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MICROBIOLOGY

Methane Generation in Permafrost Sediments

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Discovery of viable microorganisms in permafrost [1–4] raised the problem of their metabolic status. It is uncertain whether or not the microorganisms stored in permafrost sediments remain metabolically active. The use of radioactively labeled compounds is one of a few methods of detection of metabolic processes in permafrost sediments. It was shown [5] that microbial lipids could be synthesized in permafrost sediments.

The goal of this work was to study methane evolution by a natural community of methanogenic bacteria in permafrost.

Studies were carried out in the Kolyma Lowland (155–160° E, 67–70° N). The experimental samples were obtained by drilling. The extracted cores were handled in compliance with all necessary requirements intended to provide their integrity and sterility [1, 6]. The age of the loamy peat soil collected at a depth of 1 m from the permafrost top was estimated as 2920 ± 40 years (GIN-10876) (Table 1). The permafrost temperature at this depth ranges from –5°C in summer to –15°C in winter.

The critical temperature of supercooling of samples studied was –3°C. This critical level corresponded to a stepwise temperature increase to the phase transition temperature (–0.8°C) (Fig. 1).

After placing the samples with positive initial temperature into a cryostat at a temperature of –1.8°C, the samples remained unfrozen (supercooled) for three weeks of incubation (Fig. 2). If samples with a negative initial temperature (–10°C) were placed into the cryostat at the same temperature (–1.8°C), they remained frozen upon warming up to the cryostat temperature. In other words, if identical samples were stored at the same temperature (–1.8°C), they remained either frozen or supercooled according to their temperature history.

The rate of $^{14}CH_4$ formation was measured as described in [7]. The substrate radioactivity level was 10 μCi. The molar activities of $NaH^{14}CO_3$ and $Na^{14}CH_3CO_2$ were 19.3 and 43.2 kCi/mol, respectively. Concentrations of added substrates (bicarbonate and acetate) were 51.8 and 23.1 mM, respectively. Samples were incubated for 21 days at temperatures of +5, –1.8, –5, –10, or –16.5°C. At least five replicates were assayed at each incubation temperature and each substrate. All experimental results were compared with corresponding controls, in which microorganisms had been killed by autoclaving before radioactively labeled substrates were added.

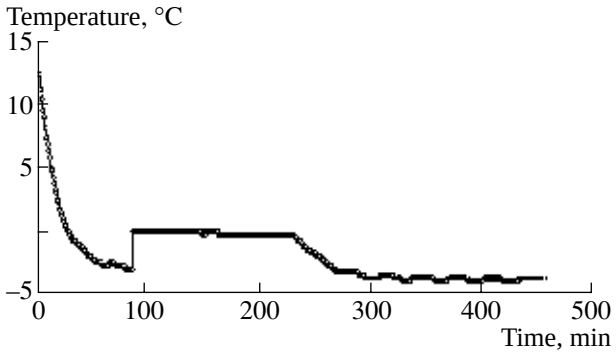

Fig. 1. Temperature changes in freezing sample: supercooling before phase transition and further temperature decrease to a cryobath temperature level.

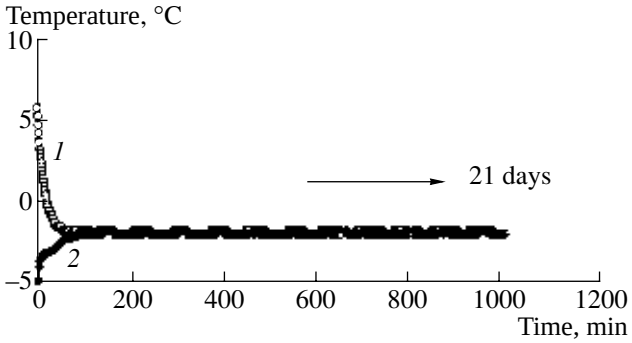

Fig. 2. Phase state of sample as a function of its temperature prehistory: (*1*) supercooled; (*2*) frozen.

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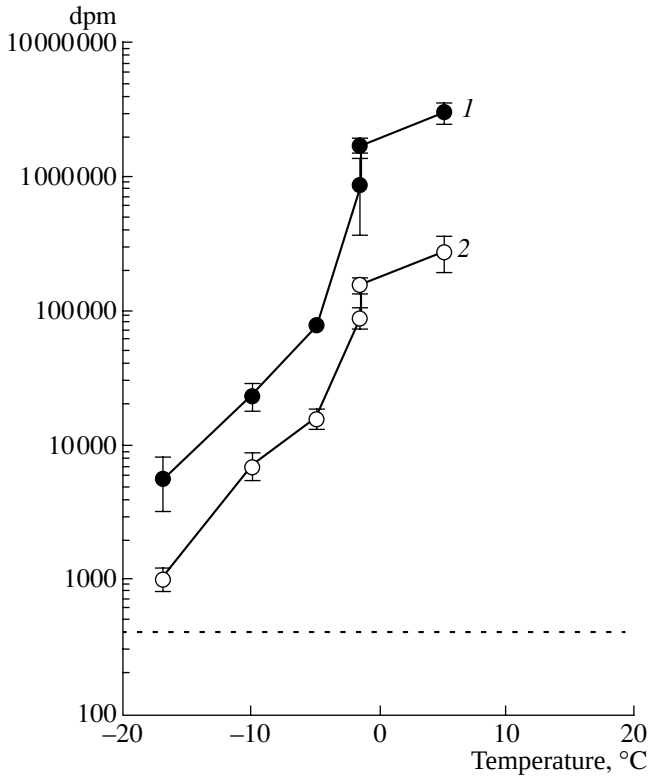

Fig. 3. Methane production from (*1*) $H^{14}CO_3^-$ and (*2*) $^{14}CH_3CO_2^-$ at different temperatures. Dashed line shows the count level in sample subjected to autoclaving (killed control).

Experimental results demonstrated that the rate of methane evolution at 5°C was only an order of magnitude lower than that value in modern bog tundra soils incubated at the same temperature [8]. The rate of methane generation in samples incubated a temperature of –1.8°C was found to be almost the same in frozen and supercooled samples (Table 2, Fig. 3).

A very low content of carbonate and absence of acetate were specific features of permafrost sediments studied in this work (Table 1). This caused an insignificant isotope dilution of samples. As a result, the count of decays per minute (dpm) was relatively high. These factors allowed the rate of methane production from $H^{14}CO_3^-$ and $^{14}CH_3CO_2^-$ to be measured at temperatures as low as –16.5°C (Fig. 3).

A temperature decrease from +5 to –1.8°C was accompanied by an almost twofold decrease in the rate of methane evolution, whereas further temperature decrease from –1.8 to –16.5°C caused a 100-fold decrease in the rate of the process (Fig. 3). According to the Arrhenius equation [9], the activation energy of the reaction of methane production from bicarbonate or acetate within the negative temperature range was higher than within the positive temperature range by factors of 3 or 1.5, respectively (Figs. 4a, 4b).

The enrichment culture of methanogenic bacteria grown on acetate contained sarcins and rod-shaped bacilli. Ultraviolet light microscopy showed that microbial cells emitted green luminescence, which is typical of methanogenic bacteria.

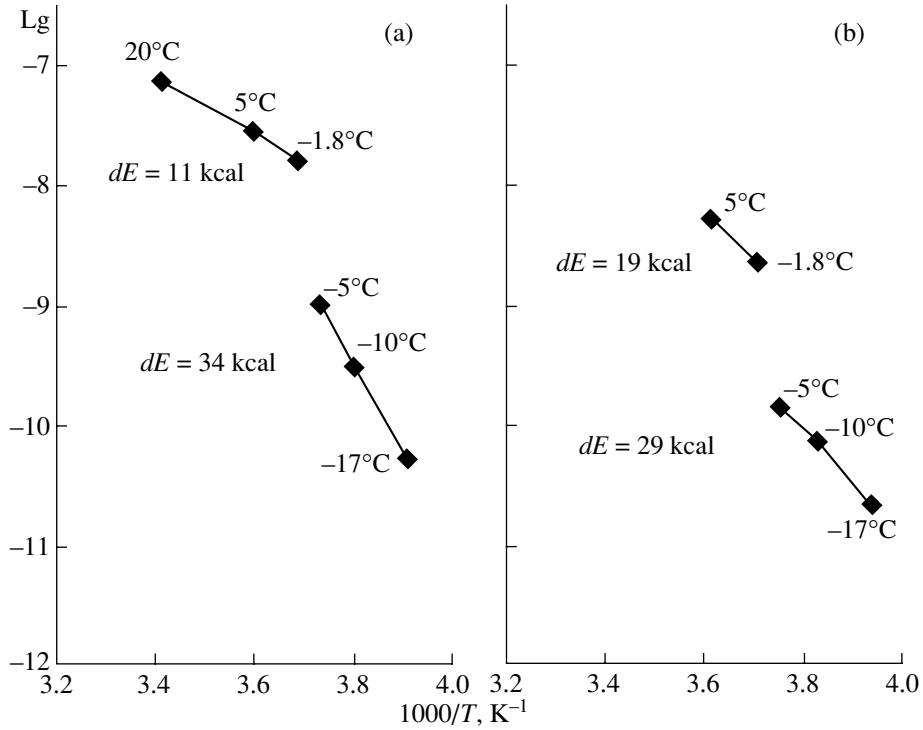

Fig. 4. Rate constants and activation energy (*dE*) of the process of methane production from (a) $H^{14}CO_3^-$ and (b) $^{14}CH_3CO_2^-$ by a community of methanogenic bacteria within different temperature ranges.

Table 1. Characteristics of samples

Test	Value
Ice content, %	83.5
C_{org}, %	9.5
Content of CH_4, μmol/kg	250
CO_3^{-2}, μmol per kg soil	–
HCO_3^-, μmol per kg soil	120
Water extract	
pH	5.7
dry residual, mg/l	49

As noted above, the rate of methane generation at –1.8°C in frozen samples was only insignificantly less than in supercooled samples. This fact can be explained taking into consideration an important role of unfrozen water in energy exchange, mass exchange, and the resulting metabolic activity of permafrost microorganisms. The relative amount of unfrozen water at –1.8°C is about 10% [10], and its energy- and mass-exchange properties differ little from similar properties of free water in supercooled samples. Therefore, exposure to negative temperatures itself does not exclude the possibility of metabolic activity. Both the activity of metabolic processes and the amount of unfrozen water depend on temperature [11]. Unfrozen water is the liquid water that is strongly bound to soil particles, and, at temperatures below –15°C, its content approaches asymptotically the level of 1–2% [10, 11]. Although permafrost can be regarded in this context as a low-temperature extremely dry environment, it is still capable of providing necessary conditions for biogeochemical processes. Based on the results of this work, it may be concluded that redox reactions mediated by bacteria do take place in permafrost.

In the case of permafrost thawing, the paleomicrobial community is expected to be even more actively involved in biogeochemical processes, including generation of greenhouse gases [12]. The results of this work are also promising for exobiology. Viable chemolithotrophic community of anaerobic permafrost microorganisms is a model of potential life providing unique mechanisms of assimilation of carbon dioxide and other compounds on planets of cryogenic type.

ACKNOWLEDGMENTS

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Table 2. Rate of methane production from different substrates in peat sediments at different temperatures

T, °C	Substrate			
	$^{14}HCO_3^-$		$^{14}CH_3CO_2^-$	
	dpm (decay per minute)	μmol CH_4 per kg per day	dpm (decay per minute)	μmol CH_4 per kg per day
+5	2990620	0.6708	1229850	0.1230
–1.8 Supercooled	1736581	0.3895	537140	0.0537
–1.8 Frozen	850513	0.2758	390749	0.0391
–5	77015	0.0250	32020	0.0032
–10	23260	0.0075	17314	0.0017
–16.5	5635	0.0013	4904	0.0005

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