Microbiology, Vol. 74, No. 2, 2005, pp. 221–229. Translated from Mikrobiologiya, Vol. 74, No. 2, 2005, pp. 261–270.

EXPERIMENTAL ARTICLES

The Processes of Methane Production and Oxidation in the Soils of the Russian Arctic Tundra

Yu. Yu. Berestovskaya, I. I. Rusanov, L. V. Vasil'eva, and N. V. Pimenov

Winogradsky Institute of Microbiology, Russian Academy of Sciences, pr. 60-letiya Oktyabrya 7, k. 2, Moscow, 117312 Russia

Received December 29, 2003

Abstract—Methane emission from the following types of tundra soils was studied: coarse humic gleyey loamy cryo soil, peaty gleyey soil, and peaty gleyey midloamy cryo soil of the arctic tundra. All the soils studied were found to be potential sources of atmospheric methane. The highest values of methane emission were recorded in August at a soil temperature of 8–10°C. Flooded parcels were the sources of atmospheric methane throughout the observation period. The rates of methane production and oxidation in tundra soils of various types were studied by the radioisotope method at 5 and 15°C. Methane oxidation was found to occur in bog water, in the green part of peat moss, and in all the soil horizons studied. Methane production was recorded in the horizons of peat, in clay with plant roots, and in peaty moss dust of the bogey parcels. At both temperatures, the methane oxidation rate exceeded the rate of methane production in all the horizons of the mossy–lichen tundra and of the hillock tundra with flat-bottom depressions. Methanogenesis prevailed only in a sedge–peat moss bog at 15°C. Bacterial enrichment cultures oxidizing methane at 5 and 15°C were obtained. Different types of methanotrophic bacteria were shown to be responsible for methane oxidation under these conditions. A representative of type I methylotrophs oxidized methane at 5°C, and *Methylocella tundrae*, a psychroactive representative of an acidophilic methanotrophic genus *Methylocella*, at 15°C.

Key words: greenhouse gases, methane emission, oxidation and production of CH_4, bacterial methane filter, psychroactive microorganisms.

The increase in the methane concentration in the atmosphere is due to the natural sources of this greenhouse gas rather than to anthropogenic sources [1]. Continental amphibial landscapes that spread over vast areas and which are most pronounced in countries with cold and temperate climates are the principal natural sources of methane. Biogenic methane is the result of the activity of methanogenic archaea [2, 3]. The anaerobic methanogenic and the aerobic methanotrophic communities form an interdependent system of biological methane turnover; its imbalance determines the emission of biogenic methane into the atmosphere [4]. Nowadays a vast array of data exists on methane emission from the moisturized northern soils of both the Western and the Eastern hemispheres [5–10]. Methane production rates are known to be the highest in soils of northern latitudes; they can reach 46.8 [7], 107 mg/(m^2 day), and higher values [10]. Some authors maintain that tundra soils are responsible for 25% of the total methane emission from natural sources [11]. A study of the methane cycle processes under low temperatures and of the factors affecting methane emission from tundra soils is therefore of current interest.

Investigation of the biological processes of the methane cycle was performed for West Siberian tundra soils [12]. Data exist on methane oxidation in tundra bog soil and on and on immunofluorescent determination of the species composition of methane oxidizing microorganisms [13]. Certain reconnaissance qualitative and quantitative characteristics of methane production and consumption are available for the soils of the Arctic tundra of European Russia [6]. The first known psychrophilic methanotroph *Methylobacter psychrophilus* was isolated from polar Ural tundra soil [14]. The conditions under which the ratio of the processes of the methane cycle changes in favor of its formation or consumption are as yet poorly known.

The main objective of our work was to study the functioning of the microbial communities involved in the methane cycle in Russian Arctic tundra soils and to identify the bacteria comprising the bacterial oxidative filter. Among our tasks were determination of CH_4 emission from tundra soil, measurement of the methane production and oxidation rates in different soil horizons, and obtaining enrichment cultures of methane oxidizing bacteria.

MATERIALS AND METHODS

The area of investigation was located in the Arctic tundra, dwarf birch subzone, 20 km south of Vorkuta near Tal'nik Station. This zone is characterized by pronounced topographic diversity, including elevated areas, streams, depressions, and lowland bogs. The

0026-2617/05/7402-0221

most characteristic ecotopes of this tundra zone were chosen for our work: (1) mounded tundra, (2) sedge–peat moss bog, (3) hillock tundra with flat-bottom depressions.

(1) Mounded tundra was a typical, mossy–lichen bushy parcel of dwarf birch tundra. The vegetation was dense and consisted of the bilberry *Vaccinium myrtillus*, the mosses *Polytrichum uniperinum* and *Aulacomnium turgidum*, and the lichens *Cetraria islandica* and *Cladonia rangiferina*. A hummock (parcel 1) at 67°019.807′ N and 63°044.269′ E, and a swampy hollow nearby (parcel 2) were chosen as investigation sites. The soil type of this part of the tundra was coarse humic gleyey loamy cryo soil.

(2) Sedge–peat moss bog. The vegetation consisted of the sedge *Carex* sp., the cotton grass *Eriophorum angustifolium*, and mosses, among which the peat moss *Sphagnum fuscum* dominated. The investigation sites were water-covered bog edge (parcel 3) at 67°019.797′ N and 63 063°044.278′ E; hummock (parcel 4) at 67°019.802′ N and 063°044.302′ E; and swampy hollow (parcel 5) at 67°019.802′ N and 063°044.302′ E. The soil was peaty gleyey.

(3) Hillock tundra with flat-bottom depressions. The investigation site was a domed elevation of soil covered with peat moss (*Sphagnum* sp.) on a channel slope (parcel 6) at 67°019.760′ N and 063°044.257′ E. The soil was peaty–gleyey–midloamy–cryo soil.

Analyses were performed in June, August, and September, 2002. These periods corresponded to the beginning, middle, and end of the vegetative period in the tundra and differed in weather conditions. The daily average temperature in June was 6°C, in August, it was 16°C, and in September, 10°C. The degree of mineralization of the soil solutions did not change significantly with temperature: 13–15 mg/l at 18°C and 8–10 mg/l at 6°C, thus corresponding to ultrafreshwater conditions.

Total methane emission from the soil surface was determined by the static chamber method. The chambers were made from 5-l plastic vessels. The base of the vessel was removed, and the neck was sealed with a rubber stopper and closed with a plastic screw cap with a sampling hole. The chamber was inserted 10–12 cm deep into the soil and left unstoppered for 20–30 min to remove the methane extruded during chamber insertion. Afterwards the chamber was sealed and air samples were taken with a syringe at the onset of incubation and every subsequent 30 min. Gas samples were injected into penicillin bottles filled with a 10% NaCl solution and stored until analysis. The samples were analyzed using a Khrom-3 gas chromatograph (Russia) equipped with a flame ionization detector. Methane emission was calculated from the measured methane concentration in the air of the chamber using a linear approximation.

The rates of microbial methane oxidation and production in bog water and soil samples were determined using the radioisotope method with $NaH^{14}CO_3$, and ^{14}C-acetate at 5 and 15°C. The sampling order was as follows: moss–lichen mixture; the green, chlorophyll-containing part of the moss; moss or moss–lichen dust; peated moss dust; peat; clay with plant roots; clay. From 3 to 20 cm^3 of soil from respective horizons was introduced into penicillin bottles or into cut plastic syringes and sealed with gas-tight rubber stoppers. Using syringes, 0.2 ml of sterile solutions of labeled substrates in distilled water were injected: $^{14}CH_4$, 2 μCi; $NaH^{14}CO_3$, 10 μCi; ^{14}C-acetate, 10 μCi. The samples were incubated directly in soil for one day. During radioisotope experiments, in June and August, the average daily soil temperature was 5 and 15°C, respectively. After incubation, the samples were fixed with 2 N KOH solution. The subsequent sample treatment was performed according to the scheme described previously [15, 12].

The temperature, pH, and redox potential of the soil horizons were determined using a WTW-330 portable ionometer (Germany).

Soil temperature was determined 10 cm below the surface.

The level of ground water was determined as the distance between the soil surface and the top of the water horizon.

The total amount of mineral salts in soil solutions was measured using a DIST WP 1 salinometer.

For enrichment cultures, soil samples were collected as monoliths from the vegetation and down to 10 cm below the top of the gley layer, cooled to 4°C, and brought to the laboratory.

The ability of soil microflora to oxidize methane was studied with soil samples at 5 and 15°C. Soil suspensions were prepared from samples of the soil monolith layers, according to the scheme of in situ study of bacterial activity: green moss; moss dust; moss–lichen mixture; peated moss–lichen and moss dust; peat; clay with plant roots; clay. Soil samples were transferred to 500-ml vials and covered with low-mineral-salt medium [16]. The medium-to-sample ratio was 10 : 1. The vials were hermetically sealed, evacuated and filled with a (30 : 70) air–methane mixture. The methane concentration in the gas phase was monitored during incubation.

Methane was determined using an LKhM-80 gas chromatograph equipped with a catharometer. Argon was used as the carrier gas, and Porapak Q was used as the sorbent.

Enrichment cultures of methanotrophic bacteria were obtained from soil samples for which active methane oxidation had been determined. Cell morphology was studied using an Amplival phase-contrast microscope (Germany). Ultrathin sections were prepared after fixation by the standard procedure.

Identification of methanotrophic bacteria in enrichment cultures was performed by hybridization with 16S rRNA–targeted specific fluorescent oligonucleotide probes according to the procedure described in [17].

Table 1. Methane emission from tundra soils at the beginning, middle, and end of the vegetation season

Object	June			August			September		
	Soil temperature, °C	Ground water level, cm	CH_4 emission, mg/(m^2 h)	Soil temperature, °C	Ground water level, cm	CH_4 emission, mg/(m^2 h)	Soil temperature, °C	Ground water level, cm	CH_4 emission, mg/(m^2 h)
Mounded tundra Soil: coarse humic gleyey loamy cryo soil									
Hummock	2.6	20	0.05	9	40	0.15	10	70	ND
Swampy hollow	6.5	6	0.07	8	26	0.23	8	50	0.12
Sedge–sphagnum bog Soil: peaty gley soil									
Bog edge	8	F	0.13	9	F	1.27	10	F	1.21
Hummock	5.6	27	0.07	10	37	0.38	10	57	ND
Swampy hollow	5.8	F	0.08						
Hillock tundra with flat-bottom depressions Soil: peaty gleyey mid-loamy cryo soil									
Slope	3.9	5	0.06	9	20	0.21	9	30	0.12

Note: ND, emission not detected; F, completely flooded parcels.

The group-specific probes developed for detection of type I and II methanotrophs, M-84+M-705 and M-450, respectively [18], as well as some species-specific probes: Mcell-1026, Mcaps-1032, and Mcell-143, which were developed for detection of the acidophilic methanotrophs *Methylocella palustris*, *Methylocapsa acidiphila*, and *Methylocella tundrae* [17, 19], were used for hybridization. The Cy3 dye was used as a fluorescent label. The samples were analyzed using an Axioplan-2 epifluorescent microscope (Germany).

RESULTS AND DISCUSSION

To estimate the intensity of microbial processes of the methane cycle in tundra soils, CH_4 emission was measured, the concentration of methane and its homologs in different soil horizons was determined, and the rates of methane production and oxidation by the microflora of soil samples were estimated. Methane emission was measured in June, August, and September. These periods differed in weather conditions, in temperature, and in the groundwater level (Table 1). In June, at an average daily temperature of 6°C, methane emission was insignificant in all parcels. Under soil temperatures of 2.6–6.5°C and high level of ground waters, methane emission on hummocks, on the slope, and in the swampy hollow was 0.05–0.08 mg CH_4/(m^2 h). The highest value of 0.13 mg CH_4/(m^2 h) was recorded in a completely flooded parcel of a sedge–sphagnum bog at 8°C. In August, when the average daily temperature was 16°C and the soil temperature increased to 8–10°C, the level of the water horizon decreased and the rate of methane emission to the atmosphere increased in all parcels: three- to fivefold on hummocks and swampy hollows and by an order of magnitude at the edge of the bog. Compared to August, the soil temperature in September hardly changed. The groundwater level was at the lowest mark for the entire period of investigation; the sedge–sphagnum bog, however, remained completely flooded. Under these conditions, CH_4 emission decreased twofold in the tundra swampy hollow and on the slope of the hillock tundra with flat-bottom depressions; on the hillocks it was absent altogether. In the bog, the methane emission rate did not change significantly between August and September. The results of direct measurements of methane flow to the atmosphere using the chamber method have thus demonstrated that the tundra soils studied, i.e., coarse humic–gleyey loamy–cryo soil, peaty–gleyey soil, and peaty–gleyey–midloamy–cryo soil, can act as sources of atmospheric methane. The rate of CH_4 emission from these soils was determined in situ by the conditions of temperature and moisture. The highest rates of methane emission were observed in August, when the soil temperature was 8–10°C and the groundwater level was high. The flooded parts of the sedge–sphagnum bog remained methane sources throughout the entire period of investigation. Decreased methane emission in September was probably a result of the activation of the methane oxidizing community of the bacterial filter under conditions of improved aeration.

In order to determine the in situ localization of the microbial communities performing methane transformations in tundra soils, the rates of methane production and oxidation by samples of the same soil horizons were measured at 5 and 15°C (Table 2). Methane oxidation was detected in bog water; in a combined sample of bog water and *Sphagnum fuscum*; in the green parts of *Sphagnum* sp. of the hillock tundra with flat-bottom depressions; in all soil horizons. No CH_4 oxidation was

Table 2. Methane oxidation and production rates in the tundra soil horizons at 15 and 5°C

Location/soil horizon	Horizon depth, cm	Horizon pH	Horizon temperature, °C	CH^4 oxidation, μl/(l day)	CH_4 production, μl/(l day)	CH^4 oxidation, μl/(l day)	CH_4 production, μl/(l day)
Experimental temperature, °C				15		5	
Mounded tundra							
Hummock (parcel no. 1)							
Moss, lichen	0–1	3.71	12.5	ND	ND	ND	ND
Peaty moss–lichen dust	2.5–12.5	3.95	8.8	0.025	ND	0.372	ND
Peat	12.5–16	4.89	2.6	0.95	0.122 (13.8)	0.118	0.001
Clay with plant roots	16–18	4.7	2.4	1.085	0.13 (3.0)	0.116	0.001
Swampy hollow (parcel no. 2)							
Moss, lichen	0–0.5	–	–	–	–	ND	ND
Peaty moss–lichen dust	0.5–1.5	–	–	–	–	0.135	ND
Peat	1.5–3	5.17	6.5	–	–	0.075	0.001
Peat with clay	3–3.5			–	–	0.029	0.003
Brown clay with plant roots	3.5–6.0	5.16	5.5	–	–	0.014	0.002
Gray clay	Below 6.0	4.56	4.4	–	–	ND	ND
Sedge–sphagnum bog							
Water		5.0	11.0	1.360	ND	–	–
Water with moss plants		5.2	11.5	–	–	1.305	ND
Bog edge (parcel no. 3)							
Green moss parts		4.5	11	0.139	ND	0.360	ND
Moss dust		5.1	10	0.233	ND	0.567	ND
Peaty moss dust		5.1	10	1.672	6.51 (1.3%)	1.193	0.012
Peat		–	–	2.295	31.33 (7.7%)	–	–
Clay with plant roots		4.7		4.159	53.54 (0.1%)	–	–
Hummock (parcel no. 4)							
Green moss parts	0–2	4.2	14.1	0.002	ND	0.238	ND
Moss dust	2–5	3.85	11.1	0.006	ND	0.018	ND
Peaty moss dust	5–10	4.23	6.7	0.04	ND	0.098	0.037
Peat	10–14			0.182	53.05 (5.6)	–	–
Clay with plant roots	14–18	4.46	5.6	0.074	0.930 (0.1)	0.004	0.002
Swampy hollow (parcel no. 5)							
Green moss	0–3	4.79	10.7	–	–	0.006	ND
Moss dust	5–10	4.6	6.5	0.002	ND	0.152	ND
Peaty moss dust	10–15			0.132	34.87 (1.2%)	–	–
Peat	15–29			0.237	55.94 (6.3%)	–	–
Hillock tundra with flat-bottom depressions							
Hummock (parcel no. 6)							
Green moss	0–1	3.8	15.4	–	–	0.003	ND
Moss dust	1–13	3.5	13.0	–	–	0.004	ND
Peaty moss dust	13–18	3.5	10.5	–	–	0.1	0.002
Peat	18–20	3.9	2.2	–	–	0.028	0.018

Note: In parentheses, the contribution of aceticlastic methanogenesis (%) to the total methane production is indicated. "–," not determined; ND, not detected.

detected in the samples of lichens *Cetraria islandica* and *Cladonia rangiferina* and the mosses *Polytrichum* sp. and *Aulacomnium* sp. of the moss–lichen tundra. Methane production occurred in the layers of peat, peat with clay, clay with plant roots; in the bogey parcels, it also occurred in peaty moss dust. The highest rates of the processes of the methane cycle were found in the peat layer and in clay with plant roots.

In all of the studied soil horizons of the parcels of mounded tundra and of hillock tundra with flat-bottom depressions, methane oxidation rates exceeded the rates of methane production, both at 5 and 15°C. Methanogenesis prevailed only in the boggy parcels and only at 15°C. The rates of methane oxidation in bog water and in the combined water–sphagnum sample were approximately the same at 5 and 15°C, namely, 1360 and 1305 μl CH_4/(l day), respectively.

Thus, microbial production and consumption of methane occurred in the tundra soil horizons at temperatures of 2.2–15.4°C, pH 3.5–5.2, and mineral salt concentrations of 8–15 mg/l. At 5°C, methane oxidation prevailed over methane production in all horizons of tundra soil, indicating the existence of a psychrophilic bacterial oxidative community. High rates of methane oxidation, as compared to methane production rates, can be due to the oxidation of both newly produced methane and the methane deposited in soil. The methane-oxidizing community is probably first activated after defreezing of tundra soils and it consumes the methane released from the soil. For methanogenic archaea to become active, a considerable decrease of the medium redox potential (Eh) is required. At soil temperatures of 2.6–6.5°C, Eh values were positive for all the sampling sites and varied from +100 to +460 mV. With the warming of the soil to 8–10°C and enhancement of oxidative microbial processes, the redox potential at a depth of 10–15 cm decreased to –50 or –100 mV, thus promoting the activation of anaerobic processes, including methanogenesis. It is noteworthy that, unlike 5°C, at 15°C not only methane formation from carbon dioxide, but also aceticlastic methanogenesis, was detected; its contribution to the net methane production was the highest in the peat layer.

The presence of methane and its homologs was studied in soil samples from different soil horizons (Table 3). Methane was detected in all of the samples, including samples of the mosses *Polytrichum* sp. and *Aulacomnium* sp., and of the lichens *C. islandica* and *C. rangiferina*, although methanogenic activity was absent in these horizons. Together with methane, its heavier homologs, ethane and a propane–butane mixture, were also detected. The presence of homologs usually indicates an abiogenic origin of methane and its penetration from deeper layers. The highest levels of ethane, butane, and propane, however, were found in the horizons of peat and peaty moss–lichen dust. The concentrations of C_2 and $C_{3–4}$ gases decreased in the lower gley horizon. Clay with its dense structure and small pore volume is probably less capable of accumulating gases released from soil than are the porous peat and moss dust. Biogenic processes leading to the formation of methane homologs can be another possible explanation to the elevated concentrations of the C_2–C_4 gases in these horizons; the mechanism of these processes requires special investigation.

To determine the principal bacterial agents responsible for methane oxidation in the tundra soil horizons at 5 and 15°C, methane oxidation by soil samples was investigated, and enrichment cultures of methane-oxidizing bacteria were obtained.

Both at 5 and at 10°C, methane oxidation was detected in suspensions prepared from samples of moss dust, moss–lichen dust, of peaty moss dust, peaty moss–lichen dust, peat, and clay with plant roots (Table 4). These findings are in good accordance with the results of field investigations. No methane oxidation occurred in clay samples, unlike clay with plant roots; this may be an indirect indication of the bacterial relations with the root system. Unlike radioisotope experiments, no methane oxidation was detected in vitro in the green parts of *Sphagnum* sp. and *Sphagnum fuscum*; this is in accordance with our previous results [20] and may be due to differences in sample treatment and to the high sensitivity of the radioisotope method. In the case of in situ analysis, sphagnum was impregnated with water, and, actually, it was its methane-oxidizing activity that was detected.

At both temperatures, the methane-oxidizing activity of the soil samples in vitro was considerably higher than that of the soil horizons as measured in situ. This is possibly a result of the in vitro experimental procedure, when all the incubation mixtures were supplemented with approximately the same amount of methane, much higher than its natural concentration. This setup demonstrates the potential methane-oxidizing ability of the community. It should be mentioned that soil suspensions oxidize methane more actively at 5°C than at 15°C.

The most active soil samples were used for the isolation of methanotrophic bacteria. Two enrichment cultures of psychrotolerant methane-oxidizing bacteria were obtained, NK-1 and NK-2. The NK-1 enrichment culture was obtained from a clay and plant root suspension. During culture growth, flocks were formed, containing both a morphologically characteristic methanotrophic organism and rosette-forming rod-shaped bacteria (Fig. 1a). The methanotrophic cells were big cocci, 2–2.5 μm (Fig. 1b). Small stacks of membranes located in the peripheral zone of the cytoplasm were found in ultrathin sections (Fig. 1c, 1d). We earlier detected organisms of similar morphology in tundra sphagnum peats [20]. The enrichment culture NK-1 oxidized methane both at 5 and 15°C, although with different rates and dynamics (Fig. 2a). While at 5°C culture growth and methane oxidation began on the third–fourth day, at 15°C the lag phase duration before

Table 3. Concentrations of hydrocarbon gases in the tundra soil horizons

Location; soil horizon	Depth, cm	Amount, μl/l		
		Methane	Ethane	Propane and butane
Mounded tundra				
Hummock				
Moss, lichen, moss–lichen dust	0–2.5	4.13	0.92	0.69
Peaty moss–lichen dust	2.5–12.5	5.85	7.34	0.69
Peat	12.5–16	4.02	4.25	0.17
Gley layer	16–19	2.07	0.34	0.00
Swampy hollow				
Moss and lichen	0–0.5			
Moss–lichen dust	0.5–1.5			
Peat	1.5–3	3.90	3.10	0.34
Peat and clay	3–5	2.07	0.57	0.00
Brown clay	5–8	1.95	0.23	0.00
Gray clay	5–8	6.31	0.34	0.00
Sedge–sphagnum bog				
Water and sphagnum	0–1	0.96	0.11	0.00
Water	10	0.77	0.08	0.00
Hummock				
Moss	0–5	2.18	0.80	0.46
Moss dust	5–10	2.07	0.57	0.23
Peaty moss dust	10–14	1.95	0.57	0.12
Brown clay	14–17	2.64	0.57	0.23
Swampy hollow				
Moss with water	0–3	5.62	1.26	0.34
Moss	10–12	9.87	0.46	0.12
Hillock tundra with flat-bottom depressions				
Moss	0–1	2.3	1.61	0.34
Moss dust	1–13	1.84	0.80	0.12
Peaty moss dust	13–18	2.07	1.26	0.46
Peat	18–20	2.64	2.07	0.34

the onset of growth and methane oxidation was 12–15 days. At 5°C, the maximum rate of methane oxidation was 0.18 mmol CH_4/(l h), and the total amount of oxidized methane was 143 mmol CH_4/l culture. At 15°C, the maximum rate of methane oxidation was twice as less, and the amount of oxidized methane under these conditions was 52.4 mmol CH_4/l culture. Culture NK-1 oxidized methane in a wide pH range, 4.8–7.5. The temperature of 5°C and pH range 6.2–6.8 were optimal for this consortium. It retained stability in the course of numerous transfers, but the standard techniques did not result in isolation of a pure culture of the methanotrophic component of this community.

In order to identify the methanotrophic component of this enrichment culture, we used fluorescent oligonucleotide probes designed for the detection of type I and II methanotrophs and for a number of acidophilic methanotrophs (see Materials and Methods). Only a combination of probes M-84 + M-705, complementary to sites of 16S rRNA sequences specific for type I methanotrophs, resulted in specific staining of the coccoid cells.

The NK-2 enrichment culture obtained from peaty moss dust consisted of rods of different sizes. It growth was homogeneous. The growth of NK-2 and the dynamics of methane oxidation were different at 5 and 15°C (Fig. 2b). At both 5 and 15°C, methane consumption began without a noticeable lag phase, at rates of 0.086 and 0.07 mmol CH_4/(l h), respectively. At 5°C, however, methane oxidation became slower by the 12th day and stopped after 36 days. The rate of CH_4 oxidation by this culture at

Table 4. Methane oxidation by soil suspensions, μl/(l day)

Location	Mounded tundra				Sedge–sphagnum bog						Hillock tundra with flat-bottom depressions	
	Parcel no. 1		Parcel no. 2		Parcel no. 3		Parcel no. 4		Parcel no. 5		Parcel no. 6	
Temperature, °C	15	5	15	5	15	5	15	5	15	5	15	5
Green part of moss					ND	ND	ND	ND	ND	ND	ND	ND
Moss and lichen	ND	ND	ND	ND								
Moss dust					23.2	20.4	14.5	15.1	12.3	28.1	17.4	19.9
Moss–lichen dust	13.5	17.8	10.7	19.0								
Peaty moss dust					25.6	33.6	13.6	19.8	10.8	39.5	14.7	20.3
Peaty moss–lichen dust	20.4	21.9	17.8	7.3								
Peat	20.9	27.1	18.4	36.8	24.7	40.2	17.4	23.7	10.5	40.0	20.5	28.6
Clay and plant roots	15.8	31.2	13.6	34.5			14.3	21.1			19.5	27.5
Clay	ND	ND	ND	ND							ND	ND

Note: ND, not detected.

15°C increased significantly by the 24th day and by the 36th day reached the maximum level, 2.5 times higher than that at 5°C. The optimum for NK-2 was the temperature of 15°C and the pH range 5.5–6.0. Analysis of this culture with fluorescent oligonucleotide probes revealed its affiliation with *Methylocella tundrae*, a recently identified species of acidophilic methanotrophs [21].

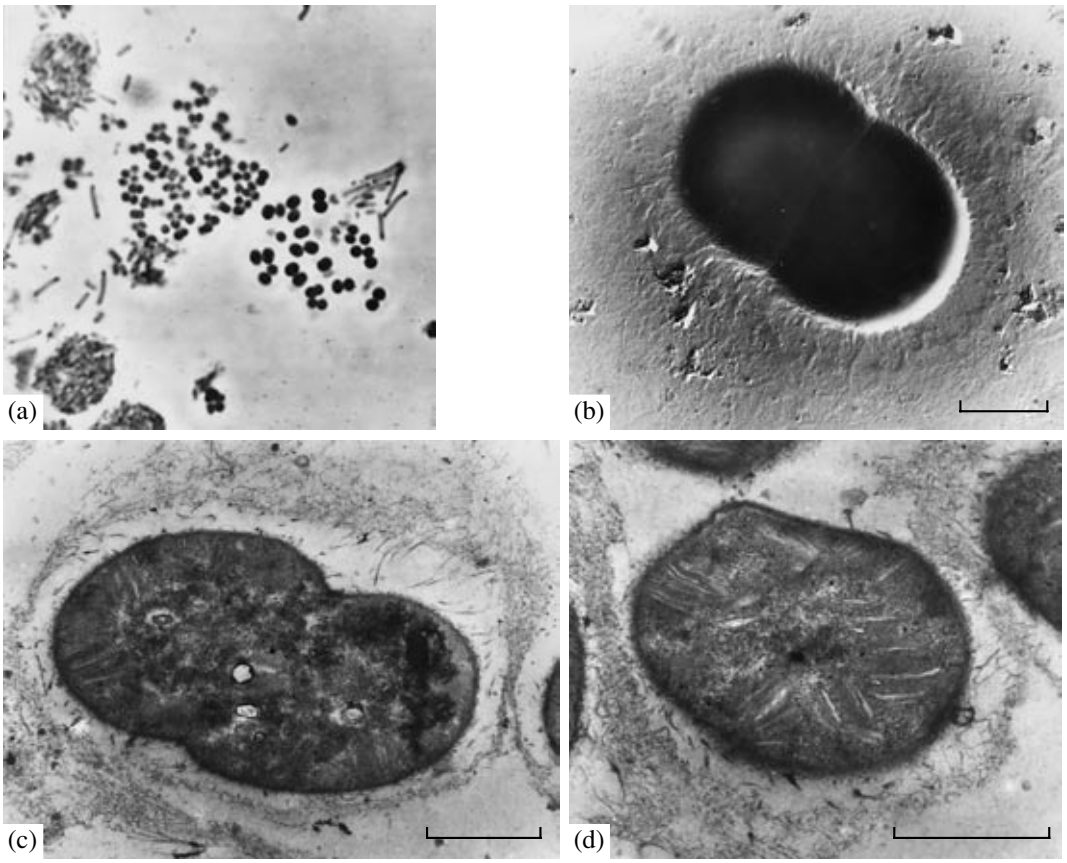

Fig. 1. Methanotrophic component of enrichment culture 1: (a) in consortium with rod-shaped bacteria (1100×); (b) a methanotrophic cell (chromium spray-coating; bar, 1 μm); (c, d) ultrathin sections of the methanotroph (bar, 1 μm).

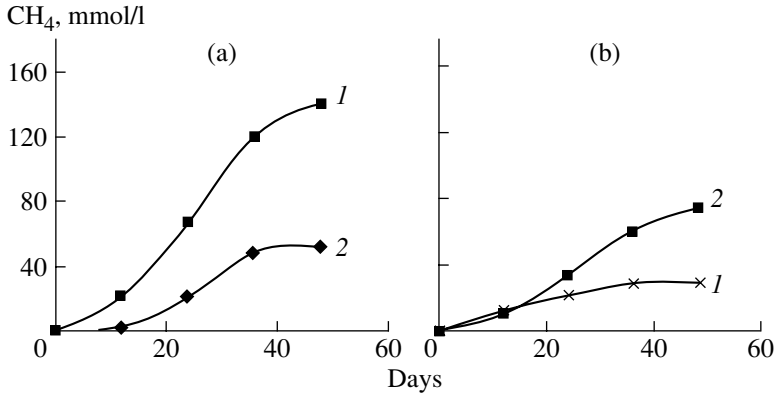

Fig. 2. Methane oxidation by methanotrophic enrichment cultures: (a) NK-1; (b) NK-2 (*1*) at 5 and (*2*) 15°C.

The present paper demonstrates that the bacterial community of tundra soil includes representatives of various groups of methanotrophic bacteria, which are responsible for methane oxidation at different values of pH and temperature. Type I methanotrophic organisms oxidized methane at 5°C and neutral acidity values of the soil solution. At 15°C and under acidic conditions, methane oxidation was carried out by a psychrotolerant representative of acidophilic methanotrophic bacteria of the genus *Methylocella, Methylocella tundrae.*

ACKNOWLEDGMENTS

The authors thank researchers from the Institute of Microbiology, Russian Academy of Sciences, S.N. Dedysh for her assistance in the analysis of the composition of methanotrophic communities using fluorescently labeled oligonucleotide probes and for her comments in the course of discussing experimental results, and L.L. Mityushina for ultrathin cell sectioning.

This work was supported by the Federal Special Program "Research and Development in Priority Fields of Science and Technology" on the topic "Biogenic Sinks, Sources, and Reservoirs of Greenhouse Gases" and by the Russian Foundation for Basic Research, project no. 02-04-49296.

REFERENCES

1. Zavarzin, G.A. and Vasil'eva, L.V., The Methane Cycle on the Territory of Russia, in *Krugovorot ugleroda na territorii Rossii* (The Carbon Cycle on the Territory of Russia), Moscow: Minist. Nauki Tekhnol. Ross. Feder., 1999, pp. 202–230.
2. Microorganisms and the Composition of the Atmosphere, *Rol' mikroorganizmov v krugovorote gazov v prirode* (The Role of Microorganisms in the Gas Cycling in Nature) Zavarzin, G.A., Ed., Moscow: Nauka, 1979, pp. 5–35.
3. Zavarzin, G.A., *Bakterii i sostav atmosfery* (Bacteria and the Composition of the Atmosphere), Moscow: Nauka, 1984.
4. Zavarzin, G.A., The Psychrophilic Sohngen Cycle, *Ekol. Khimiya*, 1995, vol. 4, p. 3.
5. Whalen, S.C. and Reeburgh, W.S., Consumption of Atmospheric Methane by Tundra Soils, *Nature*, 1990, vol. 12, p. 346.
6. Slobodkin, A.I., Panikov, N.S., and Zavarzin, G.A., The Production and Consumption of Methane by Microorganisms in the Marshes of Tundra and Middle Taiga, *Mikrobiologiya*, 1992, vol. 61, no. 4, pp. 683–691.
7. Christensen, T.R. and Jonasson, S., Spatial Variation in High-Latitude Methane Flux along a Transect across Siberian and European Tundra Environments, *J. Geophys. Res.*, 1995, vol. 100, pp. 21 035–21 045.
8. Christensens, T.R., Prentice, I.C., Kaaplan, J., Haxeltine, A., and Sitch, S., Methane Flux from Northern Wetlands and Tundra, *Tellus*, 1996, vol. 48B, pp. 652–661.
9. Christensens, T.R., Jonasson, S., Callaghan, T.V., Havstrom, M., and Livens, F.R., Carbon Cycling and Methane Exchange in Eurasian Tundra Ecosystems, *Ambio*, 1999, vol. 28, no. 3, pp. 239–244.
10. *Zemel'nye resursy Rossii* (The Soil Resources of Russia), CD-ROM of NASA and Russian Academy of Sciences, 2002.
11. Fung, I., John, J., Lerner, J., Matthews, E., Prather, M., Steele, L.P., and Fraser, P.J., Three-Dimensional Model Synthesis of the Global Methane Cycle, *J. Geophys. Res.*, 1991, vol. 96, pp. 13033–13065.
12. Gal'chenko, V.F., Dulov, L.E., Kramer, B., Konova, N.I., and Barysheva, S.V., Biogeochemical Processes of Methane Cycle in the Soils, Bogs, and Lakes of Western Siberia, *Mikrobiologiya*, 2001, vol. 70, no. 2, pp. 215–225.
13. Vecherskaya, M.S., Galchenko, V.F., Sokolova, E.N., and Samarkin, V.A., Activity and Species Composition of Aerobic Methanotrophic Communities in Tundra Soils, *Curr. Microbiol.*, 1993, vol. 27, pp. 181–184.
14. Omel'chenko, M.V., Vasil'eva, L.V., Zavarzin, G.A., Savel'eva, N.D., Lysenko, A.M., Mityushina, L.L., Khmelenina, V.N., and Trotsenko, Yu.A., A Novel Psychrophilic Methanotroph of the Genus *Methylobacter, Mikrobiologiya*, 1996, vol. 65, no. 3, pp. 384–389.
15. Rusanov, I.I., Savvichev, A.S., Yusupov, S.K., Pimenov, N.V., and Ivanov, M.V., Production of Exometabolites in the Microbial Oxidation of Methane in Marine

Ecosystems, *Mikrobiologiya*, 1998, vol. 67, no. 5, pp. 710–717.

16. Berestovskaya, Yu.Yu., Vasil'eva, L.V., Chestnykh, O.V., and Zavarzin, G.A., Methanotrophs of the Psychrophilic Microbial Community of the Russian Arctic Tundra, *Mikrobiologiya*, 2002, vol. 71, no. 4, pp. 538–544.
17. Dedysh, S.N., Derakshani, M., and Liesack, W., Detection and Enumeration of Methanotrophs in Acidic Sphagnum Peat by 16S rRNA Fluorescence In Situ Hybridization, Including the Use of Newly Developed Oligonucleotide Probes for *Methylocella palustris, Appl. Environ. Microbiol.*, 2001, vol. 67, pp. 4850–4857.
18. Eller, G., Stubner, S., and Frenzel, P., Group Specific 16S rRNA- Targeted Probes for the Detection of Type I and Type II Methanotrophs by Fluorescence In Situ Hybridization, *FEMS Microbiol. Letts.*, 2001, vol. 198, pp. 91–97.
19. Dedysh, S.N., Dunfield, P.F., Derakshani, M., Stubner, S., Heyer, J., and Liesack, W., Differential Detection of Type II Methanotrophic Bacteria in Acidic Peatlands Using Newly Developed 16S rRNA-Targeted Fluorescent Oligonucleotide Probes, *FEMS Microbiol. Ecol.*, 2003, vol. 43, pp. 299–308.
20. Vasil'eva, L.V., Berestovskaya, Yu.Yu., and Zavarzin, G.A., Psychrophilic Acidophilic Methanotrophs from a Sphagnum Wetland in the Permafrost Area, *Dokl. Akad. Nauk*, 1999, vol. 368, no. 1, pp. 125–128.
21. Dedysh, S.N., Berestovskaya, Y.Y., Vasylieva, L.V., Belova, S.E., Khmelenina, V.N., Susina, N.E., Trotsenko, Y.A., Liesack, W., and Zavarzin, G.A., *Methylocella tundrae* sp. nov., a Novel Methanotrophic Bacterium from Acidic Tundra Peatlands, *Int. J. Syst. Evol. Microbiol.*, 2004, vol. 54, pp. 151–156.