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EXPERIMENTAL ARTICLES

Methanotrophs of the Psychrophilic Microbial Community of the Russian Arctic Tundra

Yu. Yu. Berestovskaya*, L. V. Vasil'eva*, O. V. Chestnykh, and G. A. Zavarzin***

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

***Center of Forest Ecology and Productivity, Russian Academy of Sciences, Moscow*

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Abstract—In tundra, at a low temperature, there exists a slowly developing methanotrophic community. Methane-oxidizing bacteria are associated with plants growing at high humidity, such as sedge and sphagnum; no methanotrophs were found in polytrichous and aulacomnious mosses and lichens, typical of more arid areas. The methanotrophic bacterial community inhabits definite soil horizons, from moss dust to peat formed from it. The potential ability of the methanotrophic community to oxidize methane at 5°C enhances with the depth of the soil profile in spite of the decreasing soil temperature. The methanotrophic community was found to gradually adapt to various temperatures due to the presence of different methane-oxidizing bacteria in its composition. Depending on the temperature and pH, different methanotrophs occupy different econiches. Within a temperature range from 5 to 15°C, three morphologically distinct groups of methanotrophs could be distinguished. At pH 5–7 and 5–15°C, forms morphologically similar to *Methylobacter psychrophilus* predominated, whereas at the acidic pH 4–6 and 10–15°C, bipolar cells typical of *Methylocella palustris* were mostly found. The third group of methanotrophic bacteria growing at pH 5–7 and 5–10°C was represented by a novel methanotroph whose large coccoid cells had a thick mucous capsule.

Key words : psychrophily, methane oxidation, bacterial filter, greenhouse gases, microbial communities.

The major sources of atmospheric methane, an important greenhouse gas, are on the continents [1]. Amphibian landscapes contribute much to methane emission. In Russia, water-logged and peaty areas make up 369 million hectares, three fourths of which are in the permafrost zone, permanently under low temperature; these areas contribute significantly to biogeochemical processes [2]. The flux of methane to the atmosphere depends on the ratio of two microbial processes, methane production and methane oxidation [3]. In situ analyses of methane emission from water-logged soils showed that bogged soils are natural biofilters for methane [4–7]. Methane formed in the anaerobic zone is oxidized by the soil methanotrophic community. At low temperatures, methane oxidation was shown to occur in both neutral and acidic ambiance. Psychrophilic acidophilic methanotrophs have been found, which are closely associated with sphagnum [8, 9]; however, only two psychrophilic organisms have so far been isolated in pure cultures: the freshwater *Methylobacter psychrophilus* growing within a temperature range from 3.5 to 10° with an optimum at 6°C [10] and *Methylosphaera hansonii* requiring a seawater salinity value and having a growth optimum at 10 to 13°C and a growth maximum at 16–21°C [11]. The biogeochemical activity of methane-oxidizing bacteria in soils of northern areas remains poorly studied. In the present work, we studied the psychrophilic methane-oxidizing bacterial community of tundra soils, its association with vegetation, and distribution along the soil profile, as well as the temperature and pH dependence of the diversity of methane-oxidizing bacteria.

MATERIALS AND METHODS

Tundra soil was sampled near the Tal'nik station (67°–20' N, 62°44' E), 20 km south of Vorkuta, in July–August 1999. Soil samples were taken in brush-and-lichen tundra (plot 1) and sedge-and-moss bog (plot 2), which differed in moisture content, acidity (pH), and in the species composition of vegetation covering. The tundra soil was characterized by a steep temperature gradient along its profile. At the soil surface of plot 1, the temperature was 19°C, whereas at a depth of 5 and 10 cm from the surface it was 15.4 and 8.3°C, respectively. In soil of the sedge bog, a temperature drop was

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even more abrupt: from 20.2°C at the surface to 4.0°C at a depth of 10 cm. The pH values of soil solutions derived from plot 1 and 2 were 5.5 and 4.5, respectively.

Soil samples were excised in the form of monoliths, from the surface to the mineral horizon. In the laboratory, each monolith was separated into layers as follows: vegetation covering (lichen, moss, and sedge); moss dust; mos dust undergoing transformation into peat; and peat. Two to five samples of each layer were taken. Dominant plants, namely lichens of the genera *Cetraria* and *Cladonia*, mosses of the genera *Sphagnum*, *Polytrichum*, and *Aulacomnium* and the sedge *Carex* sp. (Table 1), were examined to reveal the association between methane-oxidizing bacteria and the representatives of the vegetation covering. In lichens, the thalli were examined. Mosses were divided into the upper green portion containing chlorophyll and the chlorophyll-deprived plant basis, which formed moss-dust layers varying in thickness. In sedge, the upper (stem, leaves) and underground (roots, rhizome) portions were examined.

Weighed sample portions were put in 500-ml serum bottles and in 125-ml flasks and poured with mineral medium in a weight/volume ratio of 1 : 10 so that the incubation mixture made up 10% of the flask volume. The flasks were closed hermetically, and the gas phase was replaced by a mixture of methane and air. Methane accounted for 30 to 50% of the gas phase.

A medium with low mineralization was specially developed to cultivate microorganisms from ultrafresh habitats, (mg/l): NH_4SO, 500; $MgCl_2$, 40; KH_2PO_4, 70; and a solution of microelements, 1 ml/l [8]. The total amount of mineral salts comprised 0.6 g/l, which was similar to that in the soil solution in the sites of sampling.

The values of pH from 4.0 to 7.0 were obtained by using 0.1 N solutions of HCl and NaOH.

Since we were interested in the processes occurring in soil horizons at a low temperature, all experiments were conducted at temperatures ranging from 5 to 20°C.

The content of methane in the gas phase was measured on an LKhM-80 gas chromatograph equipped with a katharometer. Argon was the carrier gas, and Porapak Q served as the sorbent.

Cell morphology was examined under an Amplival phase-contrast microscope (Germany).

RESULTS AND DISCUSSION

No methanotrophic bacteria were found among the microflora of the upper green portion of moss and lichen thalli. In samples prepared from sedge stems and leaves, methane oxidation was detected after two months of cultivation. The methane-oxidizing activity was retained in culture transfers, which suggests that sedge serves as a methane filter similarly to that of rice [12]. The population density of methane-oxidizing bacteria (microscopic observations) and methane-oxidizing activity increased in plants growing at high humidity (sedge and sphagnum). There were no methanotrophs associated with plants growing in arid areas (polytrichous and alacomnious mosses and lichens). The population density of methanotrophs appeared to increase in the zones of methane formation, namely in flooded anaerobic regions with intense methanogenesis [4]. These results disagree with the common notion suggesting that in tundra the atmospheric methane is primarily consumed in well-aerated, high, and dry areas [13].

Table 1. Methanotroph association with vegetation

Plant	Presence of methanotrophs		
	5°C	10°C	15°C
Lichens:			
Cetraria nivalis	–	–	–
Cetraria islandica	–	–	–
Cladonia rangiferina	–	–	–
Cladonia arbuscula	–	–	–
Mosses (chlorophyll-containing portion of the plant):			
Sphagnum sp.	–	–	–
Politrichum sp.	–	–	–
Aulacomnium sp.	–	–	–
Sedge (*Carex* sp.):			
leaves and stems	–	+	+
roots and rhizomes	–	–	–

Methanotrophic bacteria proved to be associated with various soil horizons: moss dust, peat formed from the moss dust undergoing transformation to peat, and peat, regardless of their thickness and the depth of occurrence. Microscopic examination of the microbial communities of the enrichment cultures obtained from these samples revealed methanotrophs, which could be easily identified because of their typical cell morphology. We also confirmed our previous data on the association of methanotrophic bacteria with the hyaline cells of sphagnum [8]. Under conditions of low buffer capacity, a favorable pH value can probably be maintained only in the presence of sphagnum plants, and this may account for the close association between certain methanotroph species and sphagnum.

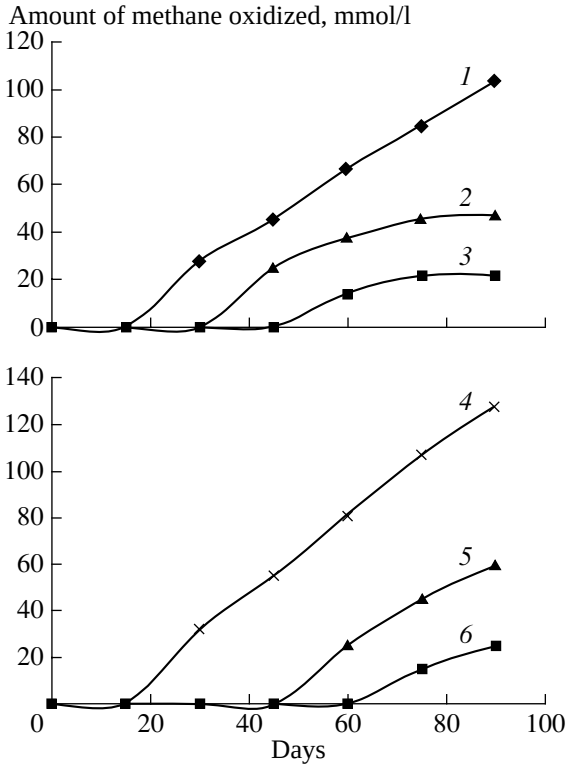

Fig. 1. Methane oxidation at 5°C by the bacterial communities isolated from soil samples taken at various depth: (*1*) 18–20, (*2*) 6–8, (*3*) 3–4, (*4*) 8–10, (*5*) 4–5, and (*6*) 2–3 cm.

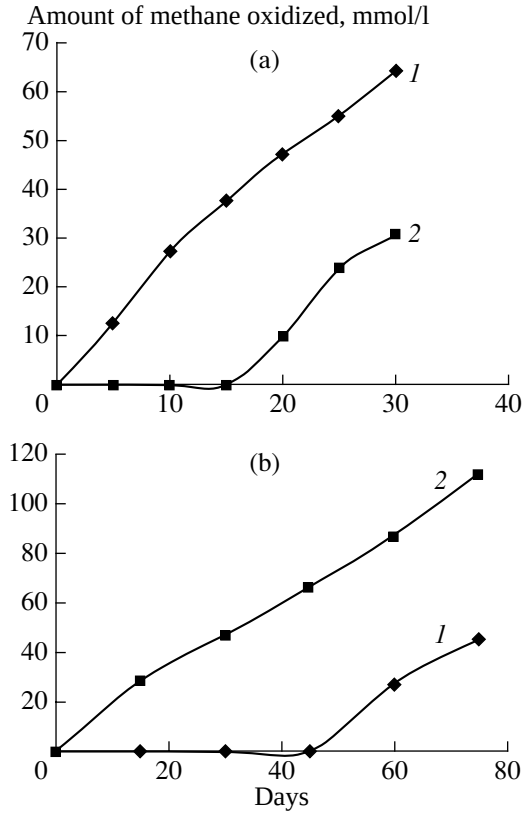

Fig. 2. Methane oxidation by the bacterial communities at different temperatures: (a) 10°C and (b) 5°C: (*1*) bacterial community isolated from a soil horizon with a temperature of 10°C; (*2*) bacterial community isolated from a soil horizon with a temperature of 5°C.

In the enrichment cultures, the methane-oxidizing activity at 5°C correlated with the depth of sampling (Fig. 1). At this temperature, the amount of methane utilized by a microbial community increased with the depth of occurrence of the sampled soil horizon. The pattern of methane consumption also changed: as the permafrost horizon was approached and the soil temperature decreased, the lag phase preceding methane consumption reduced.

Table 2. Presence of various morphotypes in methanotrophic communities as dependent on pH and temperature

pH	Temperature, °C	Morphotype 1	Morphotype 2	Morphotype 3
4	5	–	–	–
	10	–	–	–
	15	–	–	–
5	5	+	+	–
	10	+	+	+
	15	–	–	+
6	5	+	+	–
	10	+	+	+
	15	+	–	+
7	5	+	+	–
	10	+	+	–
	15	+	–	–

The adaptability of methane-oxidizing communities to various temperatures was also studied (Fig. 2). The bacterial community isolated from a soil horizon with a temperature of 4–5°C utilized methane at 5°C from the very beginning of cultivation, whereas under incubation at 10°C methane utilization began as late as on the 10th to 15th day of cultivation. The bacterial community from the soil layer with a temperature of 10°C oxidized methane without any noticeable lag phase at 10°C, whereas at 5°C the adaptation period comprised 40–45 days. Thus, under in vitro conditions, the psychrophilic methane-oxidizing community shows slow adaptation to temperature changes.

Different methane-oxidizing agents appear to be present in soil samples, as judged from the adaptation period preceding methane oxidation at various temperatures and from the dependence of methane consumption on temperature and pH. Microscopic examination

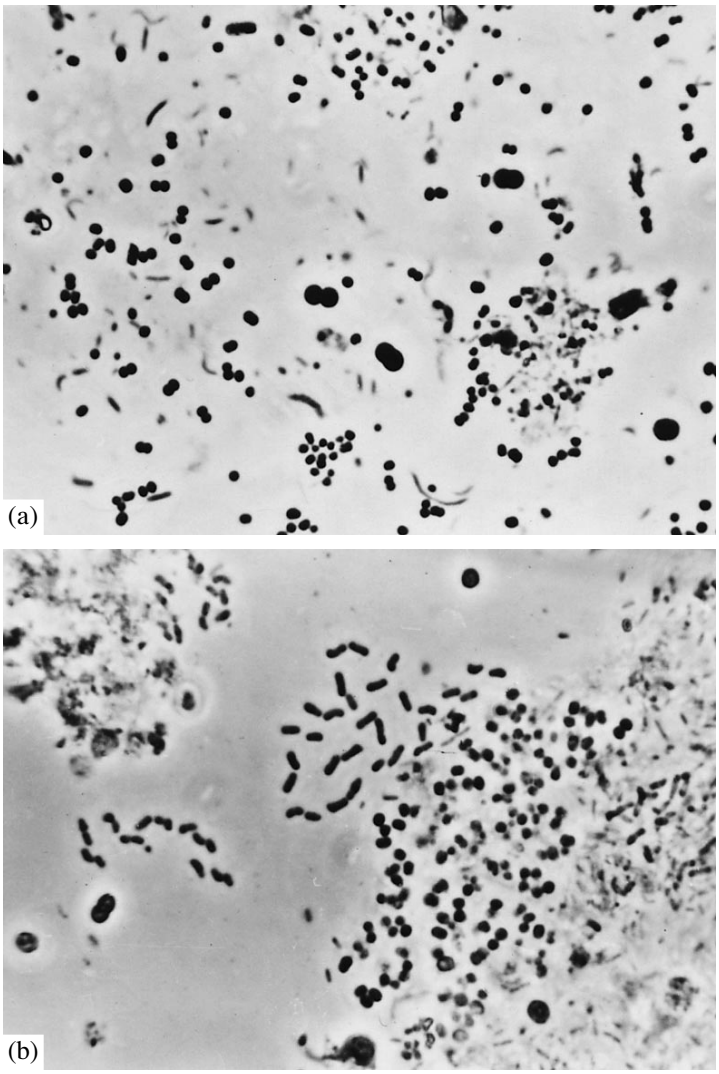

Fig. 3. Morphological types of methanotrophs contributing to the methane-oxidizing bacterial community: (a) coccoid bacteria (950×); (b) rod-shaped bacteria (800×).

of the microbial communities developing at different temperatures revealed various methanotrophic bacteria, which were readily differentiated due to their specific morphology (Fig. 3). In the samples studied, at least two coccoid forms of methanotrophs were identified, which differed in cell size and either contained vesicles or not (Fig. 3a). The cells with a diameter of 1–1.4 μm resembled those of *Methylobacter psychrophilus* described earlier; they were designated as morphotype 1; the large cocci with a diameter of 2–2.5 μm and rods morphologically similar to *Methylocella palustris* (Fig. 3b) were designated as morphotypes 2 and 3, respectively.

In the bacterial communities studied, the morphotypes altered depending on physicochemical conditions. Table 2 shows the presence of various morphotypes at the temperatures of 5, 10, 15, and 20°C and at pH from 4 to 7. At 20°C, all cultures consumed and insignificant amount of methane and, therefore, further experiments were conducted within a temperature range from 5 to 15°. At pH < 4 and temperatures of 5, 10, and 15°C, yeasts dominated the microbial communities. Yeast growth was also observed at all the temperatures studied and at pH from 4 to 6. The morphotype 1 and 2 methanotrophs were detected at temperatures of 5 and 10°C and pH 5–6. The morphotype 1 methane-oxidizing bacteria grew at 15°C and at pH higher than 5, whereas there were no morphotype 2 bacteria at that temperature at any pH values studied, suggesting that the morphotype 2 methanotrophs were actual psychrophiles. The methanotrophs similar to *Methylocella palustris* emerged at pH 5 beginning with a temperature of 10°C and grew at 10–15°C and pH lower than

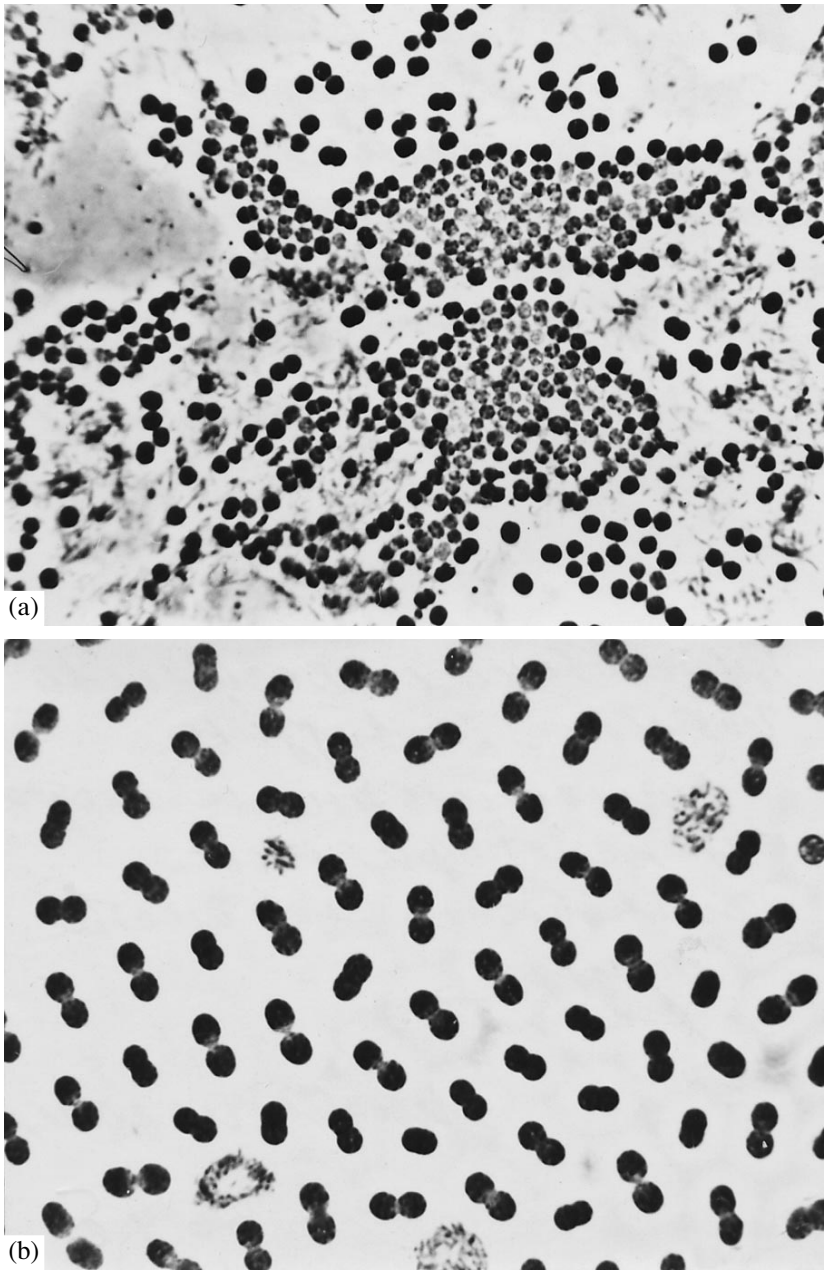

Fig. 4. Enrichment monocultures of methanotrophic bacteria: (a) morphotype 1; (b) morphotype 2. Magnification, 1120×.

7.0 [14]. At pH 5–6 and 10°C, the community was the most diverse; all three morphotypes were detectable.

Some methanotrophs were isolated in monocultures containing rod-shaped satellites (Fig. 4). These monocultures shared a common property of forming flocs throughout the entire volume of the cultivation medium. Most of the cells were arranged in aggregates with a structure shown in Fig. 5. The large morphotype 2 cells had thick mucous capsules, which prevented permeation of small bacteria and protozoa (Fig. 5a). The structured microcolonies of these cells in the form of packed spheres contained methanotrophs in the central part, whereas rod-shaped bacteria filled the space between mucous spheres (Fig. 5b). Such a community was stable after numerous culture transfers. Structured communities of microorganisms attract much attention not only because many processes are driven by them, but also because their physical organization can be studied in situ by the methods of the branch of science referred to as parahistology of microbial communities.

Thus, various representatives of methane-oxidizing bacteria, which serve as the methane filter, thrive in the

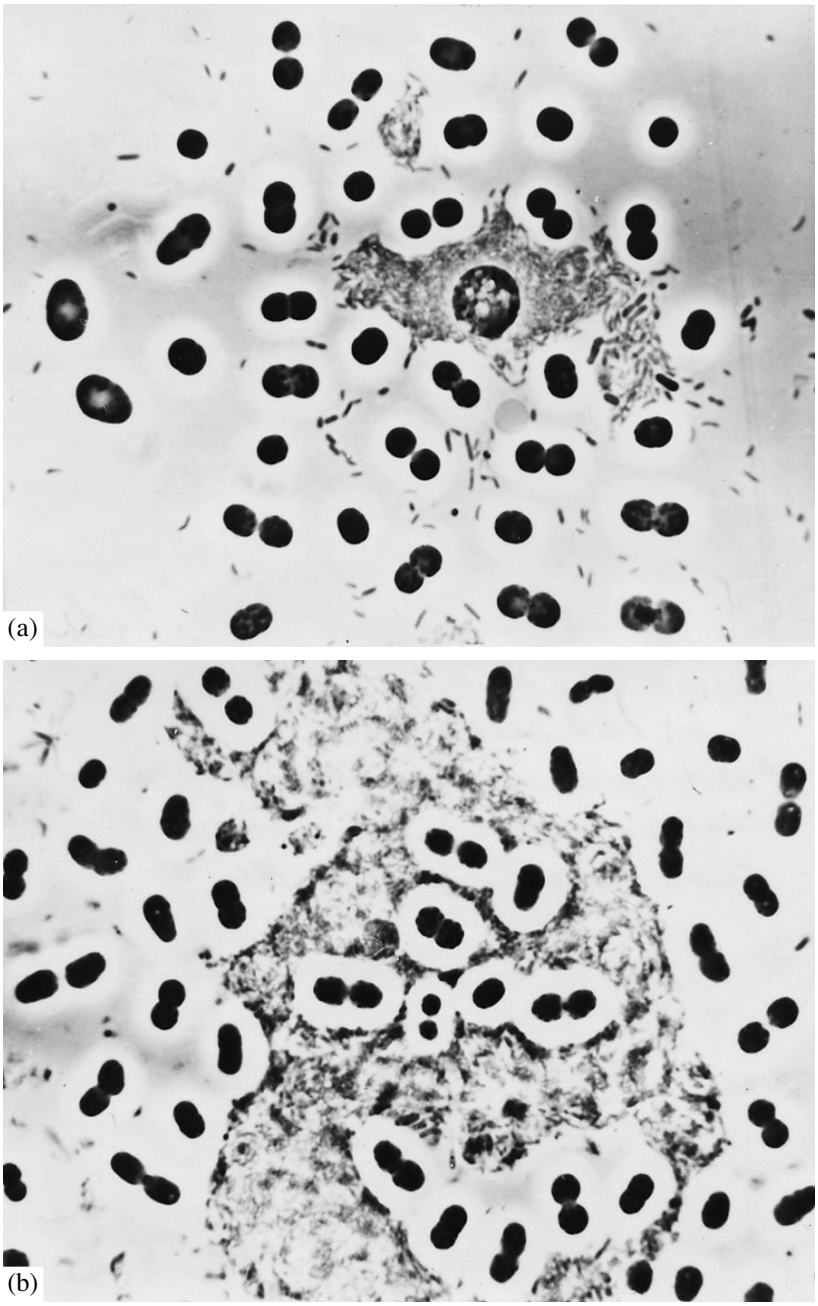

Fig. 5. Structure of the bacterial community in the enrichment monoculture (morphotype 2). Magnification, 1120×.

tundra bogged soil under extremely low mineralization of the ambiance. The morphological and physiological diversity of these organisms ensures the adaptability of the methane filter to different physicochemical conditions, pH, and temperatures. Analysis of this group of bacteria may be of paramount importance for the understanding of the factors controlling the methane flux into atmosphere from the huge Arctic terrestrial areas in Russia.

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