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EXPERIMENTAL ARTICLES

Composition of the Microbial Communities of Bituminous Constructions at Natural Oil Seeps at the Bottom of Lake Baikal

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Abstract—Microbial communities of two bituminous constructions at the bottom of Lake Baikal in the region of natural oil seeps at a depth of 900 m have been investigated. Construction 8 contained biodegraded hydrocarbons, and construction 3, through which oil seeped, contained material that experienced biodegradation to a lesser degree. The composition of the microbial communities was studied by means of pyrosequencing of 16S rRNA gene fragments. Most of the bacterial 16S rRNA gene sequences identified in both bituminous constructions were attributed to proteobacteria, along with which *Actinobacteria*, *Acidobacteria*, *Bacteroidetes*, and TM7 were revealed. About 40% of the bacterial sequences in bituminous construction 3 belonged to representatives of uncultured groups within the classes *Alphaproteobacteria* and *Betaproteobacteria* and the phylum *Bacteroidetes*. The 16S rRNA gene sequences of archaea belonged to aceticlastic and hydrogenotrophic methanogens of the orders *Methanosarcinales*, *Methanomicrobiales*, and *Methanobacteriales*. The 16S rRNA genes of various groups of bacteria carrying out aerobic biodegradation of aromatic compounds and *n*-alkanes were found; their compositions differed between the constructions. Neither known groups of denitrifying betaproteobacteria nor known groups of sulfate-reducing deltaproteobacteria capable of carrying out anaerobic degradation of *n*-alkanes were found, which agrees with the low content of nitrate and sulfate in the water. In the anaerobic zone of bituminous constructions, the processes of biodegradation of hydrocarbons are probably carried out in the absence of alternative electron acceptors by the syntrophic community, including deltaproteobacteria of the genus *Syntrophus* and methanogenic archaea.

Keywords: hydrocarbon biodegradation, bituminous constructions, Lake Baikal, oil seep, syntrophy, pyrosequencing, 16S rRNA

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Lake Baikal is of tectonic origin; it is the world's deepest lake, and contains about 20% of the terrestrial surface waters. Mud volcanoes, pockmarks, cold seeps, hydrothermal springs, natural oil and gas seeps have been found at the bottom of the lake [1, 2]. Oil seeps occur extremely rarely in lakes; among the few examples are the deep-water Lake Tanganyika on the East African Rift, which features bitumen globules and liquid oil floating up to the surface [3], and Lake Chapala in Mexico, in which bituminous masses form small floating islets [4].

In 2005, a new oil seep was discovered in a deep-water area of Lake Baikal, near Cape Gorevoi Utes [5]. About four tons of oil reaches the lake surface annually. The oil composition gives evidence of formation in the Oligocene–early Miocene from the organic matter buried in freshwater reservoir sediments [5, 6]. The bottom sediments sampled in this region contained oil, bitumen, and methane hydrates [7].

The oil seep region at the bottom of Lake Baikal near the Cape of Gorevoi Utes was explored during submersions of the deepwater submersible vehicle (DSV) *Mir* in the course of the 2008–2009 expeditions. During investigations of the bottom of the lake, bituminous hills [7] formed at the sites of oil seep were discovered. These constructions were densely inhabited by deep-water invertebrates, whose population density exceeded that in the control bottom regions by an order of magnitude [7]. Most probably, this community does not depend on the supply of organic matter from the surface layers of water, but is based on the activity of microorganisms carrying out biodegradation of hydrocarbons [7].

The aim of the present work was to investigate the phylogenetic diversity of the microbial communities of two bituminous constructions by pyrosequencing 16S rRNA gene fragments.

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MATERIALS AND METHODS

Sampling. In 2008–2009, in the course of joint expeditions of researchers from the Institute of Limnology, Siberian Branch, Russian Academy of Sciences, and the Shirshov Institute of Oceanology, Russian Academy of Sciences, two bituminous constructions (3 and 8) were sampled using the DSV *Mir* in the oil seep region near Cape Gorevoi Utes. The lake depth in this region was 870–900 m; the water temperature was almost constant (3–4°C). The bitumen samples were separated from the constructions with manipulators and lifted aboard the research ship, where they were analyzed. In addition, near-bottom water was sampled using a board bathometer (st. 1), and bottom sediments were sampled in the region of constructions using a benthic tube (st. 2) and a hand net (st. 3).

Analysis of the composition of the pore water of bottom sediments. In order to carry out chemical analysis, the pore water samples from the constructions and the bottom sediments located between them were squeezed by double centrifugation (7000 and 14000 rpm). Before analysis, the samples were kept at 4°C. The anion and cation contents in water were determined according to the methods described earlier [8].

Analysis of the composition of bituminous constructions. The solutions of the contents of the bituminous constructions in methylene chloride were analyzed on a chromato-mass-spectrometer (GC-MS, Agilent, GC 6890, MSD 5973, United States) under the following conditions: DB-17 ms column, 30 m × 0.25 mm (for polycyclic aromatic hydrocarbons, PAH) or HT-8 column, 30 m × 0.25 mm (for *n*-alkanes); helium as the carrier gas. The analysis of *n*-alkanes was carried out with column temperature programming from 50 to 300°C; the analysis of PAH, with temperature programming from 95 to 310°C at a heating rate of 10°C min^{-1}; the injector temperature was 290°C; the quadruple temperature, 150°C; the sample volume introduced into the injector was 2 μL. The chromatographic peaks were recorded with full scanning of the mass spectra in the *m/z* range from 50 to 600. The peaks were identified using the LIB2NIST v1.0.0.8 mass spectral data software package, which includes a database of mass spectra of 150000 compounds.

When *n*-alkanes were quantitatively determined, the peaks in the chromatograms were recorded in the mode of monitoring selected ions with *m/z* 57 and 71; when PAH were determined, ions with *m/z* 128, 142, 152, 154, 166, 178, 192, 202, 228, 252, 272, and 278 were monitored. The internal standards used were a squalane solution (220 mg/mL, Supelco, United States) and a solution of deuterated PAH (naphthalene-d_8, acenaphthene-d_{10}, phenanthrene-d_{10}, chrysene-d_{12}, and perylene-d_{12}, 5 mg/mL each, Supelco, United States). The error of determination of *n*-alkanes and PAH did not exceed 10%.

Isolation of metagenomic DNA, PCR amplification and pyrosequencing of 16S rRNA gene fragments. The internal parts of the constructions were sampled for molecular-biological analyses with the observance of aseptic conditions. DNA isolation was carried out according to the technique described earlier [9]. The universal primers PRK341F (5'-CCT ACG GGR BGC ASC AG) and PRK806R (5'-GGA CTA CYV GGG TAT CTA AT) were used for the PCR-amplification of the 16S rRNA gene fragment that included the variable V3–V6 regions. Pyrosequencing of the PCR fragment obtained was carried out on a GS FLX genome analyzer (Roche, Switzerland) using the GS XLR70 Sequencing Kit.

Construction and analysis of libraries of *mcrA* and *alkB* gene fragments. The *mcrA* gene library was obtained using the MCRf (5'-TAY GAY CAR ATH TGG YT) and MCRr (5'-ACR TTC ATN GCR TAR TT) primers [10] to amplify corresponding DNA fragments. The *alkB* gene fragment library was obtained using the primers Alk3f (5'-TCGAGCACATC-CGCGGCCACCA) and Alk3r (5'-CCGTAGT-GCTCGACGTAGTT) [11]. The template was metagenomic DNA from construction 3. The PCR fragments obtained were cloned in the pGEM-T vector (Promega, United States), and the nucleotide sequences of independent clones were determined on an ABI3730 sequencer (Applied Biosystems, United States). The nucleotide sequences of the *mcrA* and *alkB* genes obtained in this work were deposited in the GenBank under the accession numbers KC525136–KC525158.

Analysis of the taxonomic composition of the community. Before carrying out the analysis, low-quality sequences, as well as those differing in the terminal sections from the primer sequences, were removed from the set of the 16S rRNA gene sequences determined by pyrosequencing. Such data filtration allows sequencing artifacts and errors to be minimized [12]. The resulting set of data contained 11952 sequences for sample 3 and 4031 sequences for sample 8.

The community composition was characterized with the RDP Classifier software package [13]. For this purpose, cluster analysis of the sequences obtained was performed. A representative nucleotide sequence corresponding to the cluster center was selected for each cluster using the Dereplicate Request program (RDP Classifier). The taxonomic identification of the sequences representing the cluster was performed by comparing them with the database of the 16S rRNA sequences in GenBank using BLASTN tool. The clusters including fewer than 20 sequences for sample 3 and fewer than 10 sequences for sample 8 were not analyzed. When a sequence with more than 97% similarity to the 16S rRNA gene of a validated microorganism was found, the cluster was assigned to the corresponding taxon. In the absence of such a

Fig. 1. Bituminous construction 3 near Cape Gorevoi Utes, depth of 830 m. The photograph was taken from board the DSV *Mir*. The white objects are flatworms and amphipods.

homolog, the taxonomic position of the cluster was determined by constructing a phylogenetic tree that included its representative sequence and a 16S rRNA gene sampling of bacteria or archaea. The nucleotide sequences were aligned using the Clustal X software [14]. The phylogenetic trees were constructed by the neighbor-joining method using the TREECONW software package [15].

RESULTS

Bituminous constructions and their chemical composition. Two bituminous constructions were studied. Construction 8 was 10 m high and about 50 m in diameter. At the construction periphery, there were "torches" giving evidence of the evolution of gas. The survey from aboard the DSV *Mir* showed that the construction was colored light brown, had a very irregular surface and ragged edges. The upper 1–2 cm of the construction substance was represented by hard and brittle material; deeper layers, by dark brown plastic substance having the properties of bitumen.

Construction 3 (Fig. 1) was at a distance from bituminous construction 8, in the region of the gas jets. It was cone-shaped, and had smaller dimensions: 1 m in height and 1.5 m in diameter. In contrast to construction 8, it was represented by viscous material of dark brown color; there was a vertically positioned tube at its top from which oil drops periodically oozed out.

The carbon content in the material of the constructions varied between 80 and 87%; that of hydrogen, between 12.3 and 13.3%; the mineral matter content was up to 1.1% (Table 1). Based on the carbon to hydrogen ratio (C/H = 6.7) and the presence in the IR spectra of the absorption bands of the CH_3, CH_2, and $(CH_2)_x$ groups characteristic of the structure of saturated hydrocarbons, the organic matter of the constructions was identified as paraffin petroleum bitumen. In the construction samples, the *n*-alkanes were identified as representing homolog series from *n*-C_{13} to *n*-C_{36}, and their maximums were shifted towards the high molecular homologs, as distinct from the oil sampled from the water surface. However, the compositions of the two constructions differed substantially. In

Table 1. Chemical composition of the material of bituminous constructions

Construction	Elements (%)		Mineral compounds, %	*n*-Alkane content, mg/g	PAH content, mg/g
	C	H			
3	80.3–82.4	12.3–13.4	1.1	770	0.98
8	85.8–87.2	12.3–13.0	<0.1	150	0.29

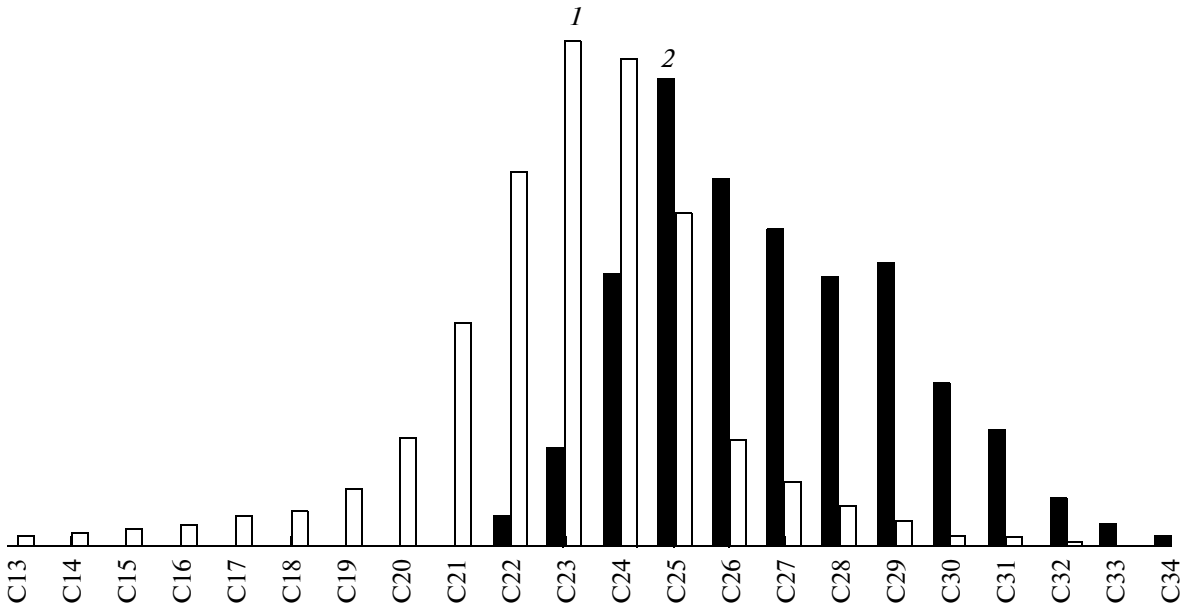

Fig. 2. Shares (%) of *n*-alkanes with different chain lengths in the construction material (*1*, construction 3; *2*, construction 8).

the material of construction 3, *n*-alkanes constituted about 77%, while in construction 8, their share accounted for only 15%, and the chromatograms showed the peak of naphthenoaromatic compounds that are formed as a result of oil biodegradation. The smaller amount of *n*-alkanes in the construction 8 material correlated with a shift in the maximum of the homolog series of *n*-alkanes towards the higher paraffins region (Fig. 2). Most probably, only long-chain *n*-alkanes, more resistant to biodegradation, were retained in construction 8.

The pore water composition was determined for bituminous construction 8 and the bottom sediments adjacent to it. By chemical composition, these are hydrocarbonate-calcium waters characterized by low mineralization (Table 2). In particular, the sulfate and, especially, nitrate concentrations in the construction pore waters were lower than in the near-bottom water, which may be indicative of the expenditure of these ions in the course of the biogeochemical processes in the construction thickness. The near-bottom water content of oxygen in the region of bituminous constructions was about 7 mg/L (Table 2), which creates conditions for aerobic oxidation of hydrocarbons of the constructions.

Diversity of bacterial 16S rRNA genes in the libraries constructed on the basis of DNA of the microbial communities of the bituminous constructions. 11952 sequences for construction 3 and 4031 sequences for construction 8 were determined by pyrosequencing of 16S rRNA gene fragments. Cluster analysis showed that the library of 16S rRNA genes of

Table 2. Composition of near-bottom water, pore water of bottom sediments and bituminous construction 8

Sample	Ion contents, mg/L								O_2, mg/L	pH
	HCO_3^-	Cl^-	NO_3^-	SO_4^{2-}	Na^+	K^+	Ca^{2+}	Mg^{2+}		
Near-bottom water (st. 1)	66.90	1.31	0.84	2.37	4.25	2.64	16.1	0.61	6.9	7.47
Pore water from construction 8	67.29	0.77	0.00	1.20	4.41	2.39	17.8	0.21	ND	ND
Pore water from the sediment between the constructions (st. 2), depth of 0–1 cm	45.90	1.33	0.00	3.71	4.67	2.58	11.2	0.32	ND	ND
Pore water from the sediment between the constructions (st. 2), depth of 8–10 cm	49.55	0.41	0.00	3.56	2.4	1.81	13.3	0.63	ND	ND
Pore water from the sediment between the constructions (st. 3), depth of 0–2 cm	24.40	0.78	0.00	8.90	2.61	1.95	8.8	0.13	ND	ND

Note: ND stands for "not determined."

the microbial community of construction 3 contained 5370 sequences of bacteria and 4520 sequences of archaea. For construction 8, the number of the identified sequences of bacteria and archaea was 2240 and 1405, respectively. The data on the phylogenetic composition of the communities are presented in Table 3.

Among the identified 16S rRNA gene sequences of the bacterial community of construction 8 (Fig. 3), the overwhelming majority belonged to proteobacteria of the classes *Betaproteobacteria* (39.7% of sequences), *Gammaproteobacteria* (19.4%), *Alphaproteobacteria* (11.2%), *Deltaproteobacteria* (5.6%), and *Epsilonproteobacteria* (4.0%). The minor components of the community belonged to *Actinobacteria* (5.8%), *Acidobacteria* (6.2%), *Caldiserica* (1.8%), *Bacteroidetes* (1.6%), TM7 (1.2%), and *Firmicutes* (1.1%).

The revealed 16S rRNA gene sequences of betaproteobacteria belonged to representatives of the families *Rhodocyclaceae* (of the genera *Zoogloea, Georgfuchsia*, and *Dechloromonas*), *Burkholderiaceae* (of the genera *Burkholderia, Cupriavidus*, and *Ralstonia*) and *Comamonadaceae* (*Acidovorax* and *Delftia*) and of the genus *Aquabacterium*. The capacity for alkane degradation under aerobic conditions has been shown for the bacteria of the genera *Burkholderia* and *Ralstonia*. Almost half the 16S rRNA genes of betaproteobacteria belonged to uncultured representatives of the family *Rhodocyclaceae*. Close 16S rRNA gene sequences (98–99% identity) were earlier discovered during analyses of the microbial communities of soils, activated sludge, subsurface waters, and hydrocarbons-contaminated sediments (accession numbers FQ659272, FQ660535, JX271909, JQ919631, JX012267).

Certain betaproteobacteria are known to be capable of anaerobic degradation of aromatic compounds and alkanes using nitrate as electron acceptor [16]. However, the known hydrocarbon-degrading denitrifying betaproteobacteria, which belong to the *Azoarcus-Thauera* group [17], were not detected.

The second most abundant group of bacterial sequences belonged to gammaproteobacteria; the overwhelming majority of them represented the genus *Pseudomonas* (17%). Many bacteria of this genus are capable of aerobic hydrocarbon degradation. For example, *Pseudomonas fluorescens* CHA0 can utilize even long-chain (C_{18}–C_{28}) *n*-alkanes [18]. The sequences of alphaproteobacteria mainly belonged to the aerobic heterotrophs of the genera *Sphingomonas* (1.1%), *Sphingobium* (0.6%), and *Caulobacter* (2.8%), which are capable of degrading aromatic hydrocarbons. One more bacterial group (about 1% of the 16S rRNA gene sequences) for which the capacity for degradation of *n*-alkanes and certain aromatic compounds was shown were the moderately thermophilic aerobic bacteria of the genus *Geobacillus*, which are often isolated from petroleum reservoirs [19].

The sequences of deltaproteobacteria constituted about 5.6% in the library of the 16S rRNA genes of the

Table 3. Phylogenetic diversity of the 16S rRNA genes of bacteria and archaea of the microbial communities of the bituminous constructions

Taxonomic group	Share in the community (%)*	
	construction 3	construction 8
Bacteria		
Alphaproteobacteria		
Acetobacteraceae	–	1.2
Bradyrhizobiaceae	6.7	3.4
Caulobacteraceae	0.4	2.8
Rhodobacteraceae	5.0	1.6
Sphingomonadaceae	1.0	1.7
Uncultured groups	13.8	0.5
Betaproteobacteria		
Aquabacterium	3.2	1.7
Burkholderiaceae	–	10.9
Comamonadaceae	6.6	2.5
Methylibium	5.2	–
Rhodocyclaceae	0.4	8.5
Uncultured groups	14.4	15.4
Others	0.7	0.7
Gammaproteobacteria		
Methylobacter	0.6	–
Pseudomonas	–	17.2
Others	5.7	2.2
Deltaproteobacteria		
Geobacter	–	0.5
Syntrophobacter	–	0.5
Syntrophus	0.6	4.6
Others	0.9	–
Epsilonproteobacteria	–	4.0
Actinobacteria		
Cellulomonas	–	3.0
Nocardia	6.6	–
Others	1.3	2.8
Bacteroidetes	13.2	1.6
Acidobacteria	1.1	6.2
TM7	3.9	1.2
Caldiserica	–	1.8
Firmicutes	–	1.1
Verrucomicrobia	1.4	–
Chlorobi	1.2	–
Spirochaetes	1.7	–
Other bacteria	4.4	2.4
Archaea		
Methanosarcinales	58.8	27.7
Methanimicrobiales	25.2	55.9
Methanobacteriales	15.4	16.4
TMEG	0.6	–

* Of all 16S rRNA gene sequences of bacteria or archaea, respectively.

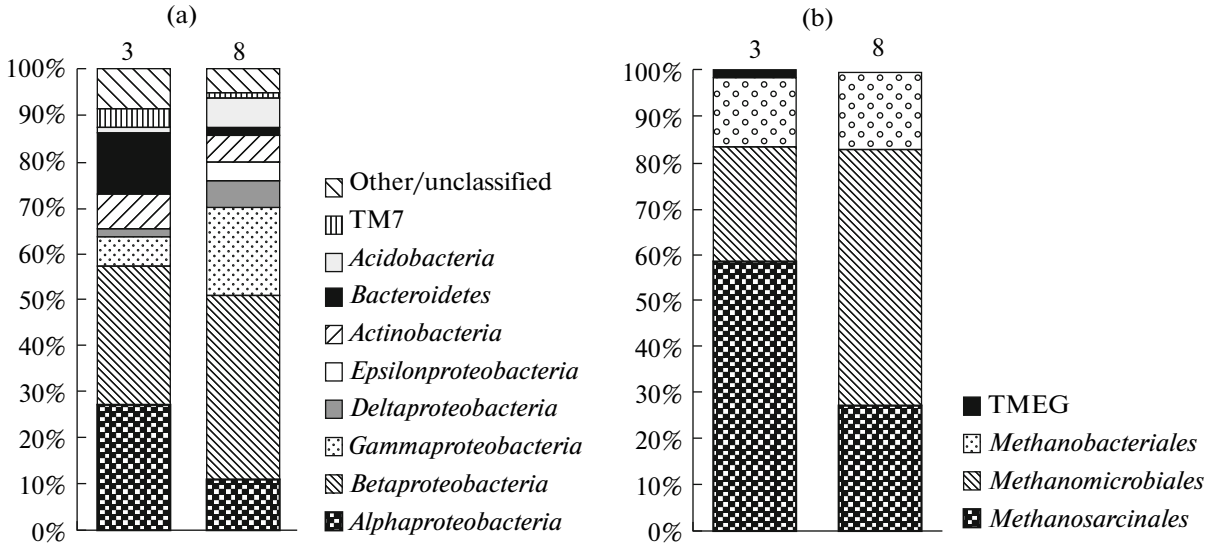

Fig. 3. Composition of microbial communities of the bituminous constructions as determined by analysis of the 16S rRNA gene sequences. (a) *Bacteria*, (b) *Archaea*.

bacterial community of construction 8. Sulfate-reducing bacteria of this class are widespread in petroleum reservoirs [20]; among them, strains also capable of anaerobic degradation of aromatic hydrocarbons and *n*-alkanes have been described [21]. However, the 16S rRNA genes of known sulfate-reducing bacteria were not found among the deltaproteobacterial sequences, most of which belonged to bacteria of the genera *Syntrophus, Syntrophobacter*, and *Geobacter*. The representatives of the first two genera are anaerobic bacteria characterized by syntrophic metabolism, in the course of which the degradation of organic compounds (fatty acids, sugars, hydrocarbons, etc.) is thermodynamically advantageous only if the concentration of the molecular hydrogen formed upon fermentation is maintained at a very low level as a result of its consumption by a partner microorganism, e.g., a methanogenic archaeon [22]. In particular, the conversion of hexadecane to methane by a consortium including *Syntrophus* bacteria and methanogenic archaea has been described [23, 24]. The bacteria of the genus *Geobacter* are capable of anaerobic degradation of aromatic hydrocarbons coupled to reduction of iron(III) oxide [25].

The 16S rRNA gene sequences of the domain *Bacteria* in the library of the microbial community of construction 3, through which oil seeped, belonged to the same main groups as in the library of the community of construction 8, but their ratio and the species composition were substantially different. 30.5% of the sequences belonged to betaproteobacteria, and almost half of them represented uncultured bacteria present also in the construction 8 library. However, representatives of the families *Burkholderiaceae* (which includes bacteria carrying out aerobic degradation of hydrocarbons, see above) and *Rhodocyclaceae* were virtually absent from the sequences of the betaproteobacteria identified; instead, 16S rRNA genes of the methylotrophic bacteria of the genus *Methylibium*, capable of oxidizing aromatic hydrocarbons, were revealed [26]. The 16S rRNA gene sequences of alphaproteobacteria constituted a considerable share in the library of the bacterial community of construction 3 (26.9%) and belonged to several uncultured lineages. In contrast to the library of construction 8, much fewer sequences of gammaproteobacteria (6.3%) were found in the library of construction 3; no *Pseudomonas* representatives were revealed. Deltaproteobacteria (6.3%, with syntrophic bacteria being in the minority) were represented in smaller numbers in the library of construction 3. *Acidobacteria* were also poorly represented (1.1%). The 16S rRNA gene sequences of *Actinobacteria* constituted 8% and belonged to the genus *Nocardia*, whose representatives are able to oxidize alkanes aerobically. The most clear distinction of the two libraries was the large share (13.2%) in the library of construction 3 of the 16S rRNA genes of bacteria of the phylum *Bacteroidetes*. Almost all of them belonged to the microorganisms of several uncultured lineages. 16S rRNA sequences close to them were retrieved during the study of the microbial communities of soils and lacustrine sediments (FR667317, AB658249, EU978785).

Diversity of the 16S rRNA genes of archaea in the libraries constructed on the basis of DNA from the microbial communities of bituminous constructions. Archaeal sequences belonging to methanogens of the orders *Methanosarcinales, Methanomicrobiales*, and *Methanobacteriales* were found in the libraries of the16S rRNA genes of both communities (Table 3). The *Methanosarcinales* sequences identified were close (98–99% identity) to those of the aceticlastic

methanogens of the genus *Methanosaeta*, which utilize acetate for methane formation. Two other groups of sequences belonged to hydrogen-utilizing methanogens [27]. The archaea of the orders *Methanosarcinales* and *Methanomicrobiales* were found earlier in the methane hydrate-bearing sediments of Lake Baikal [8, 28]. Apart from the methanogens sequences, the library of the construction 3 community contained 16S rRNA genes of representatives of the uncultured TMEG lineage of the phylum *Euryarchaeota*, also found earlier in the hydrate-bearing sediments of Lake Baikal [28].

The answer to the question of whether the identified sequences of archaea belong to methanogens or to the organisms referred to as ANME and carrying out the reverse reaction, i.e., anaerobic methane oxidation, is crucially important for analysis of the pathways of methane biotransformation. Phylogenetically, the ANME archaea belong to three groups, the first of which, ANME-1, forms an isolated branch in the phylogenetic tree of archaea, and the other two, ANME-2 and ANME-3, form separate branches within the order *Methanosarcinales*.

The 16S rRNA sequences of archaea revealed by us did not belong to the ANME-1, ANME-2, or ANME-3 groups. However, for independent confirmation of this conclusion, we carried out phylogenetic analysis of the α-subunit of methyl-coenzyme M reductase (McrA), one of the key enzymes of methanogenesis. Both methanogens and the ANME archaea possess methyl-coenzyme M reductase: it is involved it in both processes. Three groups of sequences were revealed in the library of cloned McrA gene fragments that we obtained for the bituminous construction 3 (Fig. 4). The first group (four clones) belonged to representatives of the order *Methanomicrobiales*; the second (five clones), to the archaea of the order *Methanobacteriales*; and the third group (three clones), to *Methanosaetaceae*. Thus, phylogenetic analysis of both the 16S rRNA and McrA gene sequences confirms that the archaea detected belong to methanogenic and not methane-oxidizing groups.

Identification of the alkane hydroxylase genes (*alkB*). Alkane hydroxylases are the key enzymes in the processes of aerobic oxidation of hydrocarbons [29]. Therefore, we performed an experiment to detect *alkB* genes by PCR in order to subsequently identify the corresponding microorganisms. It should be emphasized that, as distinct from *mcrA*, the *alkB* genes are more diverse, which makes the development of universal primers for their detection practically impossible [11]. However, the detection of a sequence close to the *alkB* of a known microorganism will indicate the possibility of *n*-alkanes degradation by the partucular bacteria present in the community. Of the 11 *alkB* fragment sequences obtained for the construction 3 metagenome, six exhibited high homology to the *alkB* gene of genus *Nocardia* actinobacteria, and one sequence, with *alkB* of bacteria of the genus *Burkholderia*, which may testify to the involvement of these groups in the aerobic degradation of alkanes in the bituminous constructions. The rest sequences were homologous to the *alkB* genes of uncultured bacteria or had no close homologs in public databases.

DISCUSSION

One of the features of Lake Baikal is the presence of bituminous constructions at the bottom of the lake at the sites of natural oil seeps. The material of the bituminous constructions serves as a source of carbon and energy for the microorganisms inhabiting them; however, a limiting factor in the use of *n*-alkanes is the availability of electron acceptors whose role can be played by oxygen, nitrate, sulfate, and iron and manganese oxides. Under anaerobic conditions, hydrocarbons may also be degraded by syntrophic associations in which the bacterial partner degrades hydrocarbons with the formation of acetate and hydrogen, further utilized by methanogenic archaea.

Apparently, the constructions samples taken for analysis contained, along with the anaerobic zone, sites that were in contact with oxygen-containing lacustrine water and experienced aerobic or microaerobic conditions (sites near fractures, etc.). The analysis of the 16S rRNA gene sequences of the microbial communities of both constructions revealed various groups of bacteria known to be capable of aerobic degradation of aromatic hydrocarbons and *n*-alkanes. The compositions of these groups differed significantly between the constructions. For example, the 16S rRNA gene sequences of *Pseudomonas* and *Burkholderia* bacteria predominated in the construction 8 library but were virtually absent from the construction 3 library, where actinobacteria of the genus *Nocardia* were revealed. This was probably due to the difference between the material of the construction 8, which contained the most degradation-resistant long-chain *n*-alkanes, and the material of construction 3, where the hydrocarbon biodegradation processes were less pronounced. However, in both libraries, less than half the 16S rRNA gene sequences belonged to the bacteria for which the capacity for aerobic *n*-alkane oxidation is documented. It is possible that the uncultured representatives of alphaproteobacteria, betaproteobacteria and *Bacteroidetes*, accounting for about 40 and 16% of the bacterial 16S rRNA gene sequences in the construction 3 and construction 8 libraries, respectively, are involved in the process of aerobic degradation of hydrocarbons (Table 3). This suggestion is evidenced by the detection of the similar 16S rRNA sequences of uncultured bacteria in various hydrocarbon-contaminated ecosystems, as well as by the detection of the *alkB* genes not assigned to known groups of bacteria.

Only a small fraction of the identified bacterial 16S rRNA gene sequences belonged to the groups known to display the capacity for anaerobic degradation of

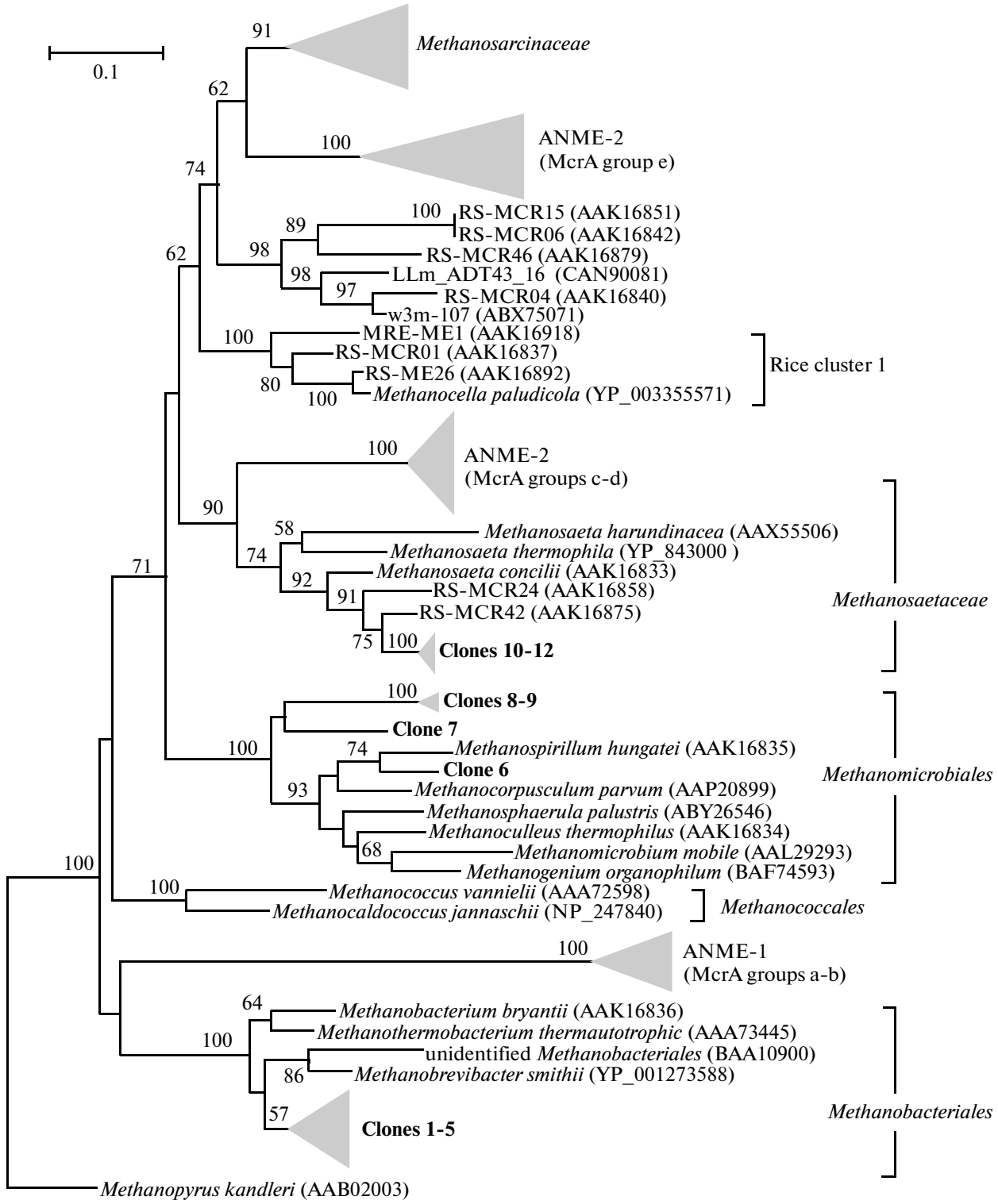

Fig. 4. Phylogenetic tree of the amino acid sequences of McrA gene fragments obtained in this work (Clones 1–12) and of representatives of known methanogenic and ANME lineages of archaea. The tree was constructed using the neighbor-joining method; the *Methanopyrus kandleri* McrA was used as the outgroup. The numbers at the branching points indicate bootstrap support (100 replicas); only values higher than 50% are shown. The scale bar corresponds to 0.1 substitution per site. The GenBank accession numbers are indicated in parentheses after the names of organisms or clones.

hydrocarbons. This is likely to be the consequence of the low availability of nitrate and sulfate, the electron acceptors alternative to oxygen (Table 1). No 16S rRNA gene sequences of known sulfate-reducing bacteria or of betaproteobacteria of the *Azoarcus–Thauera* group [17] capable of degrading hydrocarbons were discovered. The 16S rRNA genes of the *Georgfuchsia* betaproteobacteria (0.9 and 0.4% in the libraries of constructions 8 and 3, respectively) and of *Geobacter* deltaproteobacteria, which are capable of anaerobic degradation of aromatic hydrocarbons coupled to iron(III) oxide reduction, were detected in minor amounts. In the absence of alternative electron acceptors in the anaerobic zone of bituminous constructions, the processes of hydrocarbon degradation may be carried out by the syntrophic community that includes deltaproteobacteria of the genus *Syntrophus* and methanogenic archaea. It has been assumed that syntrophy may be the main mechanism of hydrocarbons biodegradation in subsurface petroleum reservoirs [30]. Our findings show that similar processes may also occur in bituminous constructions at the bottom of Lake Baikal.

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