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Microbiology and Gas Emission at Low Temperatures : Some Field and Experimental Results (Extended Abstract)

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Permafrost is a source of greenhouse gases, thus thawing of the frozen soils affect the global carbon cycle. Organic material in thawing permafrost decays quickly, releasing carbon dioxide and methane. Permafrost soils contain microorganisms ; isolated from the world for many years, they might be still active. Fieldwork made in 2001–2002 in Eastern Siberia, Yakutsk has shown that the permafrost at temperature about $-5^{\circ}C$ contains living fungi identified as Penicillium echinulatum (Figure 1).

The members of the genus Penicillium are widespread and are found in soil, decaying vegetation, and even in the air. Fungi are responsible for biodegradation of organic material, which could be considered as one of their important practical applications.

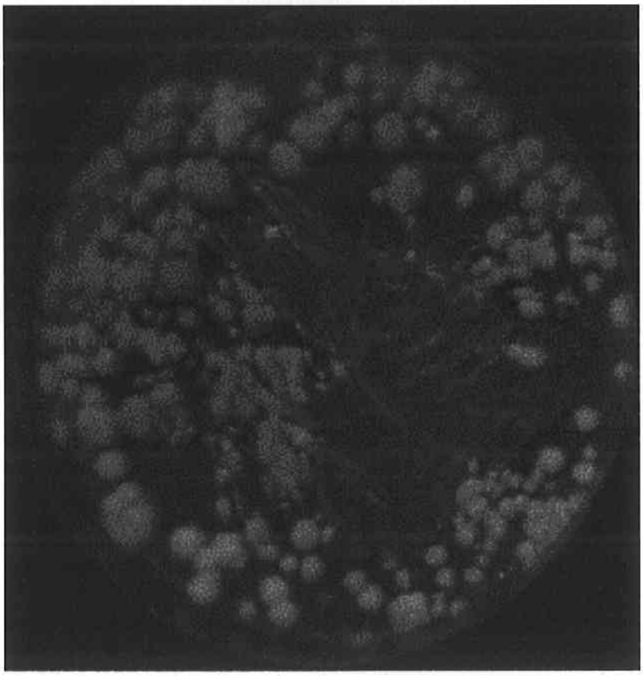

Fig. 1. Growth of Penicillium echinulatum at $-5^{\circ}C$; 7 month of incubation, PDA medium

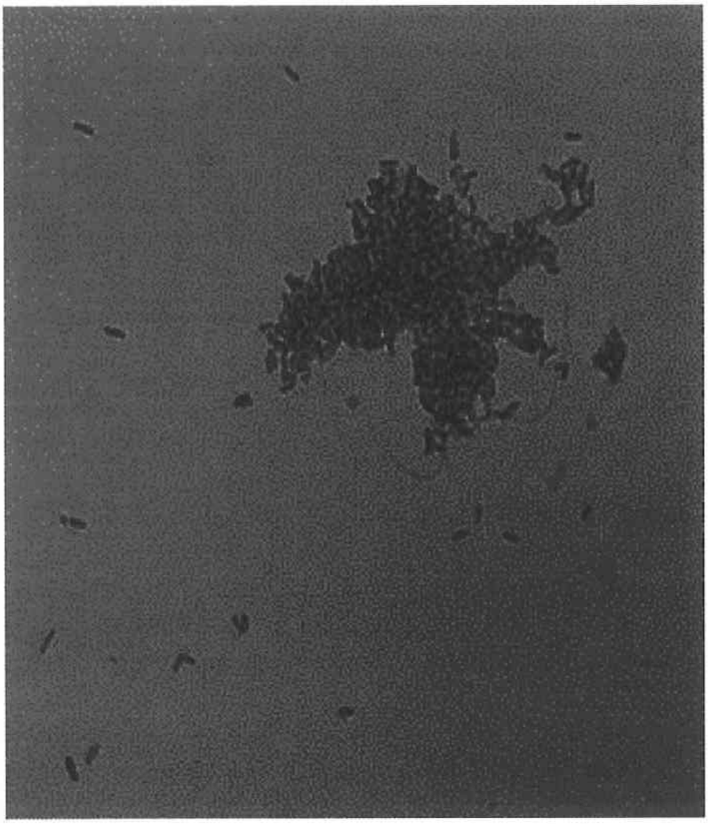

Fig. 2. Microorganisms (undetermined) from permafrost of Mammoth mountain aged about 3 million years old ; growth at temperature +20°C

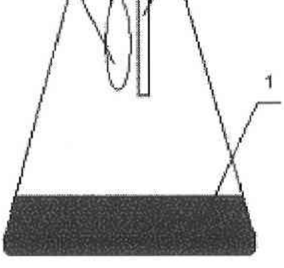

Fig. 3. Incubation flask : 1-soil sample ; 2-pipe for nitrogen input ; 3-rubber plug ; 4-tap ; 5-sampling plug ; 6-plastic bag for maintenance of the constant pressure

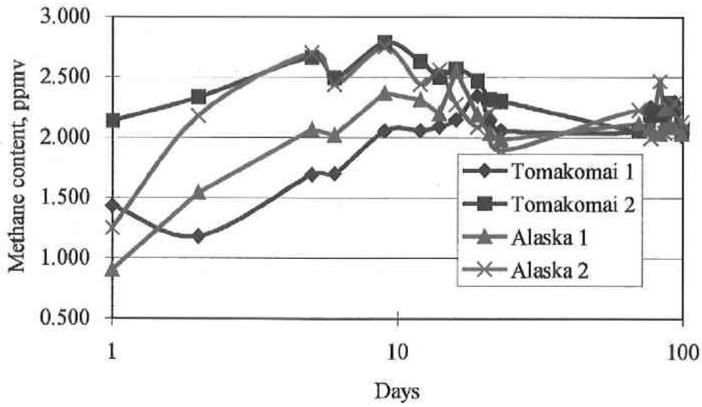

Fig. 4. Methane content in the air of flask, ppmv ; Tomakomai and Alaska water oversaturated soil, samples are numbered

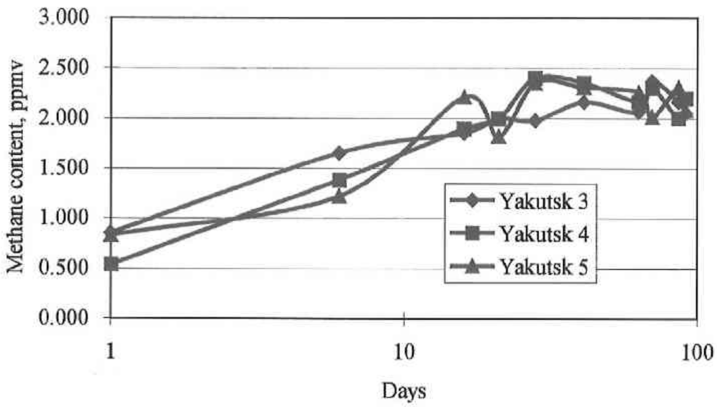

Fig. 5. Methane content in the air of flask, ppmv ; Yakutsk water oversaturated soil, samples are numbered

Some fungal species are thermophilic, others grow at low temperatures, below 0°C. However, there is no information concerning the fungi which may grow in underground permafrost at temperatures below 0°C for a long time throughout their life cycle.

The identification of permafrost fungi as Penicillium echinulatum was based on morphological characteristics and a nucleotide sequence analysis of enzymatically amplified 18S rDNA, internal transcribed spacer (ITS) region including 5.8S rDNA and D1/D2 at the 5′ end of the large subunit (26S) rDNA. The fungi were able to grow at positive and negative temperatures. Optimum temperature for the growth of P. echinulatum was estimated as 15-20°C, yet they were able to grow at the negative temperature of −5°C (Figure 1). Fungi colonies on MEA grow rather rapidly, attaining a diameter of 20 to 35 mm in 7 to 9 days at 20°C.

Sampling of permafrost exposures in Eastern Siberia, in Aldan river valley, in one

Table 1. Change of methane content (ppmv) of in the air of flask during the incubation experiment, ppmv ; sterilized, water oversaturated and air-dry soil ; nitrogen (N2) and nitrogen with carbon dioxide (CO_2) in the air of flasks

Days	Tomakomai soil			Yakutsk soil		
	N_2	N_2, Sterilized	With CO_2	N_2	N_2, Vacuumed	N_2, Air-dry
0	1.09	1.43	1.20	0.85	0.54	1.97
22	1.80	1.47	5.47	2.00	2.00	—
50	1.89	1.36	5.80	2.07	2.16	3.12

of the areas of the oldest permafrost in the Earth was done. Blocks of frozen soil and ice sized about 20×20×20 cm were cut from exposures and taken frozen to Sapporo. Frozen wood aged about 3 million years old was found and sampled.

Microorganisms were discovered (Figure 2). They were able to grow both at aerobic and anaerobic conditions.

For the sequence analysis, DNA was extracted using ISOPLANT set (Nippon Gene) and following manufacturer's instruction. Sequencing and identification of the micro-organisms need to be done.

Microorganisms might be also responsible for microbial methane formation in permafrost : according recent studies methane and gas hydrates are widely distributed in there and appear to be of a biogenic origin Long-term soil incubation experiments in flasks (Figure 3) have shown a slow production of methane in different frozen soils at −5°C. The results of study of methane production are shown in Table 1 and on Figures 4 and 5.

There was an increase of methane content in the air of flasks, especially at the first 20-50 days of the experiments.

The change of methane content occurred according logarithmical law in samples of modern soils from Alaska, Yakutsk and Hokkaido ; the rates of methane production decrease in time.

Colonies of microorganisms survived the incubation lasting more then 1 year at −5°C were found ; they were able to anaerobic growth at room temperature in GYP medium.

However, it is difficult to calculate the amount of produced methane for thousands of years based on the short-term experiments. The production could be stopped at some conditions. The temperature is also not stable : it could be suggested that methane production is larger at higher temperatures. Type of soil, organic material and water content is also essential for microbial activity.

In spite of the obvious possibility of methane producing at low temperatures, long-term forecast of methane content in frozen soils is still problematical.