Microbiology, Vol. 74, No. 3, 2005, pp. 314–320. Translated from Mikrobiologiya, Vol. 74, No. 3, 2005, pp. 370–377.

EXPERIMENTAL ARTICLES

The First Results of an Investigation into the Phylogenetic Diversity of Microorganisms in Southern Baikal Sediments in the Region of Subsurface Discharge of Methane Hydrates

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Received May 5, 2004; in final form, January 17, 2005

Abstract—Phylogenetic analysis of the bacterial communities in Lake Baikal bottom sediments in the region of subsurface methane hydrate discharge has been carried out using data on 16S rRNA sequences. The composition of these microbial communities is shown to be different in different horizons. Methanotrophic bacteria are found in the surface layer (0–5 cm), and uncultured bacteria constitute a great portion of this population. In deeper sediment layers (92–96 cm), a change in the microbial community occurs; specifically, a decreased homology with the known sequences is observed. The new sequences form separate clusters on a phylogenetic tree, indicating the possibly endemic nature of the bacteria revealed. Organisms related to the genus *Pseudomonas* constitute the main portion of the population. An archaea-related sequence was found in a horizon containing gas hydrate crystals (100–128 cm). Uncultured bacteria remain predominant.

Key words: Lake Baikal, bottom sediments, biodiversity, 16S rRNA.

The research carried out during previous decades has demonstrated the presence of a massive gas hydrate (GH) layer at the bottom of Lake Baikal [1]. It occupies a relatively large area in the southern and central Baikal [2]. Evidence exists of GH discharge into the water column via channels in the lake sediments, which has been registered by BSR (apparent reflecting boundary on seismic records, logging characteristics) [3, 4]. The first GH samples were obtained by deep-water drilling in southern Baikal. An isotopic analysis revealed that they had a high content of biologically produced methane [5, 6]. We obtained methane hydrates (MHs) (Fig. 1), which decompose with fuel gas formation, in March 2000 by sampling from the ice cover and, in subsequent years, by sampling from the research vessel *Vereshchagin* [3].

A number of works have been published dealing with the phylogenetic diversity of microorganisms inhabiting hydrate-containing sediments of marine ecosystems [7, 8]. The data presented in them were obtained by sequencing 16S rRNA genes and by fluorescent in situ hybridization (FISH). They showed the presence of specific bacterial communities involved in methane production and oxidation under anaerobic conditions [9]. It was found that the latter process may occur via syntrophic interaction of archaea (the ANME-1 and ANME-2 groups) and sulfate reducers [10]. Further investigations demonstrated that anaerobic methane oxidation can occur without sulfate reducers [11] and that archaea play the main role in this process. The state of GHs in marine ecosystems is believed to be directly related to the microorganisms present both in the crystals themselves and in the surrounding sediments [7]. Lake Baikal is, at present, the only freshwater ecosystem where MHs have been found. Measurement of the rates of bacterial methane production and oxidation in Baikal provides evidence of its high potential in relation to anaerobic bacterial methane production and oxidation [12].

We performed comprehensive studies of the microbial communities of GH-containing sediments in one of the zones of Lake Baikal. The present paper reports the results of an investigation into the phylogenetic diversity of the bottom-sediment microorganisms, performed on the basis of 16S rRNA sequence analysis.

MATERIALS AND METHODS

Materials. The samples for analysis were collected in the summer of 2002 in the vicinity of an underwater structure in the southern part of Baikal (assumed name, crater Malenki; coordinates, 51°55,200′ N, 105°38,105′ E; water depth, 1380 m). A large gravity tube was used for sampling. The maximum length of the MH-containing core was 128 cm. After lifting, the core was opened

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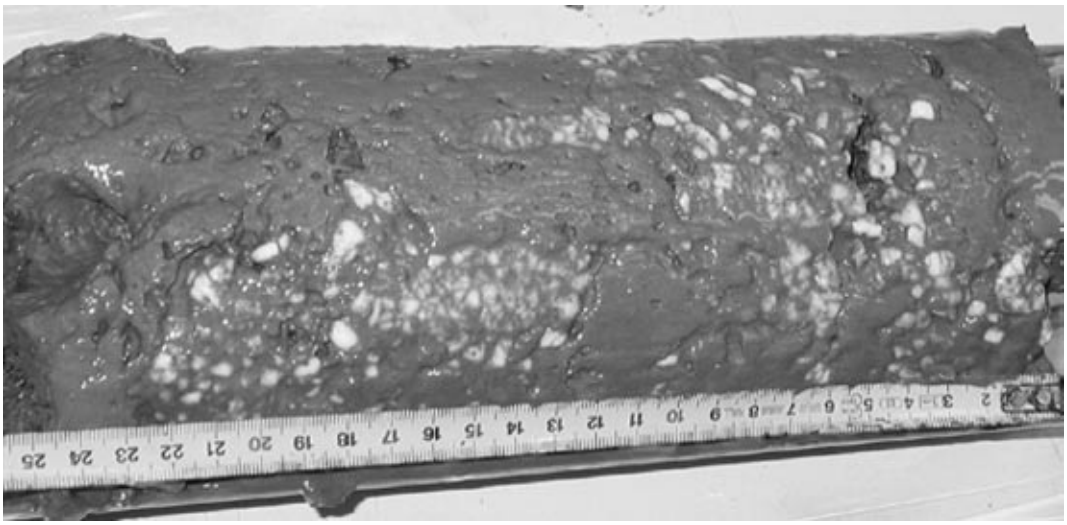

Fig. 1. Methane hydrates in bottom sediments from Lake Baikal bottom sediments at the crater Malenki station.

as quickly as possible. Half of it was placed in sterile bags and stored in liquid nitrogen in preparation for molecular biological work under laboratory conditions. The second half was used to determine the physicochemical characteristics.

DNA extraction from the bottom sediments. A modification of the Marmur's method [13] was used for DNA extraction. The sediment (2 to 5 g) was homogenized in an agate mortar with SiC (Carborundum) for 5–10 min. Then, polyvinylpyrrolidone (PVP) (0.3 g per gram sediment), TE buffer (100 mM Tris–HCl (pH 8.0) and 10 mM EDTA), and lysozyme (the final concentration was 15 μg/ml) were added, and the mixture was incubated for 1 h at 37°C and stirred every 10 min. After the addition of a 10% sodium dodecyl sulfate solution to a final concentration of 1%, the lysate was incubated for 15 min at 37°C. Three consequent freezing–thawing cycles followed (10 min at –20°C and 10 min at 56°C). The mixture was then centrifuged at 8000 *g* for 15 min. For more compete sorption of humic acids, PVP was once more added to the supernatant, as described in [14]. After mixing and extraction with an equal volume of phenol, the mixture was centrifuged to separate the phases. The upper phase was collected and extracted with an equal volume of chloroform. Then, DNA was precipitated with a double volume of ethanol and a 0.1 volume of 3 M sodium acetate. The precipitate was washed twice with 70% ethanol, dried, and dissolved in 100 μl of bacteria-free water. The presence of the isolated DNA was checked by a PCR amplification of eubacterial 16S rDNA with universal primers and a subsequent analysis in 1% agarose gel.

Amplification of the DNA fragment corresponding to the 16S rRNA gene. A polymerase chain reaction (PCR) was performed with every DNA sample isolated from a certain horizon. For this purpose, 500L and 1350R oligonucleotide primers, which are complementary to the most conservative regions of the eubacterial 16S rRNA gene (their annealing regions are in the vicinity of nucleotides 500 and 1350 in *Escherichia coli* numbering) were used. Amplification was performed using a PROGENE thermal cycler in the mode providing for the highest presentation of diverse sequences: 94°C, 4 min (one cycle); 94°C, 1 min; 48°C, 1.1 min; 72°C, 1.3 min (five cycles); 92°C, 1 min; 50°C, 1.1 min; 72°C, 1.3 min (ten cycles); 92°C, 1 min; 52°C, 1.1 min; 72°C, 1.1 min (ten cycles); 92°C, 1 min; 54°C, 1.1 min; 72°C, 1.3 min (ten cycles); 72°C, 10 min; 4°C, 10 min. The reaction mixture (15 μl) contained 16 mM Tris–HCl buffer; 0.1% Tween 20; 2.5 mM $MgCl_2$; deoxynucleotide triphosphates, each at a concentration of 0.2 μM; primers (500L: CGTGCCAGCAGCCGCGGTAA and 1350R: GACGGGCGGTGTGTACAAG), each at a concentration of 0.1 μM, and 0.05 U of *Taq* DNA polymerase.

Cloning of PCR products and clone analysis. The pGEM®-T Vector System I (Promega) kit was used for ligation of the PCR products of the 16S rRNA gene fragment. Ligation and transformation were performed using standard procedures [15]. Colonies that exhibited the lac$^-$ phenotype on a selective medium containing the β-galactosidase chromogenic substrate *X*-gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) were selected. White colonies were transferred to Eppendorf tubes with 50 μl of bacteria-free water, boiled for 5 min, and then frozen. Clones containing DNA insertions of an expected size were revealed by PCR analysis using 1 μl of the boiled cell suspension as the template and standard primers homologous to the plasmid polylinker ends (40R: CAGCTATGACCATGATTAC and 297F: TTGTAAAACGACGGGCAGC). The reaction conditions were as follows: 94°C, 5 min; 57°C, 1.1 min; and 72°C, 1.3 min (1 cycle); 94°C, 1 min; 57°C, 1.1 min; and 72°C, 1.3 min (30 cycles); 72°C,

Table 1. The number of clones obtained from the bottom sediment core taken at the crater Malenki station and the phylogenetic groups found

Horizon, cm	Number of clones		Phylogenetic groups
	obtained	sequenced	
0–5	20	10	CF and γ- and β-proteobacteria
92–94	85	9	γ- and β-proteobacteria
94–96	103	6	γ- and δ-proteobacteria
100–128	186	7	Archaea and γ-proteobacteria

10 min (1 cycle); and 4°C, 10 min. The reaction products were analyzed in 1% agarose gel. After staining with ethidium bromide, the bands corresponding in size to the insertions of the 16S rRNA gene fragment were excised. The gel fragments were placed in an electroelution chamber, and the DNA was eluted for 40 min with a saturated ammonium acetate solution. The eluted material was precipitated with a double volume of ethanol, with glycogen as the coprecipitator; stored overnight at –20°C; centrifuged at 8000 *g*; washed twice with 70% ethanol; dried; and dissolved in 15 μl of bacteria-free water.

Sequencing of gene fragments. Single-letter sequencing (a reaction with ddGTP only) was performed in order to reveal identical clones. The clones with different patterns of single-letter sequences were sequenced using the Applied Biosystems 373 (United States) automatic nucleic acid sequencer. The nucleotide sequences thus obtained were analyzed by searching for homologous sequences in three databases, GenBank, EMBL, and DDBJ, using the BLASTN software package. In order to establish homologies among the novel sequences, the CLUSTAL W software package was used. The novel sequences were sent to GenBank (accession numbers from AY739914 to AY739938).

Phylogenetic analysis was performed by means of the TREECON W software package, and phylogenetic trees were constructed using the neighbor joining method.

RESULTS AND DISCUSSION

We extracted total DNA from four core horizons: the layer containing MHs (100–128 cm), the two layers above it (92–94 and 94–96 cm), and the surface layer (0–5 cm). The amplification products of these DNAs obtained using highly conservative bacterial primers were cloned, and the insertions containing the 16S rRNA gene fragment were sequenced. From the four samples of the core taken near crater Malenki, 394 clones of 16S rRNA gene fragments of Baikal bottom sediment microorganisms were obtained; for 32 of them, the nucleotide sequences were determined (Table 1).

For each of the sequences thus obtained, a search for the closest homologue was performed using the BLASTN software package. The data on the sequences most closely related to the Baikal sequences are presented in Tables 2–5. The BLASTN searches showed that all of the clones investigated were phylogenetically different and belonged to the following groups and subgroups: archaea; *Cytophaga–Flavobacterium* (CF); and gamma-, beta-, and delta-proteobacteria. The group of proteobacteria was the most voluminous (22 clones) and was represented in all of the sediment layers investigated. This group is known to be very diverse and widespread in nature; its representatives have already been found in the vicinity of gas hydrate discharge in the Gulf of Mexico [7].

Analysis of the nucleotide sequences of the Baikal microorganisms from the surface sediment layer revealed that they all belonged to different clusters, and most of them were closest to uncultured bacteria (Fig. 2).

Table 2. The bacteria from the GenBank database most closely related, according to 16S rRNA gene sequences, to bacteria from Baikal bottom sediments in the region of methane hydrate subsurface discharge (crater Malenki station; sediment depth, 0–5 cm)

Clone no.	Closest relative	% similarity and GenBank no.	Phylogenetic group	Source of the closest relative
M 3-1	Uncultured *Bacteroides*	98, AF529117	Flavobacteria	Natural sample
M 3-4	*Ralstonia* sp.	96, AY191856	β-proteobacteria	Sediments
M 3-7	Uncultured soil bacterium	96, AY326597	β-proteobacteria	Natural sample
M 3-13	Uncultured bacterium	98, AJ535221	γ-proteobacteria	Natural sample
M 3-11	*Methylobacter psychrophilus*	96, AF152597	γ-proteobacteria	Polar tundra
M 3-20	Uncultured *Bacteroides*	98, AF388891	Flavobacteria	Natural sample
M 3-3	Uncultured eubacterium WCHA1-52	96, AF050594.1	β-proteobacteria	Natural sample
M 3-6	Uncultured bacterium from Lake Michigan	96, AF320969	β-proteobacteria	Sediments, Lake Michigan
M 3-12	Uncultured soil bacterium	96, AF128684	β-proteobacteria	Natural soil sample
M 3-9	Phenanthrene-degrading bacterium	97, AY177375	β-proteobacteria	Natural soil sample

Table 3. The bacteria from the GenBank database most closely related, according to 16S rRNA gene sequences, to the bacteria from Baikal bottom sediments in the region of methane hydrate subsurface discharge (crater Malenki station; sediment depth, 92–94 cm)

Clone no.	Closest relative	% similarity and GenBank no.	Phylogenetic group	Source of the closest relative
M 1-92	*Pseudomonas* sp.	96, AF098466	γ-proteobacteria	Arctic soils
M 2-92	Uncultured bacterium AT425	96, AY053484.1	γ-proteobacteria	Natural water sample
M 3-92	*Pseudomonas* sp.	97, AY030314	γ-proteobacteria	Not reported;
M 5-92	*Ralstonia* sp.	98, AY216798.1	β-proteobacteria	Natural sediment sample
M 12-92	Uncultured bacterium	92, AF428742	Δ-proteobacteria	Natural sample
M 13-92	*Pseudomonas* sp.	97, AY030314	γ-proteobacteria	Laboratory strain
M 14-92	*Staphylococcus capitis*	97, AY030 321	Low G+C Gram-positives	Laboratory strain
M 15-92	*Pseudomonas* sp.	96, AF098466	γ-proteobacteria	Arctic soils
M 16-92	*Pseudomonas flavescens*	98, PFL308320	γ-proteobacteria	Not reported

Table 4. The bacteria from the GenBank database most closely related, according to 16S rRNA gene sequences, to the bacteria from Baikal bottom sediments in the region of methane hydrate subsurface discharge (crater Malenki station; sediment depth, 94–96 cm)

Clone no.	Closest relative	% similarity and GenBank no.	Phylogenetic group	Source of the closest relative
M 2-94	Uncultured δ-proteobacterium	94, AF424235	Δ-proteobacterium	Natural sample
M 3-94	*Pseudomonas fragi*	98, AY195842	γ-proteobacterium	Laboratory strain
M 7-94	*Pseudomonas flavescens*	97, PFL308320	γ-proteobacterium	Laboratory strain
M 8-94	Uncultured γ-proteobacterium	97, AB074733	γ-proteobacterium	Sediments
M 9-94	*Pseudomonas flavescens*	98, PEL308320	γ-proteobacterium	Laboratory strain
M 10-94	*Pseudomonas* sp.	96, AY014813	γ-proteobacterium	Natural sample

The Baikal bacterial sequences M 3-20 and M 3-1 fell into the *Cytophaga–Flavobacterium* group, with a similarity at the species level (98%) [7]. Representatives of this phylogenetic group have also been found in gas hydrates in the Gulf of Mexico [7]. Clone M 3-3 exhibited the highest homology with the representatives of the phylogenetic group of gram-positive bacteria (96% similarity). The sequence of clone M 3-11, also found in the surface layer of the sediment, indicated the presence therein of DNA closely related to type I methylotrophic bacteria. This clone demonstrated a 96% homology with an uncultured species related to *Methylobacter psychrophilus*. It should be noted that the sequences predominant in this sediment layer (five sequenced clones out of ten)

Table 5. The bacteria from the GenBank database most closely related, according to 16S rRNA gene sequences, to the bacteria from Baikal bottom sediments in the region of methane hydrate subsurface discharge (crater Malenki station; sediment depth, 100–128 cm)

Clone no.	Closest relative	% similarity and GenBank no.	Phylogenetic group	Source of the closest relative
M 2-128	Uncultured bacterium	95, AF245405	Low G+C Gram-positives	Methanogenic community
M 7-128	Uncultured archaeon	96, AB056054	Archaea	Anaerobic layer of rice paddy soil
M 11-128	Uncultured bacterium	96, AF419682	Low G+C Gram-positives	Anaerobic methanotrophic community, hydrothermal sediments
M 12-128	Uncultured bacterium	87, AY133358	Low G+C Gram-positives	Antarctic sediments
M 13-128	*Pseudomonas* sp	97, AF513431	γ-proteobacteria	Arctic ice
M 17-128	*Pseudomonas* sp.	93, PSP391194	γ-proteobacteria	Natural sample, Adriatic Sea
M 16-128	*Pseudomonas* sp.	96, AY214345	γ-proteobacteria	Deep-sea sediments

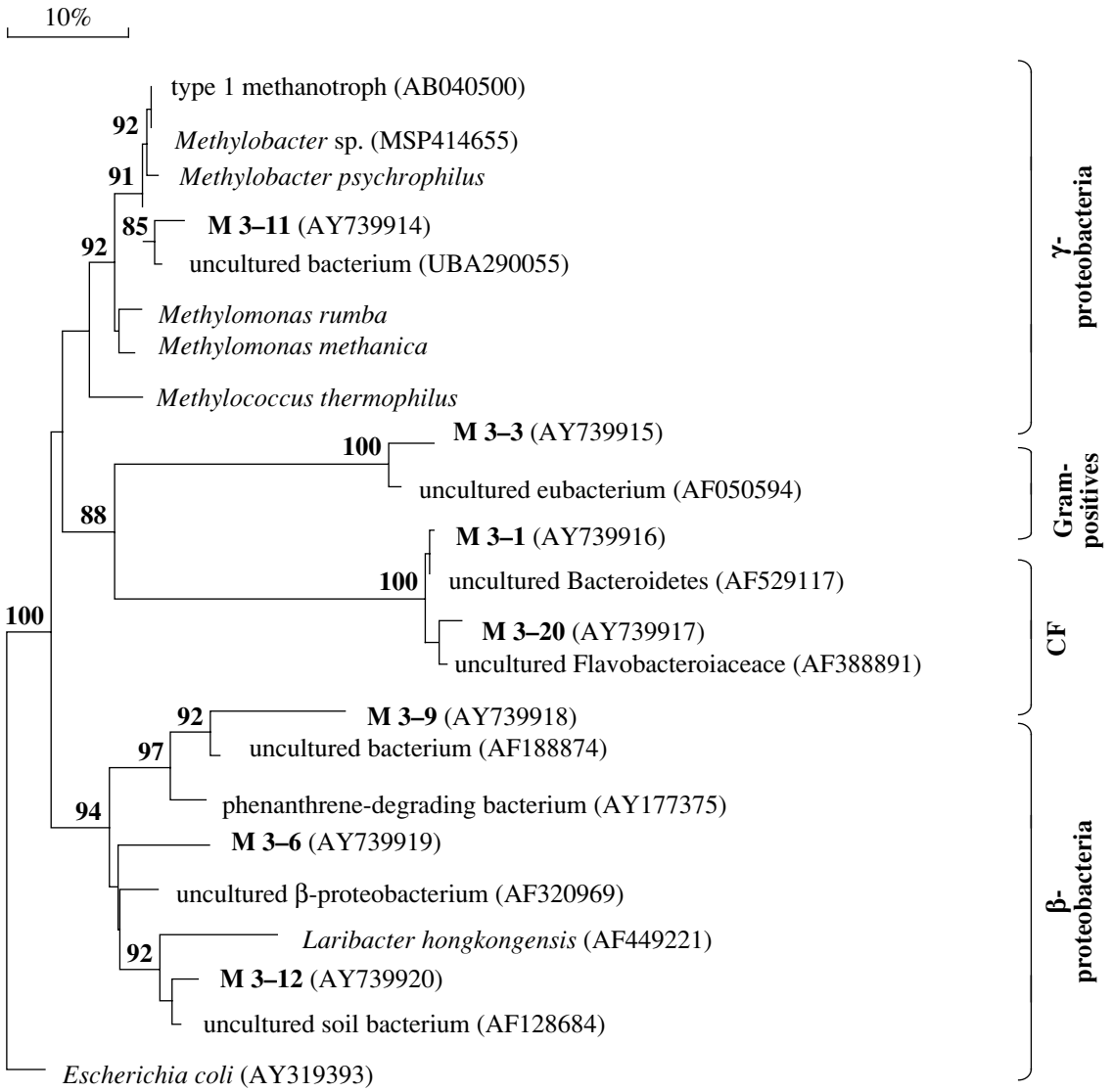

Fig. 2. The phylogenetic tree inferred from 16S rRNA gene fragment sequences of Baikal bacteria (surface sediment layer, 0–5 cm) and the GenBank sequences closest to the Baikal ones. M stands for crater Malenki station and the number following corresponds to the clone number.

belonged to the group of beta-proteobacteria and were related to the sequences of uncultured species found in the sediments of Lake Michigan. One nucleotide sequence of this group (clone M 3-9) exhibited the highest similarity with a phenanthrene-degrading bacterium (97%).

The composition of the bacterial communities changed in the deeper layers of the core, with sequences related to gamma-proteobacteria becoming the most numerous (seven clones). The sequences from the 92–94 cm layer formed a compact cluster on the phylogenetic tree adjacent to the *Pseudomonas* and *Ralstonia* bacterial genera (Fig. 3). Baikal sequence M 5-92 had a high homology with a representative of *Ralstonia* (98%). In the sediments two centimeters deeper (94–96 cm), representatives of gamma-proteobacteria predominated. One sequence from this horizon was most closely related to a clone of uncultured delta-proteobacteria (94%). In the sediments containing GH crystals (100–128 cm), seven sequences of Baikal bacteria were analyzed, three of which were similar to representatives of the genus *Pseudomonas* (93–97% homology). *Pseudomonas* representatives have also been found in the community associated with gas hydrates in the Gulf of Mexico [7, 9]. The other four sequences were most closely related to the uncultured bacteria found in sediment and ice samples from a variety of ecosystems: Antarctic sediments, Arctic ice, hydrothermal sediments, and deep-sea sediments. One of these sequences was structurally closest to archaea. Analysis of this sequence revealed that it occupied an intermediate position between true methanogens and

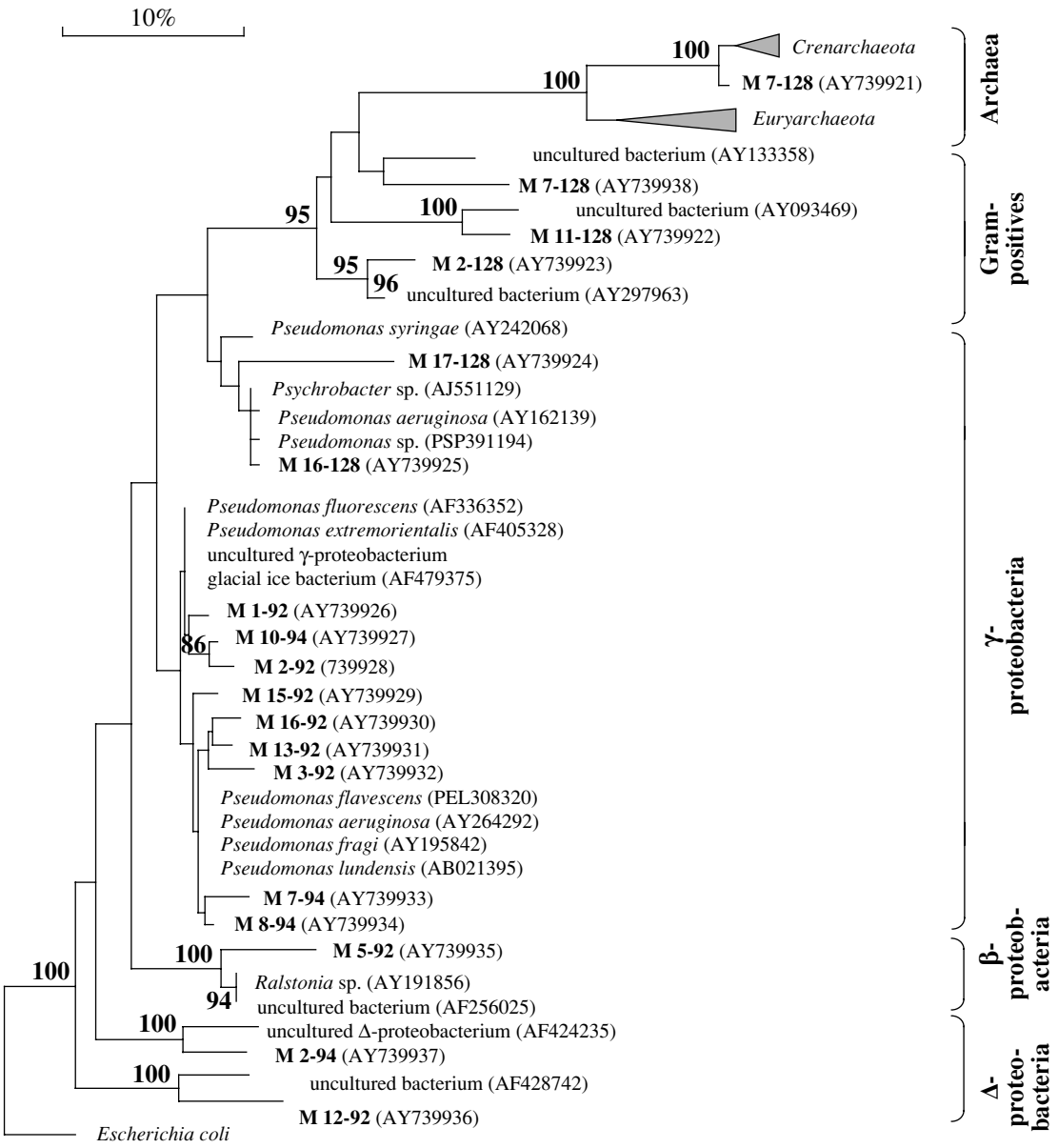

Fig. 3. The phylogenetic tree inferred from 16S rRNA gene fragment sequences of Baikal bacteria (sediment layers 92–94, 94–96, and 100–128 cm) and the GenBank sequences closest to the Baikal ones. M stands for crater Malenki station and the number following corresponds to the clone number.

those capable of oxidizing methane under anaerobic conditions (the ANME-1 and ANME-2 groups) [11].

Thus, a phylogenetic analysis of a small number of the clones representing Baikal bacteria has demonstrated the existence of a fairly diverse microbial community.

In all of the studied horizons of the sediment near crater Malenki, the majority of the clones analyzed were found to be closest to the proteobacterial group. This group is known to constitute the major part of the planktonic bacterial population of Baikal. According to results obtained using methods similar to those in the present study, alpha-proteobacteria predominate in the Baikal water column [16], while representatives of the gamma and beta subgroups prevail in the sediments of this area.

Our search of the above-mentioned public databases revealed the absence therein of sequences identical to Baikal bacteria. The highest homology among the Baikal sequences analyzed was recorded with the genera *Pseudomonas* and *Ralstonia* (1–2% nucleotide substitutions). For all the of analyzed clones from the surface layer of the bottom sediments, a rather high homology was found (not less than 96%); in contrast, in the deeper horizons adjacent to the gas hydrate layer, sequences were revealed that exhibited a much lower homology with those already known (87–92%).

This fact is in accordance with the available data on the diversity of deep-water organisms of the Baikal water column, wherein Bel'kova *et al.* also reported the low homology of the clones representing aquatic microorganisms with the known sequences [17].

ACKNOWLEDGMENTS

This work was supported by the Russian Foundation for Basic Research, project no. 03-05-65289; by the integration project of the Siberian Division of the Russian Academy of Sciences, no. 147; and by a Scientific School grant, 2195.003.4.

O.M. Khlystov acknowledges the support of the Siberian Division of the Russian Academy of Sciences provided by a grant for young researchers.

REFERENCES

1. Hutchinson, R.D., Lee, M.N., Scholz, C.A., Golmshtok, A.J., and Johnson, A.H., Lake Baikal: Gas Hydrate Character in an Active Rift Lake, *EOS*, 1995, vol. 76, p. 163.
2. Golmshtok, A.Ya., Duchkov, A.D., Hatchinson, D.R., Khanukaev, S.B., and El'nikov, A.I., Evaluation of the Heat Flux at Lake Baikal by Seismic Data on the Lower Boundary of the Gas-Hydrate Layer, *Geol. Geofiz.*, 1997, no. 10, pp. 1677–1691.
3. Klerks, Ya., Zemskaya, T.I., Matveeva, T.V., Khlystov, O.M., Grachev, M.A., Namsaraev, B.B., Dagurova, O.P., Golobokova, L.P., Vorob'eva, S.S., Pogodaeva, T.P., Granin, N.G., Kalmychkov, G.V., Ponomarchuk, V.A., Shoji, H., Mazurenko, L.L., Kaulio, V.V., and Solov'ev, V.A., Methane Hydrates in the Surface Layer of Deep-Water Sediments of Lake Baikal, *Dokl. Akad. Nauk*, 2003, vol. 393, no. 6, pp. 822–826.
4. Egorov, V.N., Polikarpov, G.G., Gulin, S.B., Artemov, Yu.G., Stokozov, N.A., and Kostrova, S.K., Modern Concepts of the Ecological Role of Methane Seeps at the Bottom of the Black Sea, *Tez. Dokl. XV Mezhdunar. Shkoly Morskoi Geologii* (XV Int. School on Marine Geology, Abstracts), 2003, vol. 1, pp. 315–316.
5. Kuz'min, M.I., Kalmychkov, G.V., Geletii, V.A., Gnilusha, V.A., Goreglyad, A.V., Khakhaev, B.N., Pevzner, L.A., Kawai, T., Yoshida, N., Duchkov, A.D., Ponomarchuk, V.A., Kontorovich, A.E., Bazhin, N.M., Makhov, G.A., Dyadin, Yu.A., Kuznetsov, F.A., Larionov, E.G., Manakov, A.Yu., Smolyakov, B.S., Mandelbaum, M.M., and Zheleznyakov, N.K., First Finding of Gas Hydrates in Bottom Sediments of Lake Baikal, *Dokl. Akad. Nauk*, 1998, vol. 362, no. 4, pp. 541–543.
6. Kuz'min, M.I., Kalmychkov, G.V., Duchkov, A.D., Geletii, I.F., Gol'mshtok, A.Ya., Karabanov, E.B., Khakhaev, B.N., Pevzner, L.A., Yoshida, Kh., Bazhin, N.M., Dyadin, Ya.A., Larionov, E.G., Manakov, A.Ya., Mandel'baum, M.M., and Vashchenko, I.F., Methane Hydrates in Bottom Sediments of Lake Baikal, *Geol. Rudn. Mestorozhd.*, 2000, vol. 42, no. 1, pp. 20–32.
7. Lanoil, B.D. and Sassen, R., La Duc M.T., Sweet S.T. Bacteria and Archaea Physically Associated with Gulf of Mexico Gas Hydrates, *Appl. Environ. Microbiol.*, 2001, vol. 67, pp. 5143–5153.
8. Nauhaus, K., Boetius, A., Kruger, M., and Widdel, F., *In Vitro* Demonstration of Anaerobic Oxidation of Methane Coupled to Sulphate Reduction in Sediment from a Marine Gas Hydrate Area, *Environ. Microbiol.*, 2002, vol. 4, pp. 296–305.
9. Mills, H.J., Hodges, C., and Wilson, K., MacDonald, I.R., and Sobesky, P.A., Microbial Diversity in Sediments Associated with Surface-Breaching Gas Hydrate Mounds in the Gulf of Mexico, *FEMS Microbiol. Ecol.*, 2003, vol. 46, pp. 39–52.
10. Boetius, A., Raveschlag, K., Schubert, C.J., Rickert, D., Widdel, F., Gieseke, A., Amann, R., Jorgensen, B.B., Witte, U., and Pfannkuche, O., A Marine Microbial Consortium Apparently Mediating Anaerobic Oxidation of Methane, *Nature*, 2000, vol. 407, pp. 623–626.
11. Orphan, V.J., Hinrichs, K.U., Ussler, W., Paull, I., Taylor, L.T., Sylva, S.P., Hayes, J.M., and DeLong, E.F., Comparative Analysis of Methane-Oxidizing Archaea and Sulfate-Reducing Bacteria in Anoxic Marine Sediments, *Appl. Environ. Microbiol.*, 2001, vol. 67, pp. 1922–1934.
12. Dagurova, O.P., Namsaraev, B.B., Kozyreva, L.P., Zemskaya, T.I., and Dulov, L.E., Bacterial Processes of the Methane Cycle in Bottom Sediments of Lake Baikal, *Mikrobiologiya*, 2004, vol. 74, no. 2, pp. 248–257.
13. Marmur, J., A Procedure for the Isolation of Deoxyribonucleic Acid from Microorganisms, *J. Mol. Biol.*, 1961, vol. 3, pp. 208–218.
14. Rochelle, P.A., Ery, J.C., Parkes, R.J., and Weightman, A.J., DNA Extraction for 16S rRNA Gene Analysis To Determine Genetic Diversity in Deep Sediment Communities, *FEMS Microbiol. Lett.*, 1992, vol. 100, pp. 59–66.
15. Sambrook, J., Frisch, E.F., and Maniatis, T., *Molecular Cloning. A laboratory manual*, *Cold Spring Harbor: Cold Spring Harbor Lab.*, 1989, vol. 2, pp. 53–72.
16. Denisova, L.Ya., Bel'kova, N.L., Tulokhonov, I.I., and Zaichikov, E.F., Bacterial Diversity at Various Depths in the Southern Part of Lake Baikal as Revealed by 16S rDNA Sequencing, *Mikrobiologiya*, 1999, vol. 68, no. 4, pp. 547–556.
17. Bel'kova, N.L., Denisova, L.Ya., Manakova, E.N., Zaichikov, E.F., and Grachev, M.A., Species Diversity of Deep-Water Microorganisms of Lake Baikal as Revealed by 16S rRNA Sequences, *Dokl. Akad. Nauk*, 1996, vol. 348, no. 5, pp. 692–695.