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EXPERIMENTAL ARTICLES

Aerobic Methanotrophic Communities in the Bottom Sediments of Lake Baikal

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Abstract—The results of the first systematical investigation into the aerobic methanotrophic communities inhabiting the bottom sediments of Lake Baikal have been reported. Use of the radioisotopic method revealed methane consumption in 12 10- to 50-cm-long sediment cores. The maximum methane consumption rates (495–737 μl/(dm^3 day) were recorded in sediments in the regions of hydrothermal vents and oil and gas occurrence. Methane consumption was most active in the surface layers of the sediments (0–4 cm); it decreased with the sediment depth and became negligible or absent at depths below 20 cm. The number of methanotrophic bacteria usually ranged from 100 to 1000 cells/cm^3 of sediment and reached 1 million cells/cm^3 in the regions of oil and gas occurrence. The seventeen enrichment cultures obtained were represented mainly by morphotype II methanotrophs. Phylogenetic analysis of the enrichment cultures in terms of the amino acid sequence of the α subunit of the membrane-bound methane monooxygenase (MMO) revealed the predominance of methanotrophs of the genus *Methylocystis.* The results obtained suggest the presence of an active aerobic methanotrophic community in Lake Baikal.

Key words: Lake Baikal, rate of methane oxidation, methanotrophs, membrane-bound methane monooxygenase, *Methylocystis.*

Aerobic methanotrophs are a specialized group of gram-negative bacteria that utilize methane and methanol as their sole sources of carbon and energy. Methanotrophs are widespread in nature and contribute significantly to the global cycle of carbon [1]; they oxidize up to 80% of the methane produced and form a strong bacterial filter that prevents methane emission into the atmosphere [2]. Freshwater sediments are among the main sources of methane production, accounting for 40–50% of the methane flux into the atmosphere [3].

The deepest freshwater lake on our planet, Lake Baikal, includes regions characterized by methane emission from methane hydrates present in its bottom sediments [4, 5]. This phenomenon is of special interest for understanding the processes of methane formation and oxidation. The bottom sediments of Lake Baikal have previously been studied mainly with regard to the processes of sulfate reduction and methanogenesis, but methane oxidation has received less attention [6–8]. The key role played by methanogens at the terminal stages of the degradation of organic matter has been established. The methane formed is actively oxidized in the bottom sediments and water column of Lake Baikal; in most cases, the oxidation of methane prevails over its formation [7]. However, until recently, the methanotrophic communities involved in methane oxidation in Lake Baikal had been little studied, which impelled us to undertake a systematical investigation of them.

The aim of this work was to look for aerobic methanotrophic communities in the bottom sediments of Lake Baikal and to provide their primary characterization.

MATERIALS AND METHODS

Samples of bottom sediments were taken with the use of a dredger and benthos tubes in August and September 2003 during an expedition on board the *G.Yu. Vereshchagin* research vessel organized by the Limnological Institute, Siberian Division, Russian

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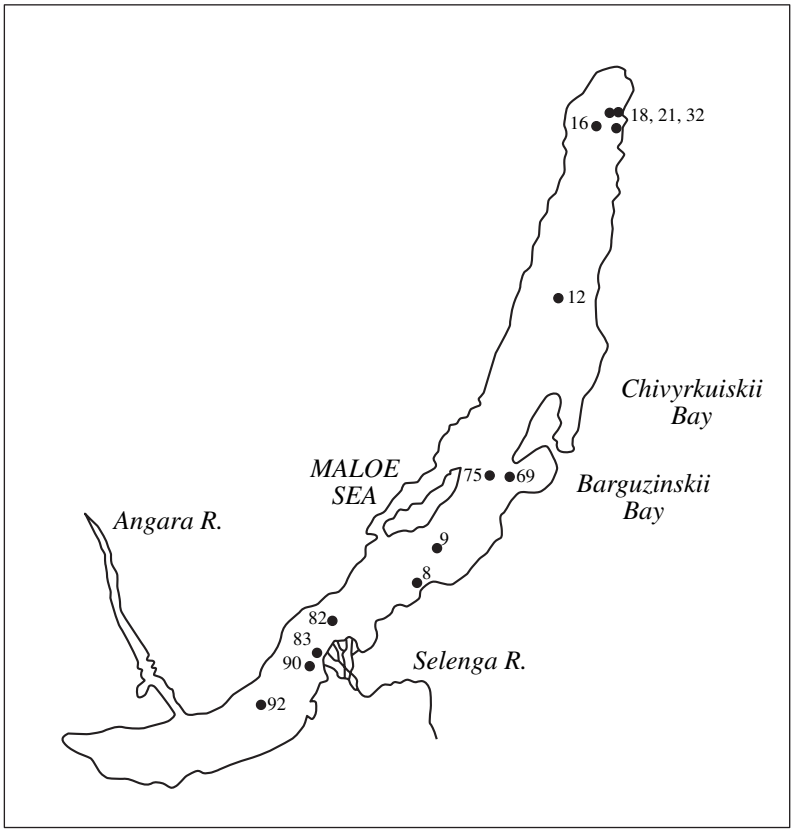

Fig. 1. Sites of sampling of Lake Baikal bottom sediments: (8) Cape Tolstyi, area of oil occurrence; (9) Ukhan–Tonkii section; (12) Elokhin–Davsha section; (16) Tyya–Nemnyanka section; (18, 21, 32) Frolikha Bay, area of hydrothermal vents; (69) outlet of Barguzinskii Bay (methane seep); (75) central basin of Lake Baikal; (82) Anga–Enkhaluk section; (83) Delta of the Selenga River (methane seep); (90) Proval Bay, thermal areas; (92) southern Baikal (area of gas hydrates).

Academy of Sciences. The regions of sampling are shown in Fig. 1. They were located at the central stations of the standard Baikal sections (Anga–Enkhaluk, Ukhan–Tonkii, Elokhin–Davsha, and Tyya–Nemnyanka), in the central basin of Baikal (background regions), in the regions of hydrothermal vents (Frolikha Bay) and a gas hydrate field (Southern Baikal), and in areas of gas and oil occurrence (Table 1). Labelled methane was added to the samples immediately after their lifting. Sediment samples used to obtain enrichment cultures and to determine the cell number of methanotrophs were stored in a refrigerator at 4°C for 1.5 months.

Measurements of the potential rate of methane consumption and enumeration of methanotroph cells. The rate of methane consumption was determined by the radioisotope method [9, 10]. Samples (1 cm^3) were withdrawn from every layer of the sediment core and put into sterile 12-ml vials. These were then completely filled with sterilized Lake Baikal water and sealed with gas-impermeable stoppers and aluminium covers. $^{14}CH_4$ (0.5–2.0 μCi, V/O Izotop, Russia) was injected into the vials with a syringe and the vials were then incubated at 4–6°C for 35–68 h. The process was terminated by the addition of 1 ml of a saturated solution of NaOH. Radioactivity of the biomass was measured on an Intertechnique SL-30 scintillation counter (France). The physical sorption of CH_4 was determined in samples that had been pretreated with a saturated solution of NaOH. To determine the cell number of methanotrophs, serial tenfold dilutions of the sediment samples were inoculated into liquid 0.5P medium (twofold diluted P medium [11]) and incubated aerobically with radioactive methane for 6–10 days. The presence and activity of methanotrophs was judged from the consumption of $^{14}CH_4$.

Isolation of enrichment cultures. The sediment samples were inoculated (about 5 vol %) into tubes with 5 ml of 0.5P medium., and The pH was adjusted to 6.8–7.2, in accordance with to the acidity of natural samples. The tubes were incubated in a methane–air (1 : 1) atmosphere at 25°C for 2–5 weeks. After the formation of bacterial films or cell precipitates, 2-ml aliquots of cell suspension were transferred to 750-ml flasks with 50 ml of the medium and incubated in a methane–air (1 : 1) atmosphere on a shaker (140 rpm) at 25°C. In further transfers of most of the enrichments, we increased the portion of methane in the gas mixture to 80%. The growth of methanotrophic bacteria was monitored with the use of a Jenaval light microscope (Germany).

Table 1. Characteristics of the samples of Lake Baikal bottom sediments

Region of sampling	Coordinates	Station	Depth of sampling, m	Horizon, cm	Sediment type
Cape Tolstyi, area of oil occurrence	N 52°38′185″	8	72	0–10	Dark brown silt
	E 107°21′765″			30–40	
Ukhan–Tonkii section	N 52°54′014″	9	1635	0–5	Dark gray clay
	E 107°31′989″			30–40	Dark gray clay with hydrotroilite interlayers
Elokhin–Davsha section	N 54°26′654″	12	890	0–62	Reddish arenaceous material
	E 109°04′054″			0–23	Gray arenaceous material
Tyya–Nemnyanka section	N 55°345′14″	16	675	5–10	Gray clay
	E 109°35′010″				
Frolikha Bay	N 55°31′301″	18	206	0–10	Black silt with plant debris
	E 109°50′340″				
	N 55°31′460″	21	330	0–10	Black silt with plant debris
	E 109°46′380″				
	N 55°31′380″	32	150	0–5	Gray silt
	E 109°46′840″				
Barguzinskii Bay, seep	N 53°25′687″	69	150	0–4	Brown–gray sand
	E 108°42′968″			5–15	Gray clay
Central basin of Lake Baikal	N 53°25′215″	75	1690	0–10	Friable brown silt
	E 108°16′011″				
Anga–Enkhaluk section	N 52°36′903″	82	1100	0–5	Brown silt
	E 105°50′522″			250–260	
Selenga River delta, methane seep	N 52°17′665″	83	100	0–6	Gray–brown silt
	E 106°12′874″				
Proval Bay, thermal areas	N 52°10′982″	90	23	0–10	Brown silt
	E 106°07′060″				
Malen'kii Crater, area of gas hydrates	N 51°55′205″	92	1370	0–6	Gray clay
	E 105°38′157″			80–90	Gray arenaceous material

Electron microscopy. Cell fixation, preparation of ultrathin sections with a Reichert ULTRACUT System ultramicrotome (Austria), and their examination under a Jeol JEM 100B electron microscope (Japan) were performed as described in [12].

Isolation of DNA and PCR amplification. Isolation of DNA from the enrichment cultures and PCR amplification were carried out, by using oligonucleotide primers specific to the functional genes of soluble (*mmo*X) and membrane-bound (*pmo*A) MMO [13]. The nucleotide sequences of the PCR products were determined on a CEQ 2000XL Beckman Coulter automatic sequencer (United States) with the use of a Big Dye Terminator Cycle Sequencing kit (Perkin-Elmer, United States) in accordance with the manufacturer's protocol.

Phylogenetic analysis. The amino acid sequences deduced from the sequenced fragments (460 bp) of the *pmo*A genes were compared with GenBank sequences using NCBI BLAST programs (http// www.ncbi.nlm.nih.gov/Blast) and aligned with the use of the Clustal W (version 1.6) program. A phylogenetic tree was constructed with the aid of the Treecon (version 1.3) program. The nucleotide sequences were deposited with GenBank (accession numbers, DQ078495–DQ078511).

RESULTS

Intensity of methane consumption. The rates of methane consumption were determined layer by layer in 12 sediment cores 10 to 50 cm long (Table 2). Methane consumption was observed in all the cores studied. In sediments from the background regions (i.e., central stations of the sections and the central basin of Lake Baikal), the rate of methane oxidation ranged from 15 to 62 μl CH_4/(dm^3 day); in sediments taken from the areas of gas hydrate fields and gas occurrence (stations 83, 90, and 92), the methane oxidation rate was higher (135–200 μl CH_4/(dm^3 day)). The maximum rate of methane consumption (495–737 μl CH_4/(dm^3 day))

was observed in sediments taken at stations 8, 18, and 32, i.e., in the areas of oil occurrence and at a hydrothermal vent.

As can be seen from Table 2, the pattern of variation in the methane consumption rate within the sediments was virtually the same in all the cores studied, regardless of their length. The highest rate of methane consumption was found in the surface layers (0–4 cm); it decreased in the 6–16 cm horizons and became negligible or zero below 20 cm.

A major portion of methane in the studied samples of bottom sediments was oxidized to CO_2. The portion of carbon from $^{14}CH_4$ incorporated into the biomass amounted to 48.7% on average in sediments from areas with extra methane emission; in the background regions, this value was lower (31%). In most of the sediment cores, the incorporation of methane radiocarbon into the biomass proceeded more actively in the upper horizons, where oxygen inflow may occur from the near-bottom water. In the deeper horizons, almost all of the ^{14}C was found in carbon dioxide. Similar results for $^{14}CH_4$ were earlier reported for sediments from the northwestern shelf of the Black Sea [14].

Description of enrichment cultures. Aerobic methanotrophs occurred in sediment samples taken at all the stations studied in an amount of at least 100–1000 cells/(cm^3 wet sediment) (Table 3). The maximum number of methanotrophs (1 million cells per 1 cm^3) was revealed in the sediments from the areas of oil and gas occurrence.

In total, 17 enrichment cultures were isolated from the bottom sediments of Lake Baikal. They contained difficult-to-separate associations of methanotrophs and heterotrophic satellites. Most of the cultures were colorless. Some of the cultures (9.2, 16, 32, and 92.1) grew well only on a semiliquid medium containing 0.3% agar; in a liquid medium, their growth was slow or almost absent. Methanotrophs formed few colonies on an agar medium, even if, according to microscopic examinations, they prevailed in the liquid medium. Two-week-old colonies were usually cream-colored (occasionally, they were white or pink), round, and bright; their diameter ranged from 0.5 to 3.0 mm.

The cells had the shape of small, rounded, quite often curved, rods (Fig. 2), which is typical of morphotype II methanotrophs. The methanotrophs from enrichment cultures 9.1, 9.2, 75, and 90 formed rosettes and conglomerates. As can be seen in the ultrathin sections, most of the cells contained intracytoplasmic membranes (ICMs) located at the cell periphery, which is also typical of type II methanotrophs (Figs. 3a–3d, 3g–3i). Only in enrichment cultures 83 and 90, were cells with type I ICMs revealed (Figs. 3e, 3f). Interestingly, culture 90 proved to be binary: it contained methanotrophs with type I ICMs and methanotrophs with poorly developed but recognizable type II ICMs (Fig. 3g). The cells with type II ICMs formed thick slimy capsules (Fig. 3d). The methanotroph cells from

Table 2. Rates of methane consumption in the samples of Lake Baikal bottom sediments

Station	Depth of sampling, m	Horizon, cm	Methane consumption, μl CH_4/(dm^3 day)	
			Oxidation to CO_2	Assimilation*
8	72	0–1	127	610
		5–6	248	126
		15–16	67	42
		26–27	1	7
		39–40	18	0
9	1635	0–1	12	3
		5–6	2	2
		18–19	5	0
		29–30	4	1
12	890	0–1	15	0
		9–10	0	3
		19–20	3	6
		29–30	5	6
16	675	0–3	7	22
		8–9	17	0
18	206	0–1	82	413
		1–2	20	34
		19–20	25	0
		32–33	11	6
32	300	0–2	146	550
		2–4	46	528
		4–6	92	48
		6–8	97	61
		8–10	13	33
		10–12	30	18
		12–13	33	15
69	150	0–1	7	8
		5–6	13	0
75	1690	0–1	8	34
		28–29	3	0
82	1100	0–1	5	58
		29–30	2	0
83	100	0–1	20	145
		1–2	16	111
		6–7	16	10
		13–14	22	41
90	23	0–1	38	98
92	1370	0–1	27	165
		2–3	106	95
		4–5	35	95
		6–7	59	36
		14–15	28	25
		30–31	3	43
		40–41	33	18

* Incorporation into acid-resistant products.

Table 3. Cell number and some properties of methanotrophs in enrichment cultures from Lake Baikal bottom sediments

Station	Horizon, cm	Cell number, cells/dm^3 wet sediment	Enrichment culture	Color	Prevailing cell morphotype
8	0–10	10^6	8.1	Cream	Beanlike cells
	30–40	10^4	8.2	Cream	Beanlike cells
9	0–5	10^2	9.1	Cream	Vibriods
	30–40	10^3	9.2	Cream	Vibriods
12	0–6	10^3	12.1	Cream	Vibriods
	20–23	10^2	12.2	Pink	Beanlike cells
16	0–10	10^2	16	Pink	Rounded rods
18	0–10	10^2	18	Cream	Rounded rods
21	0–5	10^4	21	Cream	Rounded rods
32	0–5	10^3	32	White	Rods
69	0–4	10^3	69.1	Cream	Beanlike cells
75	0–10	10^2	75	Cream	Curved rods
82	0–5	10^2	82.1	Cream	Rounded rods
83	0–6	10^2	83	Pink	Short rods
90	0–10	10^6	90	Cream	Vibriods
92	0–6	10^5	92.1	White	Rounded rods
	80–90	10^6	92.2	White	Curved rods

enrichment culture 92.2 were characterized by a peculiar morphology (Figs. 3h and 3i).

Phylogenetic analysis of the enrichment cultures of methanotrophs. The results of PCR amplification of a *pmo*A gene fragment were positive for DNA of all the enrichment cultures studied, whereas the *mmo*X gene was revealed only in culture 8.2.

Comparison of the amino acid sequences (120 amino acid residues) deduced from the sequenced amplification products of the *pmo*A gene fragments with sequences available from GenBank revealed the predominance of type II methanotrophs closely related to the genus *Methylocystis* (Fig. 4). The methanotrophs from enrichment cultures 9.1, 18, 21, 75, 82.1, 92.1, and 92.2 showed complete identity of their MMO fragment with that of *Methylocystis echinoides*. The methanotrophs from enrichment culture 12.1 were also close to *Mcs. echinoides*. Cultures 9.2, 12.2, 16, 32, and 90 were related to *Methylocystis parvus*, whereas the methanotrophs from enrichment cultures 8.1, 8.2, and 69 were related to *Methylosinus sporium.* The only representative of type I methanotrophs was found in enrichment culture 83; it was most closely related to *Methylomonas methanica.*

DISCUSSION

Methanotrophs are important components of microbial communities. They are responsible for the return of methane carbon into the global carbon cycle and fulfill the role of primary biomass producers in benthic communities in the regions of hydrothermal vents and gas hydrate fields [7, 8, 15]. Bottom sediments are favorable ecotopes for methanotrophs due to the supply of methane, which is formed during anaerobic degradation of organic matter [11, 14, 15]. The isotopic composition of methane carbon in bottom sediments taken from different regions of Lake Baikal, including the area of gas hydrates, ranges from –52.2 to –65.1‰, which confirms the microbial origin of CH_4 [4, 16]. The methane content in Lake Baikal sediments ranges from 0.1 to 40 ml/dm^3, reaching 81.7 ml/dm^3 in sediments near an underwater thermal vent in Frolikha Bay [6]. These high concentrations of methane may indicate its additional inflow from deeper layers of sediments.

Earlier studies on methane oxidation in shallow and deep-water sediments of Lake Baikal revealed considerable variations in the rate of methane oxidation dependent on the region of sampling. The maximum rate of methane oxidation (1180 μl/(dm^3 day)) was determined in the area of a hydrothermal vent in Frolikha Bay [7], where we registered the maximum rates of methane consumption (495–696 μl/(dm^3 day)).

In the regions with an additional methane inflow, increased rates of methane consumption correlated with the higher numbers of methanotrophic bacteria (up to 1 million cells per cm^3 versus the 100 to 1000 cells per cm^3 identified in many of the other samples). Methanotrophs were also found in deeper, anaerobic, layers of sediments, where they most probably reside in a resting state. It should be noted that our data on the cell numbers of methanotrophs were obtained by

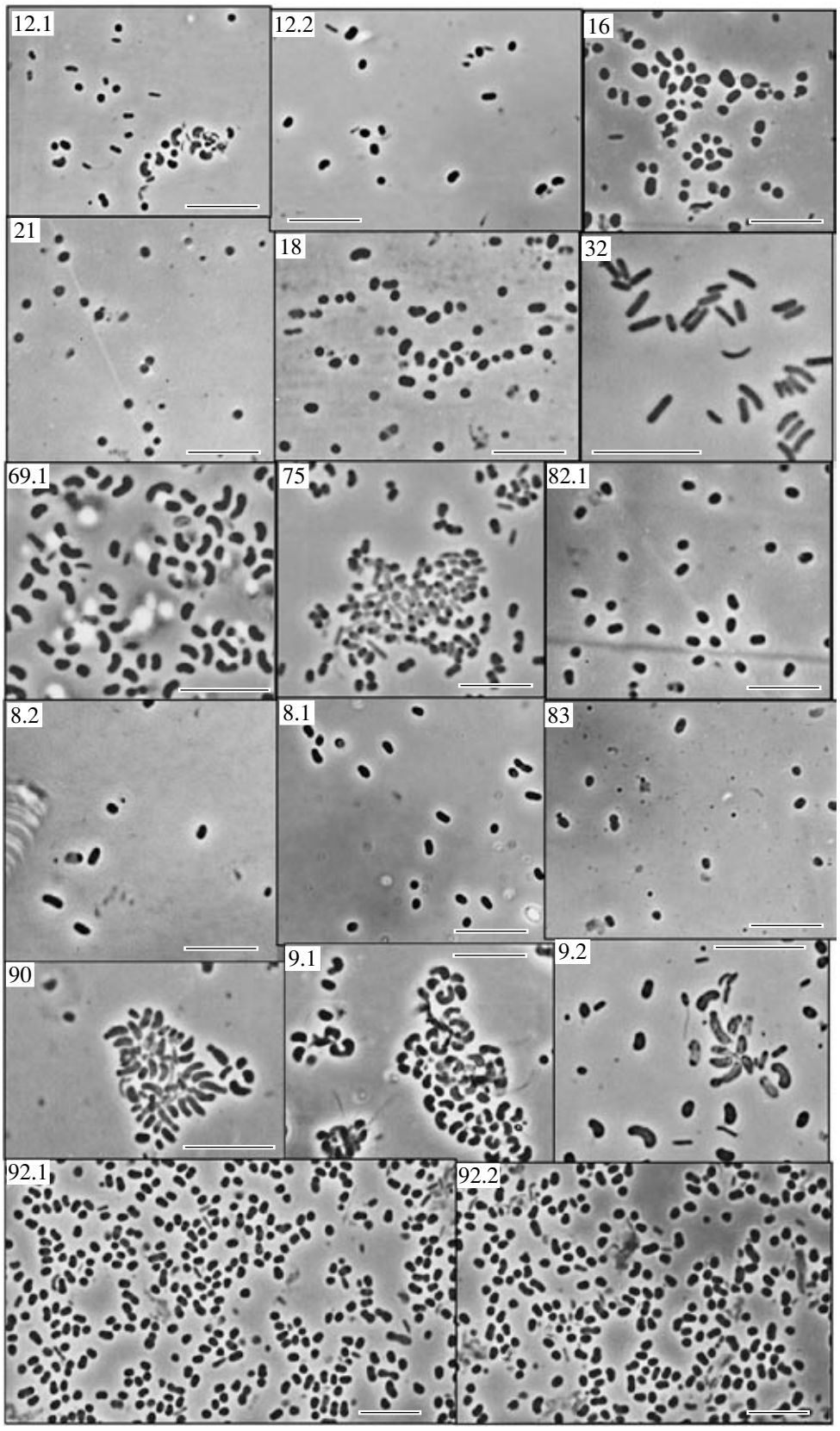

Fig. 2. Cell morphology of the methanotrophs in enrichment cultures obtained from Lake Baikal sediment samples. The bar represents 5 μm.

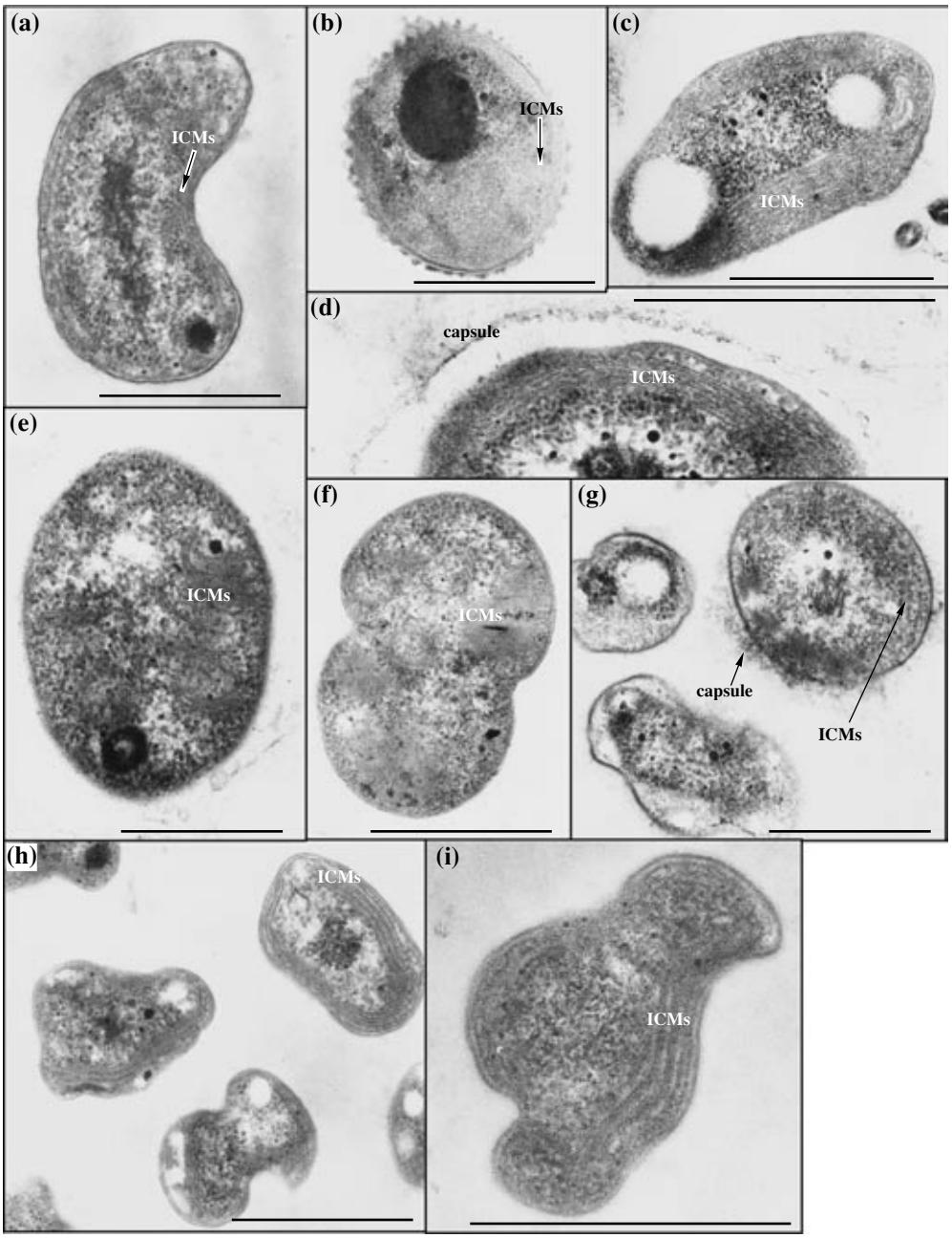

Fig. 3. Cell ultrastructure of the methanotrophs in some enrichment cultures obtained from Lake Baikal sediment samples: (a) 8.2; (b) 9.2; (c) 32; (d) 16; (e) 83; (f) 90; (g) 90; and (h and i) 92.2. The bar represents 1 μm.

inoculating sediment samples into a liquid medium after their storage in a refrigerator for 1.5 months.

We isolated methanotrophs from the surface layers of all the sediment samples and sometimes from deeper horizons. Thus, culture 92.2 was isolated from the 80- to 90-cm sediment horizon. The maintenance of some of our enrichment cultures presented certain difficulties, since they grew poorly in a liquid medium and did not grow on solid media. As a rule, the methanotrophs grew in the form of bacterial films on the bottom and walls of tubes and flasks. This could be due either to the adhesion of bacteria to a solid surface or to the high hydrophobicity of the cell walls. It is known that type II methanotrophs have a more hydrophobic cell surface than methanotrophs of type I and often form aggregates during cell growth [17]. Under in situ conditions, the hydrophobicity of the cell walls may promote the uptake of methane due to its high solubility in hydrophobic phases. In order to increase the growth rate of most of the enrichment cultures, the portion of methane in the gas phase was raised to over 80%, since type II methanotrophs prefer high p_{CH_4} and low p_{O_2} values [18].

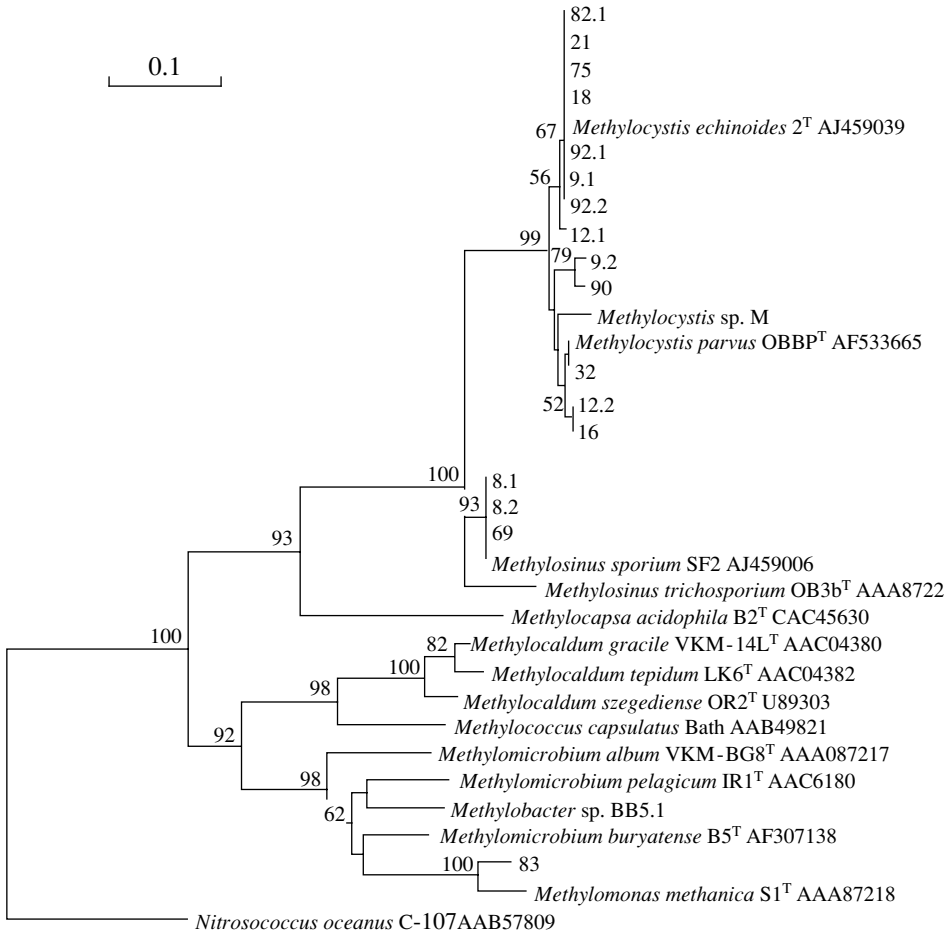

Fig. 4. Phylogenetic tree constructed on the basis of deduced amino acid sequences corresponding to the *pmoA* gene. The tree shows the phylogenetic position of methanotrophs found in the enrichment cultures isolated from Lake Baikal sediment samples. Bootstrap values higher than 50% are shown. The AmoA of *Nitrosococcus oceanus* was taken as an outgroup.

The phylogenetic position of the enrichment cultures obtained was determined by comparing the sequences of fragments of the *pmo*A gene, which encodes the α subunit of MMO. The enrichment cultures mainly represented type II methanotrophs, whereas a previously performed direct analysis of the methanotrophic communities in samples of Lake Baikal bottom sediments using species-specific immune serums revealed the presence of type I and type II methanotrophs [7]. The discrepancy between these results may be due to the medium composition and cultivation methods applied in the present study, which favored the isolation of type II methanotrophs. Bussmann *et al.* [19] also reported preferential isolation of type II methanotrophs from freshwater lakes [19].

Earlier studies on sediments from Frolikha Bay performed with the use of immune serums revealed the predominance of *Methylosinus trichosporium* and the occurrence of *Methylomonas methanica* and *Methylobacter bovis*, but no representatives of the genus *Methylocystis* were found [7]. The failure to detect the latter methanotrophs may be attributed to the difference in the compositions of the cell surface glycoproteins in the methanotrophs tested and the type strain used for serum production. We managed to isolate methanotrophs of the genus *Methylocystis* (*M. parvus* and *M. bovis*) from the samples taken in Frolikha Bay. Thus, we demonstrated that aerobic methanotrophic bacteria are widespread and active in Lake Baikal bottom sediments and that the cultivable methanotrophic community is dominated by methanotrophs of the genus *Methylocystis*.

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REFERENCES

1. Galchenko, V.F., Lein, A.Yu., and Ivanov, M.V., Biological Sinks of Methane, *Exchange of Trace Gases between Terrestrial Ecosystems and the Atmosphere*, Andreae, M.O. and Schimel, D.S., Eds., London: Wiley, 1989, pp. 59–71.
2. Reeburgh, W.S., Whalen, S.C., and Alperin, M.J., The Role of Methanotrophy in the Global Methane Budget, *Microbial Growth on C1-Compounds*, Murrell, J.C. and Kelly, D.P., Eds., Andover: Intercept, 1993, pp. 1–14.
3. Auman, A.J., Stolyar, S., Costello, A.M., and Lidstrom, M.E., Molecular Characterization of Methanotrophic Isolates from Freshwater Lake Sediment, *Appl. Environ. Microbiol.*, 2000, vol. 66, no. 12, pp. 5259–5266.
4. Klerks, Ya., Zemskaya, T.I., Matveeva, T.V., Khlystov, O.M., 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., Shodzhi, Kh., Mazurenko, L.L., Kaulio, V.V., Solov'ev, V.A., and Grachev, M.A., Methane Hydrates in Surface Layer of Deep-Water Sediments of Lake Baikal, *Dokl. Akad. Nauk*, 2003, vol. 393, no. 6, pp. 822–826.
5. Kuz'min, M.I., Kalmychkov, G.V., Geletii, V.F., Gnilusha, V.A., Goreglyad, A.V., Khakhaev, B.N., Pevzner, L.A., Kavai, T., Ioshida, 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., Mandel'baum, M.M., and Zheleznyakov, N.K., The First Finding of Gas Hydrates in the Sediments of Lake Baikal, *Dokl. Akad. Nauk*, 1998, vol. 362, no. 4, pp. 541–543.
6. Namsaraev, B.B., Dulov, L.E., Sokolova, E.N., and Zemskaya, T.I., Bacterial Methane Production in Bottom Sediments of Lake Baikal, *Mikrobiologiya*, 1995, vol. 64, no. 3, pp. 411–417.
7. Dagurova, O.P., Namsaraev, B.B., Kozyreva, L.P., Zemskaya, T.E., and Dulov, L.E., Bacterial Processes of the Methane Cycle in Bottom Sediments of Lake Baikal, *Mikrobiologiya*, 2004, vol. 73, no. 2.
8. Namsaraev, B.B., Zemskaya, T.I., Dagurova, O.P., Granina, L.Z., Golobokova, L.P., and Sitnikova, T.Ya., Bacterial Communities of the Bottom Sediments in the Zone of the Hydrothermal in the Frolikha Bay, *Geol. Geofiz.*, 2002, vol. 43, no. 7, pp. 644–647.
9. Belyaev, S.S., Laurinavichus, K.S., and Ivanov, M.V., Determination of the Rate of Microbial Methane Oxidation with the Use of $^{14}CH_4$, *Mikrobiologiya*, 1975, vol. 44, no. 3, pp. 542–545.
10. Sokolov, A.P. and Trotsenko, Y.A., Methane Consumption in (Hyper)Saline Habitats of Crimea (Ukraine), *FEMS Microbiol. Ecol.*, 1995, vol. 18, pp. 299–304.
11. Gal'chenko, V.F., *Metanotrofnye bakterii* (Methanotrophic Bacteria), Moscow: Geos, 2001.
12. Khmelenina, V.N., Kalyuzhnaya, M.G., Sakharovsky, V.G., Trotsenko, Y.A., and Gottschalk, G., Osmoadaptation in Halophilic and Alkaliphilic Methanotrophs, *Arch. Microbiol.*, 1999, vol. 172, no. 5, pp. 321–329.
13. Lin, J.L., Radajewski, S., Eshinimaev, B.Ts., Trotsenko, Y.A., McDonald, I.R., and Murrell, C.J., Molecular Diversity of Methanotrophs in Soda Lake Sediments and Identification of Potential Active Populations by Stable Isotope Probing, *Environ. Microbiol*, 2004, vol. 6, pp. 1049–1060.
14. Rusanov, I.I., Lein, A.Yu., Pimenov, N.V., Yusupov, S.K., and Ivanov, M.V., The Biogeochemical Cycle of Methane at the Northwestern Shelf of the Black Sea, *Mikrobiologiya*, 2002, vol. 71, no. 4, pp. 558–566.
15. Inagaki, F., Tsunogai, U., Suzuki, M., Kosaka, A., Machiyama, H., Takai, K., Nunoura, T., Nealson, K.H., and Horikoshi, K., Characterization of C1-Metabolizing Prokaryotic Communities in Methane Seep Habitats at the Kuroshima Knoll, Southern Ryukyu Arc, by Analyzing *pmoA, mmoX, mxaF, mcrA*, and 16S rRNA Genes, *Appl. Environ. Microbiol.*, 2004, vol. 70, no. 12, pp. 7445–7455.
16. Namsaraev, B.B. and Zemskaya, T.I., *Mikrobiologicheskie protsessy krugovorota ugleroda v donnykh osadkakh ozera Baikal* (Microbial Processes of the Carbon Cycle in the Bottom Sediments of Lake Baikal), Novosibirsk: Izd. SO RAN, filial "GEO", 2000.
17. Starostina, N.G., Koshchaev, A.G., Ratner, E.N., and Tsiomenko, A.B., Assessment of Cell Surface Hydrophobicity in Methanotrophic Bacteria by Their Adherence to Hydrocarbons, *Mikrobiologiya*, 1997, vol. 66, no. 2, pp. 185–191.
18. Amaral, J.A. and Knowles, R., Growth of Methanotrophs in Oxygen and Methane Counter Gradients, *FEMS Microbiol. Lett.*, 1995, vol. 126, pp. 215–220.
19. Bussmann, I., Pester, M., Brune, A., and Schink, B., Preferential Cultivation of Type II Methanotrophic Bacteria from Littoral Sediments (Lake Constance), *FEMS Microbiol. Ecol.*, 2004, vol. 147, pp. 179–189.