake Baikal. In plankton samples taken from the littoral zone of Lake Baikal, pyrosequencing of the 16S rRNA gene revealed 5525 sequences with a length of over 300 nucleotides, with 5031 sequences (91.1%) belonging to the domain *Bacteria* and 494 sequences (8.9%) to the domain *Eukarya*. The total length of the analyzed bacterial sequences was 2498795 nucleotides; the average length was 470 nucleotides.

The bacterial community of the water samples was characterized by high species diversity and included 888 phylotypes (OTU) belonging to 28 phyla. The rarefaction curve did not reach a plateau at the end of analysis, and the number of revealed OTU increased linearly (Fig. 1). The species richness (cluster distance of 0.03) determined with the use of the nonparametric ACE and Chao1 criteria was 2380 and 1474, respectively (Table 1). The Shannon index characterizing the species diversity was 4.67.

In the plankton, three phyla predominated in the numbers of sequences and genera: *Bacteroidetes* (32.9%, 52 genera), *Actinobacteria* (28.7%, 31 genera), and *Proteobacteria* (23.7%, 196 genera); these

phyla comprised 85.3% of the total number of bacterial sequences (Fig. 2a). The dominant phylotypes in the plankton were represented by *Planktophila* sp. and *Flavobacterium* sp. (Table 2). It should be noted that the plankton community contained, apart from bacterial genotypes, the genotypes of eukaryotic algae: *Chlorophyta*, *Cryptophyta*, *Dinophyta*, and *Ochrophyta*. Most of the eukaryotic sequences belonged to the phylum *Ochrophyta* (219 sequences assigned to 14 genera).

The phylum *Bacteroidetes* consisted of four clusters containing two cultured genera: *Flavobacterium* (*Flavobacteriales*) and *Sediminibacterium* (*Sphingobacteriales*) and two uncultured ones: EU800805 (*Flavobacteriales*) and EU800176 (*Cytophagales*). It is noticeable that the representative species of these clusters showed more than 98% similarity with the known members of the *Bacteroidetes* (Table 2). The *Flavobacterium* cluster with the representative phylotype *Flavobacterium* sp. was the largest among planktonic *Bacteroidetes* in Lake Baikal. A new genus *Sediminibacterium* was first isolated from an eutrophic reservoir in Beijing [16]; together with the other representatives of *Sphingobacteriales*, it is a usual inhabitant of natural environments. This genus is characterized by high sphingophospholipid content in the cell walls. The genotypes EU800805 and EU800176 were revealed in the marine plankton samples within the scope of the "Global Ocean Sampling" program [9].

The phylum *Bacteroidetes* includes the most diverse bacteria (both phenotypically and metabolically), mainly chemoorganoheterotrophs, inhabiting soil and water and forming symbiosis with human beings, animals, and plants. Filamentous morphotypes of *Bacteroidetes* are known to dominate in bacterial plankton of a mesotrophic lake in Germany [17]. Most of the nucleotide sequences revealed in freshwater samples were assigned to the order *Flavobacteriales* [18]. Our data also demonstrate the domination of members of this order in plankton of Lake Baikal. The population of *Flavobacterium* in subalpine lakes was shown to exhibit rapid growth during the spring phytoplankton boom [19]. The domination of the *Flavobacterium* phylotype in the plankton of Lake Baikal is possibly associated with the subglacial development of planktonic algae.

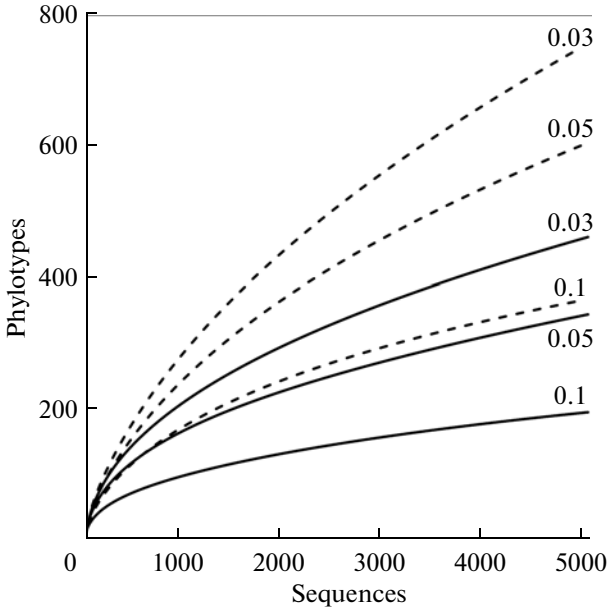

Fig. 1. Rarefaction curve at different taxonomic cutoffs (0.03, 0.05, and 0.1). Plankton —dotted lines, biofilm— solid lines.

The phylum *Actinobacteria* was characterized by two clusters including a common planktonic species *Planktophila* sp. and a uncultured phylotype EU803276, which showed 98% similarity with the sequences of a clone from freshwater Lake Gatun [9] (Table 2). By using methods of molecular biology including FISH, it has been earlier revealed that actinobacteria are among the most abundant groups of freshwater bacterial plankton amounting to up to 50% of the DAPI-stained prokaryotes [18]. Actinobacteria were also found in deeper water layers, although their number decreased with depth. They inhabit lakes with different trophicity levels (from oligo- to dystrophic) in all climatic zones. The genus *Planktophila* (*Actinomycetales*), which representatives have been recently

Table 1. Indices of species richness and diversity detected at different cluster distances

Sample	Distance	Number of sequences	Number of clusters	ACE	Chao1	Shannon
Biofilm	0	5074	2381	23011	9873	6.83
	0.03		461	1211	840	4.19
	0.05		344	916	652	3.61
	0.1		195	366	265	2.59
Water	0	5031	2527	24576	11101	7.00
	0.03		752	2380	1474	4.67
	0.05		602	1691	1145	4.26
	0.1		366	765	584	3.63

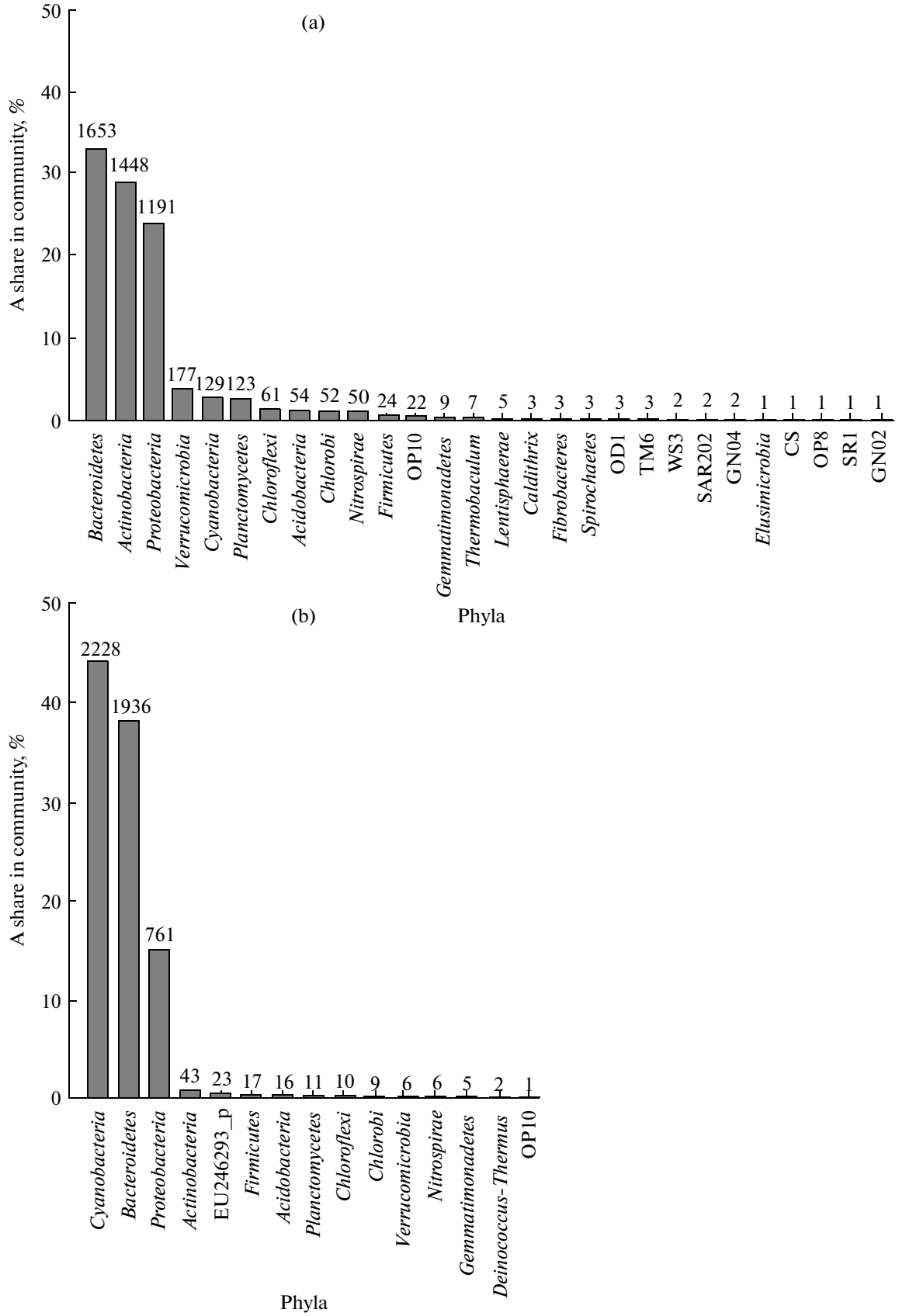

Fig. 2. Metagenomic analysis of bacterial communities of the plankton (a) and biofilm formed on an artificial substrate (b) in Lake Baikal based on the 16S rRNA gene sequencing. The numbers of detected sequences are shown above the columns.

Table 2. Composition of the water bacterial community (25% difference)

Phylum	Number of sequences	Representative sequence characterizing the cluster	Class	Share in the cluster, %	Similarity with the closest related sequence, %
Bacteroidetes	837	*Flavobacterium* sp.	*Flavobacteria*	16.6	**98.0**
	338	EU800805	*Flavobacteria*	6.7	**99.7**
	242	EU800176	*Cytophagia*	4.8	**98.0**
	232	*Sediminibacterium* sp.	*Sphingobacteria*	4.6	**98.7**
Actinobacteria	923	*Planktophila* sp.	*Actinobacteria*	18.4	**100.0**
	508	EU803276	*Actinobacteria*	10.1	**98.1**
Proteobacteria	156	*Reyranella massiliensis*	*Alphaproteobacteria*	3.1	**99.5**
	10	*Alphaproteobacteria*	*Alphaproteobacteria*	0.2	81.7
	796	*Albidiferax ferrireducens*	*Betaproteobacteria*	15.8	**97.5**
	42	AB355082	*Deltaproteobacteria*	0.8	**97.2**
	10	DQ906906	*Deltaproteobacteria*	0.2	94.5
	5	*Desulfuromonadales*	*Deltaproteobacteria*	0.1	86.6
	12	*Arcobacter cryaerophilus*	*Epsilonproteobacteria*	0.2	**99.1**
	142	DQ009153	*Gammaproteobacteria*	2.8	94.9
Cyanobacteria	134	*Synechococcus rubescens*	*Chroobacteria*	2.7	**100.0**
Verrucomicrobia	128	EU800366	*Opitutae*	2.5	**99.4**
	46	DQ415853	*Verrucomicrobiae*	0.9	95.4
Planctomycetes	73	AF418943	*Phycisphaerae*	1.4	**97.9**
	28	*Schlesneria* sp.	*Planctomycetacia*	0.6	**98.1**
	15	EU234279	*Planctomycetacia*	0.3	**98.7**
	5	AF316773	EU373996_c	0.1	**100.0**
Acidobacteria	34	FJ479430	FJ478837_c	0.7	92.5
	17	EF515323	*Holophagae*	0.3	90.4
	9	EF632805	*Solibacteres*	0.2	**98.5**
Chloroflexi	26	AF316759	*Anaerolineae*	0.5	**99.1**
	12	AJ306742	*Caldilineae*	0.2	95.4
	11	4P001458	4P001458_c	0.2	**98.3**
Chlorobi	23	AY555788	*Ignavibacteriae*	0.5	91.9
	21	EU234260	*Ignavibacteriae*	0.4	95.2
	8	DQ520175	OPB56	0.2	**98.9**

Note: Only the clusters exceeding 0.1% of the community are listed; the similarity level over 97% is shown in bold.

isolated in pure cultures, belongs to acI lineage, the most abundant actinobacterial group in bacterial plankton; however, the physiological characteristics of this genus are scarcely studied. At the same time, the affiliation of *Planktophila* with actinomycetes is indicative of their ability to utilize difficult-to-degrade substrates.

The phylum *Proteobacteria* included eight clusters containing *Alpha-, Beta-, Gamma-, Delta-*, and *Epsilonproteobacteria* (Table 2). Three out of eight representative species showed more than 97% similarity with well-known species *Reyranella massiliensis, Albidiferax ferrireducens*, and *Arcobacter cryaerophilus*. The *Betaproteobacteria* cluster was the most abundant among the proteobacteria; it was characterized by the species *A. ferrireducens*, chemoorganoheterotrophic iron-reducing bacteria. *Betaproteobacteria* are known to predominate in freshwater accounting for 60–70% of the cells (determined by the FISH method) and are the best-studied group of freshwater bacteria [18]. The number and composition of *Betaproteobacteria* varied in different lakes depending on the depth and season.

The class *Alphaproteobacteria* was a planktonic group second in abundance; these bacteria prevail in marine ecosystems and have been also found in freshwater lakes over the world, but in a smaller number than in the seas [18]. The freshwater *Alphaproteobacteria* are insufficiently studied; it is only known that they prefer oligotrophic water bodies and are able to degrade complex organic substances. In the plankton, this class was represented by the species *Reyranella massiliensis* (99.5% similarity); three strains exhibiting high oxidase activity have been recently isolated from freshwater samples in France [20]. The closely related genus *Magnetospirillum* is characterized by magnetotaxis. The occurrence of bacteria associated with the iron cycle in near-bottom zones is associated with the active functioning of this cycle in Lake Baikal [21].

The class *Gammaproteobacteria* was third in number among proteobacteria and, like the *Deltaproteobacteria*, did not contain closely related cultured strains. The least common cluster *Epsilonproteobacteria* was represented by the species *Arcobacter cryaerophilus* (99.1% similarity).

The phylum *Cyanobacteria* consisted of one cluster which included 134 sequences (2.3% of the total bacterial sequences) belonging to 20 phylotypes. Sequences of the order *Chroococcales* (88% of all cyanobacteria) with the representative species *Synechococcus rubescens* (=*Cyanobium rubescens*) prevailed. These are the smallest representatives of autotrophic picoplankton, which predominates everywhere in marine and freshwater ecosystems and may reach op to 3×10^6 cells/mL in Lake Baikal [22].

The phylum *Planctomycetes* contained four dominated by the number of sequences clusters of uncultured bacteria and the cluster *Schlesneria* (the order *Planctomycetales*), which included cultured species. The order *Planctomycetales* is represented by budding bacteria capable of degrading complex carbohydrates which were produced by the phytoplankton [18]; they are widespread in acidic swamped lakes but are rare in oligotrophic lakes.

The other clusters found in the water samples contained no relative cultured bacterial strains. However, representatives of these phyla are widespread in freshwater systems; for instance, *Verrucomicrobia* (the family Opitutaceae) were found in the plankton of 73 out of 81 European lakes [23] and reached 3.4% in Lake Baikal. The phyla *Chloroflexi*, *Acidobacteria, Chlorobi*, *Nitrospirae*, and *Firmicutes* (including bacteria with different metabolism and functions) comprised less than 5% of the planktonic microbiome. Seven minor phyla (*Gammatimonadetes*, *Thermobaculum, Lentisphaerae, Caldithris, Fibrobacteres, Spirochaetes*, and *Elusimicrobia*) and ten phantom phyla (SAR202, OP10, GN02, GN04, SR1, WS3, OP8, TM6, OD1, and CS) were revealed in the water samples.

The data obtained by metagenomic analysis of bacterial plankton in other lakes differed from our results primarily due to the different hydrophysical and hydrochemical conditions in these water bodies. In the mesotrophic lake Lac du Bourget, the phylum *Actinobacteria* dominated (about 45%), *Proteobacteria* comprised about 40%, and *Bacteroidetes* was the minor phylum (no more than 15%) [11]. In the eutrophic lake Samsonvale, which was characterized by a toxic cyanobacterial bloom, the composition and structure of the bacterial community were similar to those in lake Lac du Bourget [10].

Lake Baikal differed from the other lakes studied by metagenomic analysis in its high diversity of bacterial community (Table 3). The number of phylotypes revealed in the plankton of Lake Baikal was an order of magnitude higher than in lakes Lanier, Gatun, Samsonvale, Lac du Bourget, and Taihu [9–13]. This distinction was probably due to the fact that metagenomic analysis of the planktonic bacterial community in Lake Baikal was performed for the first time on the basis of pyrosequencing of the 16S rRNA gene fragments. Bacterial communities in the other lakes were analyzed by the whole genome shotgun sequencing or by constructing of clone libraries that considerably limited the number of revealed 16S rRNA gene sequences.

The phyla *Actinobacteria* and *Proteobacteria* dominated in Baikal, as in other freshwater lakes. Earlier, direct sequencing of the 16S rRNA gene of samples from two oligotrophic and one mesotrophic lakes revealed the predominance of *Betaproteobacteria* and *Actinobacteria* [24]. It was also shown that microorganisms inhabiting the water in Lake Baikal were represented mainly by cyanobacteria, proteobacteria, and actinobacteria; the FISH method revealed the domination of proteobacteria and "other" bacteria not hybridized with the known species probes [5].

Table 3. Comparative data on metagenomic analysis of bacterial communities in different lakes

Lake, country	Number of bacterial taxa	Number of 16S rRNA gene sequences	Dominating phyla	References
Lake Gatun, Panama	10 phyla, 20 families	174	*Actinobacteria, Proteobacteria, Acidobacteria*	[9]
Lake Samsonvale, Australia	8 phyla, 25 OTU	71	*Proteobacteria, Actinobacteria, Verrucomicrobia*	[10]
Lake Lac du Bourget, France	12 phyla, 68 OTU	288	*Actinobacteria, Proteobacteria, Bacteroidetes*	[11]
Lake Taihu, China	More than 5 phyla	No data	*Proteobacteria, Actinobacteria*	[12]
Lake Lanier, United States	17 phyla, 28 families	447	*Proteobacteria, Actinobacteria, Verrucomicrobia*	[13]
Lake Baikal, Russia	28 phyla, 888 OTU	5031	*Bacteroidetes, Actinobacteria, Proteobacteria*	This study

Thus, in the plankton, chemoorganoheterotrophic microorganisms prevailed, whereas phototrophic bacteria (cyanobacteria and green or purple bacteria), various chemolithoautotrophic, nitrifying, iron-oxidizing bacteria, etc., were found in lower amounts.

Bacterial community of the biofilm. Pyrosequencing of the metagenomic DNA from the biofilm revealed 6291 sequences of the 16S rRNA gene fragments containing over 300 nucleotides. An average length of the analyzed sequences was 430 nucleotides, whereas the total length was 2780954 nucleotides. Out of obtained sequences, 5074 (80.7%) were attributed to the domain *Bacteria* and 1217 (19.3%) to the domain *Eukarya*. Bacterial community of the biofilm contained 552 phylotypes (OTU) belonging to 15 phyla. The rarefaction curve did not reach a plateau; by the end of analysis, the number of revealed OTU increased linearly (Fig. 1). The species richness values (cluster distance of 0.03) determined with the use of nonparametric criteria ACE and Chao1 were 1211 and 840, respectively; the Shannon index at the same cluster distance was 4.19 (Table 1). Therefore, biodiversity in the biofilm was somewhat lower than in the plankton.

In the biofilm, the phyla dominated in the number of sequences and genera were represented by *Cyanobacteria* (43.9%, 27 genera), *Bacteroidetes* (38.2%, 30 genera), and *Proteobacteria* (15%, 96 genera) that comprised 97% of the total bacterial community (Table 4, Fig. 2b). The dominant genera in the biofilm were *Flavobacterium* (1027 sequences, 25 phylotypes, *Bacteroidetes*), *Chamaesiphon* (812 sequences, 3 phylotypes, *Cyanobacteria*), and *Variovorax* (84 sequences, 2 phylotypes, *Proteobacteria*).

The phylum *Cyanobacteria* was represented by one numerous cluster comprising the orders *Chroococcales, Oscillatoriales*, and *Nostocales* and unidentified groups (AB355089 and AY212703). The dominant order *Chroococcales* (1228 sequences, 55.12%) was followed by *Oscillatoriales* (937 sequences, 42.06%), and *Nostocales* was the minor component (63 sequences, 2.69%). The cyanobacterial community included 33 OTU, in which the phylotypes of the genus *Chamaesiphon* with the representative species *Chamaesiphon* sp. prevailed. The genus *Chamaesiphon* comprises cyanobacteria inhabiting cold and clean water bodies and having heteropolar cells and exospores surrounded by mucous pseudosheaths by which they attached to various substrates. Among the dominating sequences, a unique phylotype EF580987_s (385 sequences) of the order *Oscillatoriales* was revealed; it possibly belonged to the species *Heteroleibleinia pusilla* which was found in large numbers upon microscopic observation of the biofilm (data not shown).

The phylum *Bacteroidetes* was represented by seven clusters, of which four included cultured representative species (*Flavobacterium glaciei*, *Arcicella* sp., *Ferruginibacter* sp., and *Leadbetterella* sp., over 97% similarity), and three unidentified clusters belonging to the classes *Sphingobacteria, Flavobacteria*, and *Cytophagia* (Table 4). These bacteria were chemoorganoheterotrophs capable of hydrolyzing complex organic substances. The genus *Flavobacterium* (1027 sequences, 25 phylotypes) dominated among the *Bacteroidetes*: it was represented by the psychrophilic species *Flavobacterium glaciei*, which was first isolated from a glacier in China [25]. The genus *Arcicella* included cosmopolitan species found in freshwa-

Table 4. Composition of the biofilm bacterial community (25% cutoff)

Phylum	Number of sequences	Representative sequence characterizing the cluster	Class	Share in the cluster, %*	Similarity with the closest BLAST hit, %
Cyanobacteria	2225	*Chamaesiphon* sp.	*Chroobacteria*	43.8	95.0
Bacteroidetes	1107	*Flavobacterium glaciei*	*Flavobacteria*	21.8	**99.0**
	347	*Ferruginibacter* sp.	*Sphingobacteria*	6.8	**99.3**
	235	*Arcicella* sp.	*Cytophagia*	4.6	**97.2**
	108	*Leadbetterella* sp.	*Cytophagia*	2.1	**99.0**
	70	*Sphingobacteriales*	*Sphingobacteria*	1.4	87.3
	55	EF203200	*Flavobacteria*	1.1	88.4
	14	AY887012	*Cytophagia*	0.3	94.6
Proteobacteria	274	*Beijerinckia* sp.	*Alphaproteobacteria*	5.4	94.8
	477	*Curvibacter* sp.	*Betaproteobacteria*	9.4	**97.1**
	9	*Haliangiaceae*	*Deltaproteobacteria*	0.2	93.1

Note: * Only the clusters exceeding 0.1% of the community are listed; the similarity level over 97% is shown in bold.

ter biofilms [26]. The genus *Ferruginibacter* was recently isolated from the sediment of a freshwater lake in South Korea [27]. The genus *Leadbetterella* is also novel; the only described species was isolated from the substrate for mushroom cultivation in South Korea [28].

The phylum *Proteobacteria* was represented by three clusters: *Alpha-, Beta-*, and *Deltaproteobacteria* (Table 4). *Betaproteobacteria* contained the largest number of sequences with two predominant phylotypes of the genus *Variovorax* exhibiting denitrifying activity. The representative species of the *Betaproteobacteria* (*Curvibacter* sp.) and *Alphaproteobacteria* (*Beijerinckia* sp.) belonged to the nitrogen-fixing genera. Among the *Deltaproteobacteria*, an unknown genus of the family *Haliangiaceae* prevailed; it probably possessed the ability to lyse prokaryotic and eukaryotic cells that is typical of mycobacteria.

The less abundant phyla in the biofilm (comprising less than 1%) included *Actinobacteria, Firmicutes, Acidobacteria, Planctomycetes, Chloroflexi, Chlorobi, Verrucomicrobia, Nitrospirae*, and the phantomic phylum EU246293_p; the minor phyla (less than 0.1%) were represented by *Gammatimonadetes, Deinococcus–Thermus*, and the phantom phylum OP-10. Apart from these, the bacterium *Microbacterium flavescens* was revealed, several strains of which are used for oil degradation. The genotypes of *Chlorophyta*, *Criptophyta*, and *Ochrophyta* were found in the biofilm; 91.5% of the eukaryotic genotypes (1113 sequences, 10 genera) belonged to *Ochrophyta*.

The studies performed with marine ecosystems showed that biologically inert substrates (e.g., stainless steel or glass) submerged into seawater were rapidly inhabited by organisms, which formed a complex two-layer film. At the first stage, the substrates were mainly occupied by bacteria; at the second stage, after a 5-week exposition, large motile colonial and single diatoms appeared, as well as protozoa and fungi [29].

In the biofilms of drainage systems, *Proteobacteria* was the dominant group, whereas *Firmicutes*, *Actinobacteria*, *Bacteroidetes*, and *Deinococcus–Thermus* were the minor groups comprising no more than 4% [7]. In water pits, *Alphaproteobacteria* dominated (74%), whereas *Betaproteobacteria* were not revealed [8]. By using cultivation techniques and the Sanger sequencing, it has been earlier shown that biofilms from Lake Baikal contained representatives of *Betaproteobacteria, Cyanobacteria, Flavobacteria, Firmicutes, Verrucomicrobia*, and *Sphingobacteria* [6]. According to other studies, *Proteobacteria* prevailed in the biofilms formed in the regions of drink water intakes [7, 8].

Thus, the analysis of the biofilm developing in Lake Baikal revealed a great diversity of microbial species, which differed from those observed in other ecosystems both in the structure and composition. The biofilm bacterial community was dominated by photolithoautotrophs (cyanobacteria) producing a number of organic substances, which may be used as an energy

source by numerous chemoorganoheterotrophic bacteria including those involved in the nitrogen cycle.

Comparative analysis of microbial communities of the biofilm and water samples. Bacterial communities of biofilm and plankton in Lake Baikal were similar in the content of the main bacterial phyla but differed in their taxonomic composition. In the plankton, *Bacteroidetes* was the predominant phylum, whereas in the biofilm, *Cyanobacteria* prevailed. The plankton was characterized by higher species richness than the biofilm; the identified OTU comprised 888 and 552, respectively. In the plankton, the number of phyla was 1.9 times higher than in the biofilm (28 and 15, respectively); the number of phantom bacterial phyla was 15 and 2, respectively. The number of common OTU in the biofilm and water was 112 of which 72% belonged to the domains *Proteobacteria* and *Bacteroidetes*.

In both communities, the *Proteobacteria* members dominated: it is the largest bacterial phylum containing a great variety of phenotypes which occupy most ecological niches. Proteobacteria play a key role in the cycles of biogenic elements due to their high diversity, great number, and wide distribution [30].

A rank abundance curve demonstrates the irregularity of species distribution in microbiomes: in both communities, most of the taxonomic units belonged to rare organisms, which were represented by only a few sequences, whereas numerous species were few in number (Fig. 3).

The rarefaction curve reached no plateau either at the level of species, genus, or family. It indicates that the sequencing volume used in this study was insufficient for complete characterization of community diversity. New phylotypes may possibly be found if the number of analyzed sequences will be increased. The data on the number of clusters and the species richness evaluated at the difference levels of 0.03, 0.05, and 0.1 are given in Table 1. The values of species diversity both in the biofilm and water determined by the Chao1 coefficient were twofold higher than those obtained by the pyrosequencing method. A character of the rank abundance curve indicated that the largest taxa determining the community composition were already identified and the following pyrosequencing would reveal only rare species (Fig. 3).

Bacterial community of biofilm contained mainly representatives of the phyla *Cyanobacteria, Bacteroidetes*, and *Proteobacteria*; the contribution of other groups did not exceed 1%. Bacterial community of the water was more heterogeneous and included not only the dominant phyla (*Bacteroidetes, Actinobacteria*, and *Proteobacteria*), but also the members of other phyla (up to 15% of total community); the level of such phyla as *Cyanobacteria, Verrucomicrobia*, and *Planctomycetes* reached 2.5% (Fig. 2).

Thus, for the first time, taxonomic composition and distribution of different microbial groups in the biofilm and plankton of Lake Baikal were described in detail by using the pyrosequencing method. A great diversity of microorganisms was revealed in two communities. Earlier, no more than ten bacterial groups were found in the water [31], whereas the application of the pyrosequencing method allowed for determination of 28 phyla, some of which (*Chloroflexi, Chlorobi, Deinococcus–Thermus, Gemmatimonadetes*, and the phantomic phyla) were revealed in Lake Baikal for the first time. Moreover, minor groups of microorganisms containing only few sequences were revealed, which could not be recognized by other methods. A great number of sequences revealed by the pyrosequencing method had no close BLAST hits in the world database; they can represent novel species, which need further investigation.

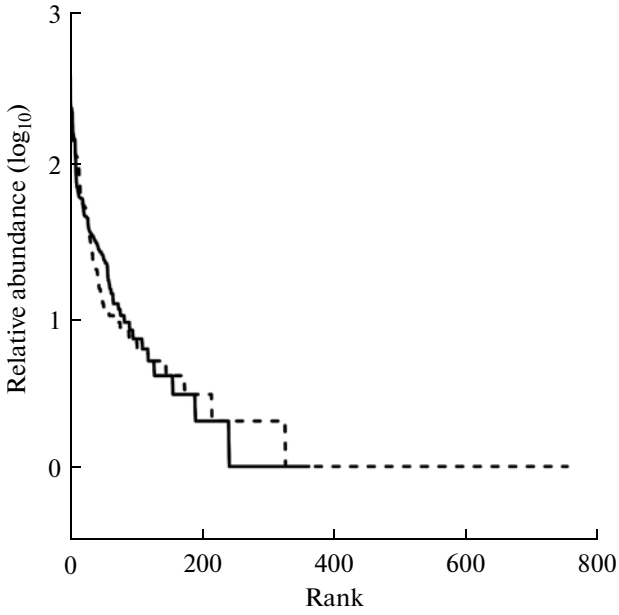

Fig. 3. Rank abundance curve. Plankton—dotted line, biofilm—solid line. The gradient indicates low species evenness in the communities: small number of numerous species and large number of rare species.

ACKNOWLEDGMENTS

This work was performed within the framework of the Federal Program VI.51.1.9. "Peculiarity of Formation and Life Strategy of the Microbial Community and Viruses of the Biofilms in Lake Baikal" and supported by the Russian Foundation for Basic Research (projects 10-05-01078-a, 10-04-01613-a, 11-04-92220 Mong_a, and 12-04-31672 mol_a).

REFERENCES

1. Zwart, G., Crump, B.C., Agterveld, M.P.K., Hagen, F., and Han, S.-K., Typical Freshwater Bacteria: An Analysis of Available 16S rRNA Gene Sequences from Plankton of Lakes and Rivers, *Aquat. Microb. Ecol.*, 2002, vol. 28, pp. 141–155.

2. Amann, R.I., Ludwig, W., and Schleifer, K.H., Phylogenetic Identification and in situ Detection of Individual Microbial Cells Without Cultivation, *Microb. Rev.*, 1995, vol. 59, pp. 143–169.

3. Gumerov, V.M., Mardanov, A.V., Beletskii, A.V., Bonch-Osmolovskaya, E.A., and Ravin, N.V., Molecular Analysis of Microbial Diversity in the Zavarzin Spring, Uzon Caldera, Kamchatka, *Microbiology*, 2011, vol. 80, no. 2, pp. 244–251.

4. *Atlas-opredelitel' pelagobiontov Baikala* (Atlas for Identification of Lake Baikal Pelagobionts), Timoshkin, O.A., Ed., Novosibirsk: Nauka, 1995.

5. Bel'kova, N.L. and Zemskaya, T.I., Molecular Investigation of Biodiversity of Lake Baikal Microbial Communities: Review of the Results of a Decade (1994–2004), in *Mikrobiologicheskie metody v ekologicheskikh issledovaniyakh: materialy vtoroi mezhdunarodnoi mikrobiologicheskoi shkoly* (Microbiological Methods in Ecological Research: Proc. 2nd Int. Microbiol. School), Vladivostok: Dal'nevost. Gos. Univ., 2007, pp. 23–35.

6. Mal'nik, V.V., Biofilm Microbial Community at the Water-Solid Interface in Lake Baikal Littoral Zone, *Extended Abstract of Cand. Sci. (Biol.) Dissertation*, Buryat State Univ., Ulan-Ude, 2010.

7. Hong, P.-Y., Hwang, C., Ling, F., Andersen, G.L., LeChevallier, M.W., and Liu, W.-T., Pyrosequencing Analysis of Bacterial Biofilm Communities in Water Meters of Drinking Water Distribution System, *Appl. Environ. Microbiol.,* 2010, vol. 76, no. 16, pp. 5631–5635.

8. Rickard, A.H., Leach, S.A., Hall, L.S., Buswell, C.M., High, N.J., and Handley, P.S., Phylogenetic Relationships and Coagregation Ability of Freshwater Biofilm Bacteria, *Appl. Environ. Microbiol.*, 2002, vol. 68, no. 7, pp. 3644–3650.

9. Rusch, D.B., Halpern, A.L., Sutton, G., Heidelberg, K.B., Williamson, S., Yooseph, S., Wu, D., Eisen, J.A., Hoffman, J.M., Remington, K., Beeson, K., Tran, B., Smith, H., Baden-Tillson, H., Stewart, C., Thorpe, J., Freeman, J., Andrews-Pfannkoch, C., Venter, J.E., Li, K., Kravitz, S., Heidelberg, J.F., Utterback, T., Rogers, Y.-H., Falcon, L.I., Souza, V., Bonilla-Rosso, G., Eguiarte, L.E., Karl, D.M., Sathyendranath, S., Platt, T., Bermingham, E., Gallardo, V., Tamayo-Castillo, G., Ferrari, M.R., Strausberg, R.L., Nealson, K., Friedman, R., Frazier, M., and Venter, J.C., The Sorcerer II Global Ocean Sampling Expedition: Northwest Atlantic through Eastern Tropical Pacific, *PLoS Biol.*, 2007, vol. 5, no. 3, pp. 398–431.

10. Pope, P.B. and Patel, B.K.C., Metagenomic Analysis of a Freshwater Toxic Cyanobacteria Bloom, *FEMS Microbiol. Ecol.*, 2008, vol. 64, pp. 9–27.

11. Debroas, D., Humbert, J.-F., Enault, F., Bronner, G., Faubladier, M., and Cornillot, E., Metagenomic Approach Studying the Taxonomic and Functional Diversity of the Bacterial Community in a Mesotrophic Lake (Lac du Bourget-France), *Environ. Microbiol.*, 2009, vol. 11, no. 9, pp. 2412–2424.

12. Li, N., Zhang, L., Li, F., Wang, Y., Zhu, Y., Kang, H., Wang, S., and Qin, S., Metagenome of Microorganisms Associated with the Toxic Cyanobacteria *Microcystis aeruginosa* Analyzed Using the 454 Sequencing Platform, *Chinese J. Oceanol. Limnol.*, 2011, vol. 29, no. 3, pp. 505–513.

13. Oh, S., Caro-Quintero, A., Tsementzi, D., DeLeon-Rodriguez, N., Luo, C., Poretsky, R., and Konstantinidis, K.T., Metagenomic Insights into the Evolution, Function, and Complexity of the Planktonic Microbial Community of Lake Lanier, a Temperate Freshwater Ecosystem, *Appl. Environ. Microbiol.*, 2011, vol. 77, no. 17, pp. 6000–6011.

14. Chun, J., Kim, K.Y., Lee, J.H., and Choi, Y., The Analysis of Oral Microbial Communities of Wild-Type and Toll-Like Receptor 2-Deficient Mice Using a 454 GS FLX Titanium Pyrosequencer, *BMC Microbiol.,* 2010, vol. 10. doi 10.1186/1471-2180-10-101.

15. Chun, J., Lee, J.H., Jung, Y., Kim, M., Kim, S., Kim, B.K., and Lim, Y.W., EzTaxon: A Web-Based Tool for the Identification of Prokaryotes Based on 16S Ribosomal RNA Gene Sequences, *Int. J. Syst. Evol. Microbiol.*, 2007, vol. 57, pp. 2259–2261.

16. Qu, J.H. and Yuan, H.L., *Sediminibacterium salmoneum* gen. nov., sp. nov., a Member of the Phylum *Bacteroidetes* Isolated from Sediment of a Eutrophic Reservoir, *Int. J. Syst. Evol. Microbiol.*, 2008, vol. 58, pp. 2191–2194.

17. Pernthaler, A., Pernthaler, J., and Amann, R., Sensitive Multi-Color Fluorescence in situ Hybridization for the Identification of Environmental Microorganisms, in *Molecular Microbial Ecology Manual*, Kowalchuk, G.A., de Bruijn, F.J., Head, I.M., Akkermans, A.D., and van Elsas, J.D, Eds., Netherlands: Springer, 2004, pp. 711–726.

18. Newton, R.J., Jones, S.E., Eiler, A., McMahon, K.D., and Bertilsson, S., A Guide to the Natural History of Freshwater Lake Bacteria, *Microbiol. Mol. Biol. Rev.*, 2011, vol. 75, no. 1, pp. 14–49.

19. Zeder, M., Peter, S., Shabarova, T., and Pernthaler, J., A Small Population of Planktonic Flavobacteria with Disproportionally High Growth during the Spring Phytoplankton Bloom in a Prealpine Lake, *Environ. Microbiol.*, 2009, vol. 11, pp. 2676–2686.

20. Pagnier, I., Raoult, D., and La Scola, B., Isolation and Characterization of *Reyranella massiliensis* gen. nov., sp. nov. from Freshwater Samples with an Amoeba Co-Culture Procedure, *Int. J. Syst. Evol. Microbiol.*, 2011, vol. 61, pp. 2151–2154.

21. Zakharova, Yu.R., Parfenova, V.V., Granina, D.Z., Kravchenko, O.S., and Zemskaya, T.I., Distribution of Iron- and Manganese-Oxidizing Bacteria in the Bottom Sediments of Lake Baikal, *Inland Water Biol.*, 2010, vol. 3, no. 4, pp. 313–321.

22. Belykh, O.I., Pomazkina, G.V., Tikhonova, I.V., and Tomberg, I.V., Characterization of Summer Phytoplankton and Autotrophic Picoplankton of Lake Baikal in 2005, *Algologiya*, 2007, vol. 17, pp. 380–396.

23. Zwart, G., van Hannen, E.J., Kamst-van Agterveld, M.P., van der Gucht, K., Lindstrom, E.S., van Wichelen, J., Lauridsen, T., Crump, B.C., Han, S.-K., and Declerck, S., Rapid Screening for Freshwater Bacterial Groups by Using Reverse Line Blot Hybridization, *Appl. Environ. Microbiol.*, 2003, vol. 69, no. 10, pp. 5875–5883.

24. Glöckner, F.O., Zaichikov, E., Belkova, N., Denissova, L., Pernthaler, J., Pernthaler, A., and Amann, R., Compar-

ative 16S rRNA Analysis of Lake Bacterioplankton Reveals Globally Distributed Phylogenetic Clusters Including an Abundant Group of *Actinobacteria, Appl. Environ. Microbiol.*, 2000, vol. 66, no. 11, pp. 5053–5065.

25. Zhang, D.-C., Wang, H.-X., Liu, H.-C., Dong, X.-Z., and Zhou, P.-J., *Flavobacterium glaciei* sp. nov., a Novel Psychrophilic Bacterium Isolated from the China No.1 Glacier, *Int. J. Syst. Evol. Microbiol.*, 2006, vol. 56, pp. 2921–2925.

26. Nikitin, D.I., Strompl, C., Oranskaya, M.S., and Abraham, W.R., Phylogeny of the Ring-Forming Bacterium *Arcicella aquatica* gen. nov., sp. nov. from a Freshwater Neuston Biofilm, *Int. J. Syst. Evol. Microbiol.*, 2004, vol. 54, pp. 681–684.

27. Lim, J.H., Baek, S.-H., and Lee, S.-T., *Ferruginibacter alkalilentus* gen. nov., sp. nov. and *Ferruginibacter lapsinanis* sp. nov., Novel Members of the Family *'Chitinophagaceae'* in the Phylum *Bacteroidetes*, Isolated from Freshwater Sediment, *Int. J. Syst. Evol. Microbiol.*, 2009, vol. 59, pp. 2394–2399.

28. Weon, H.-Y., Kim, B.-Y., Kwon, S.-W., Park, I.-C., Cha, I.-B., Tindall, B.J., Stackebrandt, E., Truper, H.G., and Go, S.-J., *Leadbetterella byssophila* gen. nov., sp. nov., Isolated from Cotton-Waste Composts for the Cultivation of Oyster Mushroom, *Int. J. Syst. Evol. Microbiol.*, 2005, vol. 55, pp. 2297–2302.

29. Marshall, K.C., Planktonic versus Sessile Life of Prokaryotes, in *The Prokaryotes*, Dworkin, M., Falkov, S., Rosenberg, E., Schleifer, K.-H., and Stackebrandt, E., Eds., New York: Springer, 2006, vol. 2, pp. 3–15.

30. Pinevich, A.V., *Mikrobiologiya. Biologiya prokariotov* (Microbiology. Biology of Prokaryotes), St. Petersburg: St. Peterb. Gos. Univ., 2007.

31. Bel'kova, N.L., Parfenova, V.V., Kostornova, T.Ya., Denicova, L.Ya., and Zaichikov, E.F., Microbial Biodiversity in the Water of Lake Baikal, *Microbiology*, 2003, vol. 72, no. 2, pp. 203–212.