Microbiology, Vol. 69, No. 4, 2000, pp. 460–465. Translated from Mikrobiologiya, Vol. 69, No. 4, 2000, pp. 553–558.

EXPERIMENTAL ARTICLES

Potential Activity of Methane and Ammonium Oxidation by Methanotrophic Communities from the Soda Lakes of Southern Transbaikal

V. N. Khmelenina, B. Ts. Eshinimaev, M. G. Kalyuzhnaya, and Yu. A. Trotsenko

Skryabin Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, pr. Nauki 5, Pushchino, Moscow oblast, 142290 Russia

Received November 15, 1999

Abstract—Radioisotopic measurements of the methane consumption by mud samples taken from nine Southern Transbaikal soda lakes (pH 9.5–10.6) showed an intense oxidation of methane in the muds of Lakes Khuzhirta, Bulamai Nur, Gorbunka, and Suduntuiskii Torom, with the maximum oxidation rate in the mud of Lake Khuzhirta (33.2 nmol/(ml day)). The incorporation rate of the radioactive label from $^{14}CH_4$ into $^{14}CO_2$ was higher than into acid-stable metabolites. Optimum pH values for methane oxidation in water samples were 7–8, whereas mud samples exhibited two peaks of methane oxidation activity (at pH 8.15–9.4 and 5.8–6.0). The majority of samples could oxidize ammonium to nitrites; the oxidation was inhibited by methane. The PCR amplification analysis of samples revealed the presence of genes encoding soluble and particulate methane monooxygenase and methanol dehydrogenase. Three alkaliphilic methanotrophic bacteria of morphotype I were isolated from mud samples in pure cultures, one of which, B5, was able to oxidize ammonium to nitrites at pH 7–11. The data obtained suggest that methanotrophs are widely spread in the soda lakes of Southern Transbaikal, where they can actively oxidize methane and ammonium.

Key words: methane oxidation, methanotrophs, alkaliphiles, nitrification.

Methane is one of the end products of the microbial degradation of organic matter in soils, bottom sediments of freshwater and saline water bodies, etc., where this gas is produced by methanogenic bacteria. Together with carbon dioxide, methane essentially contributes to the greenhouse effect [1]. The estimation of methanotrophic activity in natural habitats allows the role of methanotrophs in the reduction of methane emission into the atmosphere to be evaluated.

In soil, freshwater, and marine biotopes, methanotrophic bacteria consume up to 80% of the methane produced [2, 3]. An intense oxidation of methane was revealed in the saline water bodies of the Crimea [4] and Tuva [5]. The kinetics of methane consumption and the effect of temperature and pH on this process were studied in samples of sphagnous peaty soil [6] and slightly alkaline boreal forest soil [7].

Little is known about the methanotrophs of soda lakes, whose microbial communities are distinguished by stable trophic chains and the almost closed recycling of organic matter [8–10]. In the Tuva and Southeastern Transbaikal soda lakes, whose hydrochemical conditions are different, methane is formed primarily by the methylotrophic and hydrogenotrophic pathways, respectively [10, 11]. Acetate-utilizing methanogens barely contribute to methanogenesis in the soda lakes of Southeastern Transbaikal.

The PCR amplification analysis of mud samples showed that the Tuva and Southern Transbaikal soda lakes, such as Khilganta, Tsaidam, Nizhnii Mukei, and Barun Torei, contain methanotrophs, some of which were isolated in pure cultures [12, 13]. However, the methane oxidation rates in these lakes have not yet been estimated.

Data on the nitrifying ability of microbial communities at high pH values are scarce. At the same time, this problem is very interesting from the standpoint of nitrogen deficiency in alkaline ecosystems due to a volatilization of ammonia, which is the preferential source of inorganic nitrogen at pH values higher than 9.5 [14]. Like nitrifying bacteria, methanotrophs are able to oxidize ammonium to nitrite, albeit to a lesser degree because of the inhibitory action of high concentrations of ammonium [1].

The aim of the present work was to evaluate the rates of methane and ammonium oxidation by methanotrophic communities in water and mud samples taken from the soda lakes of Southern Transbaikal.

MATERIALS AND METHODS

Water and bottom sediment samples, as well as their characteristics, were obtained from B.B. Namsaraev (Institute of General and Experimental Biology, Siberian Division of the Russian Academy of Sciences

0026-2617/00/6904-0460$25.00 © 2000 MAIK "Nauka/Interperiodica"

Table 1. Oxidation of methane and ammonium by mud samples from the Southern Transbaikal soda lakes

Lake	pH	CH_4 consumption, nmol/(ml day)		Initial, NO_2^-, content, nmol/ml	NO_2^-, formation, nmol/(ml day)		
		label incorporation into cells	oxidation to CO_2		$-NH_4^+$	$+NH_4^+$	$CH_4 + NH_4^+$
Suduntuiskii Torom	9.7	6.4	13.34	0.5	0.12	0.38	0.04
Bulamai Nur	10.6	7.6	16.51	5.7	0.05	10.38	0
Maloe Guzhirnoe	10.0	3.87	4.12	11.6	1.73*	1.66*	1.55*
Khuzhirta**	10.2	8.2	24.96	2.0	0	45.41	0
Gorbunka	9.5	7.1	11.12	0.7	0.53	0.49	0.29
Khilganta	9.6	2.92	4.42	2.2	0	0	0
Verkhnee Beloe	9.6	4.67	8.85	0	2.97	5.73	2.94
Nizhnee Beloe	9.56	5.26	10.5	1.0	1.47	0.36	1.33
Khulga Nur	9.7	n.d.	n.d.	0	0	0.76	0.15

* Consumption rate of NO_2^- originally present in the sample.

** Total mineralization of the sample was 40 g/l.

Ulan-Ude). The samples were taken in July 1998 and August 1999.

The consumption of ^{14}C-methane in mud samples was measured radioisotopically, by incubating the samples in the presence of $^{14}CH_4$ [4] at different pH values adjusted with 1% NaOH or 1 M HCl.

Nitrification was studied as follows. Mud samples in 2-g portions were mixed with 2 ml of water and placed in three series of small serum vials. All vials, except vials of the first series, which served as the control for the formation of nitrites from endogenous substrates, were supplemented with 5 mM $(NH_4)_2SO_4$ and sealed with rubber stoppers. To study the effect of methane on nitrification, methane was injected, using a gas-tight syringe, into vials of the third series in a volume comprising 5% of the vial headspace. The vials were incubated at 20°C for 6 days under periodical shaking. The nitrite-formation rates were calculated with allowance for the initial content of nitrites in samples. Nitrites were assayed by the conventional method [21].

Detection of methanotrophs. Methanotrophs were detected by amplifying the total DNA preparations isolated from mud samples by a modified method [15]. For this purpose, 1-g mud samples were twice extracted with 0.2 M phosphate buffer (pH 8.5). The extracts were pooled and centrifuged at 6000 *g* for 20 min. The precipitate was suspended in 5 ml of lysing buffer (10 mM Tris–HCl (pH 8.0) containing 100 mM NaCl, 100 mM EDTA, and 10 mg/ml lysozyme) and incubated at 37°C for 2 h under continuous stirring. The suspension was then supplemented with RNase, proteinase K, and 2% sodium dodecyl sulfate (the latter was added as a 10% solution), incubated for the next 30 min, and subjected to three cycles of freezing (–70°C) and thawing (65°C). The resultant lysate was centrifuged at 12 000 *g* for 15 min, and the supernatant was supplemented with 1 M NaCl (added as a 5 M solution) and 1% cetyltrimethylammonium bromide (CTAB) (added as a 10% solution in 0.7 M NaCl). The supernatant was incubated at 65°C for 10 min and thoroughly mixed with an equal volume of a chloroform–isoamyl alcohol (24 : 1) mixture. The mixture was centrifuged to separate the aqueous and solvent phases, and DNA present in the upper phase was precipitated with a double volume of ethanol. The precipitate was washed with 80% and then with 70% ethanol, dried, and dissolved in 0.5 ml of TE buffer (pH 8.0). DNA was further purified by the phenol extraction method. Finally, DNA was precipitated and washed as described earlier [15] and dissolved in 50 μl of TE buffer.

PCR amplification was carried out in a reaction mixture (50 μl) containing 10 mM Tris–HCl buffer, 50 mM KCl, 1 mg/ml BSA, 5.5% Tween 20, 2.5 mM $MgSO_4$, 0.2 μM of each dNTP, 50 pmol of the respective primers, 2 U of Tth DNA polymerase, and 0.5 μg of sample DNA. PCR amplification steps were run in a PHC-2 thermal cycler (Techne, United Kingdom). The primers were synthesized as described earlier [16]. PCR amplification products were separated by electrophoresis in 1% agarose gel.

Methanotrophic enrichments. The enrichment cultures of methanotrophs of morphotypes I and II were obtained using complete medium P [17] and the same medium free of copper [18], respectively. The pH of the media was adjusted to 9.0–9.5 (typical pH value of the mud samples used) with sodium carbonate buffer. The media were sterilized by autoclaving at

Table 2. Effect of pH on the methane consumption by water samples from soda lakes

Lake	pH in situ	pH in vitro	CH_4 consumption, nmol/(ml day)	
			label incorporation into cells	oxidation to CO_2
Khulga Nur	9.7	5.1	0.04	0.1
		7	0.06	0.17
		8	0.16	0.5
		9	0.33	0.67
		10.9	0.16	0.4
Gorbunka	9.5	5.3	0.05	0.2
		7	0.12	1.06
		8	0.22	1.0
		9	0.04	0.2
		11	0.03	0.06
Khilganta	9.6	5.5	0.04	0.2
		7.1	0.09	0.55
		8	0.26	0.37
		9	0.2	0.05
		10.3	0.06	0.1

1 atm for 30 min. To obtain enrichments, mud samples (about 0.5 ml in volume) were placed in 100 ml of the respective media and incubated in a methane–air (1 : 1) atmosphere at 30°C on a rotary shaker (140 rpm) until the media became turbid (typically, 1–2 weeks). Then, 10 ml of the subculture was transferred to 100 ml of the fresh medium and again incubated in the presence of methane. The process of culture enrichment with methanotrophs was controlled using a Jenaval phase–contrast optical microscope (Germany). In some cases, the growth of protozoa in enrichments was suppressed by adding 20 μg/ml actidione. As the content of methanotrophs in the enrichments increased, the time span between two subsequent subcultures was reduced to 2–3 days.

RESULTS

The radioisotopic measurements of methane consumption by water and mud samples taken from nine Southern Transbaikal soda lakes (pH 9.5–10.6) showed an intense consumption of ^{14}C-methane by mud samples taken in June 1998 (Table 1). The rate of methane oxidation to CO_2 was 2–3 times higher than to acid-stable metabolites. Methane oxidation was most intense in mud samples taken from Lakes Khuzhirta, Bulamai Nur, Gorbunka, and Suduntuiskii Torom (Table 1), suggesting the presence of methanotrophs in these lakes.

The pH dependence of methane oxidation within the range of 5.5–11.0 was investigated using water and mud samples taken in August 1999 from Lakes Khilganta, Gorbunka, Suduntuiskii Torom, and Khulga Nur. Generally, the rates of methane oxidation by water samples were lower than those by mud samples, except for the samples taken from Lake Khilganta, for which the reverse was true (Tables 2 and 3). Optimum pH values for methane oxidation by water samples taken from Lake Khilganta and Lake Gorbunka were 7–8, whereas they were 8–9 for water samples taken from Lake Khulga Nur. The activity of methane oxidation under acidic (pH 5.1–5.5) and alkaline (pH 10.3–11.0) conditions was low. Unlike water samples, mud samples exhibited two pH ranges optimal for methane oxidation, 8.15–9.4 and 5.8–6.0. The intensity of methane oxidation by mud samples from Lake Khulga Nur was higher at pH 5.8 than at pH 9.4. As a rule, the rate of methane oxidation to CO_2 was greater than to acid-stable metabolites.

The aerobic incubation of mud samples from Lakes Khuzhirta, Bulamai Nur, Suduntuiskii Torom, Verkhnee Beloe, and Khulga Nur in the presence of 5 mM ammonium sulfate led to an increase in the nitrite content of samples. This process was almost completely inhibited by 5% methane in the gas phase (Table 1). Conversely, such incubation of mud samples from Lake Maloe Guzhirnoe lowered the initial nitrite content of these samples. In two mud samples, ammonium inhibited the formation of nitrites from endogenous substrates. In one of these samples, which was taken from Lake Nizhnee Beloe, methane partially prevented the inhibitory effect of ammonium. The formation of nitrites in the mud of Lake Khilganta was not detected.

The PCR amplification analysis of samples was performed using the group-specific primers mxaF 1003f/1561r, mmoX 882f/1403r, and pmoA 189f/682r of, respectively, methanol dehydrogenase (MDH), soluble methane monooxygenase (sMMO), and particulate methane monooxygenase (pMMO) [16]. The analysis revealed the presence of the sMMO gene in all of the samples studied (Table 4). PCR amplification analysis with the pmoA primer gave negative results with mud samples from Lake Khuzhirta and Lake Verkhnee Beloe, and PCR amplification analysis with the mxaF primer gave negative results with mud samples from Lake Khuzhirta and Lake Gorbunka.

To isolate methanotrophs of morphotypes I and II, mud samples were incubated in complete medium P and copper-free medium P, respectively. This approach is based on the finding that the growth of morphotype I methanotrophs is inhibited by copper deficiency in the medium [18]. The enrichment cultures obtained from all samples represented the difficult-to-separate associations of methanotrophs and heterotrophs. The enrichments were dominated by oval and rod-shaped bacterial cells typical of the morphotype I methanotrophs. For the most part, these cells were motile. The morphotype II methanotrophs were revealed only in an enrichment culture obtained from the mud samples of Lake

Maloe Guzhirnoe. A noticeable growth of this methanotroph was observed only after its incubation for as long as 2–3 weeks. The shape of the cells of this methanotroph corresponded to that of representatives of the genus *Mehtylocystis*.

Three morphotype I methanotrophs were isolated in pure cultures. Strain B5, which was isolated from mud from Lake Gorbunka, efficiently oxidized ammonium to nitrites under alkaline conditions (pH 7–11) with an optimum at pH 9.0–9.5. However, this strain failed to grow in a liquid medium with ammonium as the sole nitrogen source.

DISCUSSION

Analysis of ^{14}C-methane consumption by the bottom sediment samples from nine Southern Transbaikal soda lakes showed that methanotrophs are active members of the alkaliphilic microbial communities of these biotopes. The preferential oxidation of methane to CO_2 can be associated with additional energy requirements to maintain the pH of the cytoplasm at a homeostatic level. Comparing the results presented here with those obtained in the study of the neutral (pH about 6.5) saline lakes of the Crimea and Tuva [4, 5] shows that methane consumption is more intense in soda lakes. This can be explained by a higher content of methanotrophs in the microbial communities of soda lakes than in the microbial communities of saline water bodies. It should be noted that the rate of methane oxidation in the alkaline (pH > 9.0), saline Lake Mono of California (United States) was also high, reaching 48 nM/day in the anoxic zone of this lake [19].

Recent estimations have shown that the rates of methane formation in the bottom sediments of some soda lakes of Southeastern Transbaikal range from 0.14 to 6.01 μl/(kg day) [11]; i.e., they are two orders of magnitude lower than the rates of methane oxidation in bottom sediments estimated in the present study. Such a considerable difference between the rates of methane formation and oxidation is an indication of the high activity of methanotrophs in the alkaline ecosystems studied. Potentially, methanotrophs present in these ecosystems can completely consume all biogenic methane. It should, however, be noted that the methane formation and oxidation rates were estimated by Namsaraev *et al.* [11] and by our group under different redox conditions.

Estimations showed that methanotrophs dominated in the bottom sediments of Lake Khulga Nur and Lake Gorbunka but in the water of Lake Khilganta. Presumably, the growth of methanotrophs in the mud of the latter lake was limited by oxygen deficiency; this assumption is confirmed by the presence of anoxigenic photosynthetic purple bacteria and sulfate reducers in this habitat. The mud of Lake Khilganta was characterized by the lowest rates of methane consumption and oxidation.

Table 3. Effect of pH on the methane consumption by mud samples from soda lakes

Lake	pH in situ	pH in vitro	CH_4 consumption, nmol/(ml day)	
			label incorporation into cells	oxidation to CO_2
Khulga Nur	9.7	5.8	0.3	0.9
		7.4	0.13	0.9
		8.2	0.07	0.5
		9.4	0.06	0.87
		11.1	0.005	0.18
Gorbunka	9.5	6	8.5	13.95
		7.4	4.6	9.03
		8.3	12.7	18.3
		9.2	1.2	13.3
		11	0.04	0.61
Khilganta	9.6	5.9	0.21	0.18
		7.1	0.24	0.21
		8.3	0.9	0.15
		9.5	0.1	0.18
		11.1	0.3	0.14
Suduntuiskii Torom	9.7	5.9	34.3	41.3
		7.4	3.5	9.1
		8.15	59.7	89.6
		9.4	23.5	32.6
		11.5	0.14	0.6

Table 4. PCR amplification of gene fragments isolated from mud samples of the Southern Transbaikal soda lakes

Lake	PCR amplification of		
	mxaF (MDG)	mmoX (sMMO)	pmoA (pMMO)
Suduntuiskii Torom	+	+	+
Bulamai Nur	+	+	+
Maloe Guzhirnoe	+	+	+
Khuzhirta	–	+	–
Gorbunka	–	+	+
Khilganta	+	+	+
Verkhnee Beloe	+	+	–
Nizhnee Beloe	+	+	+

In all water and mud samples, methane could be oxidized in a wide range of pH values (5.1–11.0). This suggests that methanotrophs in the lakes studied have adapted to considerable changes in pH caused by seasonal variations in the water level during spring and

summer periods. Interestingly, some mud samples exhibited two peaks of methane oxidation, at acidic and alkaline pH values. The optimum pH for methane oxidation in muds from Lake Gorbunka and Lake Sudun-tuiskii Torom was one unit lower than the in situ pH of these muds.

The possibility of nitrification in soda lakes has already been reported [14], although high concentrations of ammonium were found to be toxic to ammonia oxidizers. Neutrophilic methanotrophs are able to oxidize ammonium due to the activity of nonspecific particulate methane monooxygenase close in structure to the ammonium monooxygenase of nitrifying bacteria [1]. However, it has recently been shown that the activity of methanotrophs in the alkaline, saline Lake Mono is considerably lower than the activity of nitrifiers and that methane oxidation in this lake is inhibited by low concentrations (0.3 mM) of NH_4^+ [19]; therefore, methanotrophs do not seem to be involved in the process of nitrification in Lake Mono.

Our estimations showed that the rates of ammonium and methane oxidation in the bottom sediments of the Southern Transbaikal soda lakes are comparable. In mud samples from five of the nine soda lakes studied, nitrite formation was inhibited by methane. In the mud of Lake Nizhnee Beloe, nitrite formation was also inhibited by 5 mM ammonium sulfate; this inhibition could be partially prevented by the addition of methane. The inhibition of nitrification by ammonium and methane (this could be due to their action on either nitrifying or methanotrophic bacteria) suggests a functional diversity of the microbial communities of soda lakes. The involvement of methanotrophs in nitrification processes in soda lakes is confirmed by the ability of strain B5 to oxidize ammonium to nitrite at alkaline pH values (9.0–9.5) and by the isolation of the alkaliphilic methanotroph *Methylomicrobium* sp. AMO1 from a Kenyan soda lake, which efficiently oxidized ammonium at pH 10–10.5 in the presence of 10% CH_4 in the gas phase [20].

The detection of the genes of MDH and soluble and particulate MMO by PCR amplification analysis confirmed the presence of methanotrophic bacteria in the bottom sediments of soda lakes. The negative results of PCR amplification with the pmoA primer and mud samples from Lake Khuzhirta and Lake Verkhnee Beloe and with the mxaF primer and mud samples from Lake Khuzhirta and Lake Gorbunka can be explained by the presence in these muds of methanotrophs with unusual forms of pMMO and MDH, such as the pMMO of the recently described methanotroph *Methylocella* [22]. It should be noted that all *Methylocella* strains described by now are obligately acidophilic methanotrophs.

Thus, PCR amplification analysis and the results of measurements of methane consumption by mud samples, as well as the isolation of enrichment and pure methanotrophic cultures that are able to grow at pH values close to those in situ and to oxidize ammonium at alkaline pH values, give strong grounds to consider methanotrophs to be active members of the microbial cenoses of the soda lakes of Southern Transbaikal.

ACKNOWLEDGMENTS

We are grateful to B.B. Namsaraev for providing samples from the soda lakes of Southern Transbaikal and to O.V. Petrova for technical assistance.

This work was supported by the Russian Foundation for Basic Research, project no. 98-04-48144a.

REFERENCES

1. Hanson, R.S. and Hanson, T.E., Methanotrophic Bacteria, *Microbiol. Rev.*, 1998, vol. 60, no. 2, pp. 437–471.
2. Reeburg, W.S., Whalen, S.C., and Alperin, M.J., The Role of Methylotrophy in the Global Methane Budget, *Microbial Growth on C1-Compounds*, Murrell, J.C. and Kelly, D.P., Eds., Andover: Intercept Ltd., 1993, pp. 1–14.
3. Gal'chenko, V.F., Bacterial Methane Cycle in Marine Ecosystems, *Priroda*, 1995, no. 6, pp. 35–48.
4. Sokolov, A.P. and Trotsenko, Y.A., Methane Consumption in (Hyper)Saline Habitats of the Crimea (Ukraine), *FEMS Microbiol. Ecol.*, 1995, vol. 8, no. 4, pp. 299–304.
5. Khmelenina, V.N., Starostina, N.G., Tsvetkova, M.G., Sokolov, A.P., Suzina, N.E., and Trotsenko, Yu.A., Methanotrophic Bacteria of the Saline Water Bodies of Ukraine and Tuva, *Mikrobiologiya*, 1996, vol. 65, no. 5, pp. 696–703.
6. Dedysh, S.N. and Panikov, N.S., Kinetics of Methane Oxidation in Sphagnous Peat as a Function of pH, Temperature, and Salt Concentration, *Mikrobiologiya*, 1997, vol. 66, no. 4, pp. 569–573.
7. Dunfield, P., Knowles, R., Dumont, R., and Moore, T.R., Methane Production and Consumption in Temperate and Subarctic Peat Soils: Response to Temperature and pH, *Soil Biol. Biochem.*, 1993, vol. 25, no. 3, pp. 321–326.
8. Jones, B.E., Grant, W.D., Collins, N.C., and Maatha, W.E., Alkaliphiles Diversity and Identification, *Bacterial Diversity and Systematics*, Priest, F.G. *et al.*, Eds., New York: Plenum, 1994, pp. 195–228.
9. Zhilina, T.N. and Zavarzin, G.A., Alkaliphilic Anaerobic Community at pH 10, *Curr. Microbiol.*, 1994, vol. 29, pp. 109–112.
10. Zavarzin, G.A., Zhilina, T.N., and Pikuta, E.V., Secondary Anaerobes in the Haloalkaliphilic Communities of Tuva Lakes, *Mikrobiologiya*, 1996, vol. 65, no. 4, pp. 546–553.
11. Namsaraev, B.B., Zhilina, T.N., Kulyrova, A.V., and Gorlenko, V.M., Bacterial Production of Methane in the Southeastern Transbaikal Soda Lakes, *Mikrobiologiya*, 1999, vol. 68, no. 5, pp. 683–688.
12. Khmelenina, V.N., Kalyuzhnaya, M.G., Starostina, N.G., Suzina, N.E., and Trotsenko, Y.A., Isolation and Characterization of Halotolerant Alkaliphilic Methanotrophic Bacteria from Tuva Soda Lakes, *Curr. Microbiol.*, 1997, vol. 35, no. 5, pp. 1–5.
13. Kalyuzhnaya, M.G., Khmelenina, V.N., Suzina, N.E., Lysenko, A.M., and Trotsenko, Yu.A., Novel Methan-

otrophic Isolates from the Southern Transbaikal Soda Lakes, *Mikrobiologiya*, 1999, vol. 68, no. 5, pp. 689–697.

14. Sorokin, D.Yu., About the Possibility of Nitrification under the Highly Alkaline Conditions of Soda Biotopes, *Mikrobiologiya*, 1998, vol. 67, no. 3, pp. 404–408.
15. Lee, S.Y., Bolinger, J., Bezdicek, D., and Ogram, A., Estimation of the Abundance of an Uncultured Soil Bacterial Strain by a Competitive Quantitative PCR Method, *Appl. Environ. Microbiol.*, 1996, vol. 62, no. 10, pp. 3787–3793.
16. Murrell, J.G., McDonald, J.R., and Burne, D.O., Molecular Methods for the Study of Methanotroph Ecology, *FEMS Microbiol. Ecol.*, 1998, vol. 27, no. 2, pp. 103–114.
17. Gal'chenko, V.F., Andreev, L.V., and Trotsenko, Yu.A., *Taksonomiya i identifikatsiya obligatnykh metanotrofnykh bakterii* (Taxonomy and Identification of Obligately Methanotrophic Bacteria), Kalakutskii, L.V., Ed., Pushchino: Nauchn. Tsentr Biol. Issled. Akad. Nauk SSSR, 1986, p. 96.
18. Graham, D.W., Chaudhary, J.A., Hanson, R.S., and Arnold, R.G., Factors Affecting Competition between Type I and II Methanotrophs in Two-Organism, Continuous-Flow Reactors, *Microb. Ecol.*, 1993, vol. 25, pp. 1–17.
19. Joye, S.B., Connell, T.L., Miller, L.G., Oremland, R.S., and Jellison, R.S., Oxidation of Ammonia and Methane in an Alkaline, Saline Lake, *Limnol. Oceanogr.*, 1999, vol. 44, pp. 178–188.
20. Sorokin, D.Y., Jones, B.E., and Kuenen, J.G., A Novel Obligately Methylotrophic, Methane-oxidizing *Methylomicrobium* Species from a Highly Alkaline Environment, *Extremophiles*, 2000 (in press).
21. *Manual of Methods for General Bacteriology*, Gerhardt, P. *et al.*, Eds., Washington: Am. Soc. Microbiol., 1981. Translated under the title *Metody obshchei bakteriologii*, Moscow: Mir, vol. 2, pp. 341–350.
22. Dedysh, S.N., Liesack, W., Khmelenina, V.N., Suzina, N.G., Trotsenko, Y.A., Semrau, J.D., Abing, A.M., Panikov, N.S., and Tiedje, J.M., *Methylocella palustris* gen. nov., sp. nov., a New Methane-oxidizing Acidophilic Bacterium from Peat Bogs Representing a Novel Subtype of Serine Pathway Methanotrophs, *Int. J. Syst. Evol. Microbiol.*, 2000, vol. 50, no. 3, pp. 955–969.