Living microorganisms in Siberian permafrost and gas emission at low temperatures

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Permafrost is a source of greenhouse gases. Thus thawing of the frozen soils affects conditions of the global carbon cycle. Organic material in the thawing permafrost decays quickly, releasing carbon dioxide and methane in the process.

Long-term soil incubation experiments in flasks containing soil samples and nitrogen in the air have shown a slow production of methane in different frozen soils at -5°C. Soils were over-saturated with water. There was an increase in methane content in the air of the flasks, especially in the first 20-50 days of the experiments (Brouchkov and Fukuda 2002). The change in methane content occurred according to the logarithmical law in samples of modern soils from Yakutsk, Russia and Hokkaido (Tomakomai), Japan; the rates of methane production decrease with time (Table 1).

Table 1. Change of methane content (ppmv) in the air of the flasks during an incubation experiment at -5°C

Days	Tomakomai soil	Yakutsk soil
0	1.09	0.85
22	1.80	2.00
50	1.89	2.07
390	3.15	3.10

Micro-organisms could be responsible for microbial methane formation in permafrost: according to recent studies, methane and gas hydrates are widely distributed in there and appear to be of a biogenic origin. Colonies of microorganisms were discovered that survived the incubation period lasting longer than 1 year at –5°C; they were able to undergo anaerobic growth at room temperature in GYP medium; 16S rDNA was amplified by PCR.

However, calculations of the amount of methane produced in permafrost based on short-term experiments are difficult. The production could be discontinued under certain conditions. The temperature is not stable: it could be suggested that methane production increases at higher temperatures. The type of soil, organic material and water content are also essential to microbial activity. In spite of the obvious possibility of methane production at low temperatures, long-term forecast of methane content in frozen soils is problematic.

Permafrost soils contain micro-organisms (Friedmann 1994, Gilichinsky and Wagener 1995); isolated from the world for many years, they might be still active. Techniques for sampling in permafrost for microbiological studies are not well-established. The requirement is for biological cleanliness and absolute avoidance of contamination while the sample is brought to the surface and during subsequent handling of the sample. Fieldwork done in 2001-2002 in Yakutsk, Eastern Siberia, has shown that the permafrost at temperature of -5°C contains living fungi identified as *Penicillium echinulatum* (Figure 1).

Figure 1. Growth of *Penicillium echinulatum*, new strain PF at -5°C; 11th month of incubation, PDA medium (Potato Dextrose Agar)

The genera *Penicillium* are widespread and found in soil. Fungi are responsible for the biodegradation of organic material; this could be considered as one of their important practical applications. Some fungal species 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 during 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. The 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.

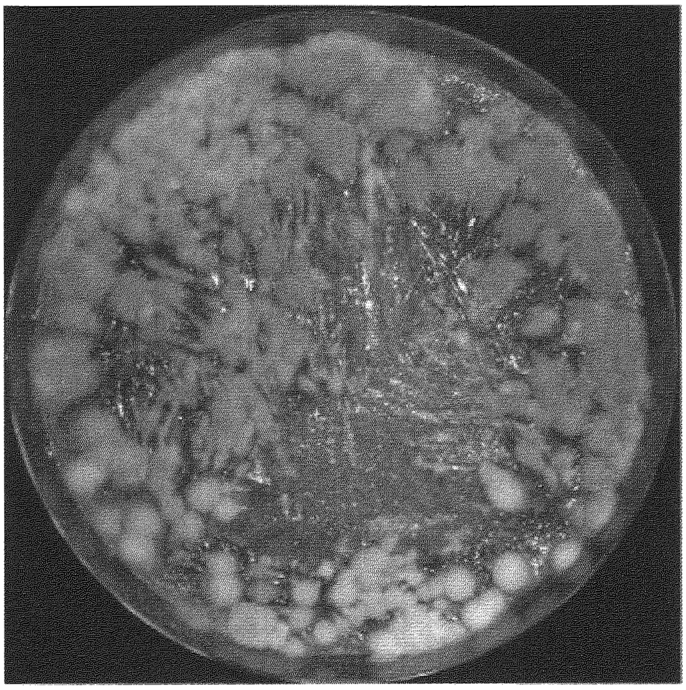

Figure 2. Micro-organisms from permafrost of Mammoth Mountain, aged approximately 3 million years; growth at temperature of +20°C

Sampling of permafrost exposures in Aldan river valley (Mammoth Mountain, Eastern Siberia), in one of the oldest regions of permafrost on Earth (possibly aged about three million years) was done. Blocks of frozen soil and ice sized approximately 20x20x20 cm in size were cut from exposures and taken frozen to Sapporo. Frozen wood of the same age was also sampled.

Micro-organisms have been discovered (Figure 2). They were able to grow at both aerobic and anaerobic conditions. For the sequence analysis DNA was extracted using the ISOPLANT set (Nippon Gene) and following the manufacturer's instructions. Preliminary results of sequencing and analysis of the evolutionary tree of the micro-organisms show that they are most related to *Bacillus anthracis*.

Understanding these permafrost organisms and their relationship to their cold environment, especially to unfrozen water common in frozen soils (Williams and Smith 1989), has immediate practical implications. The fact that viable bacteria occurring at significant depths in permafrost can be prompted to reproduce, presents imaginative possibilities, for example for the decontamination of buried contaminants. The living micro-organisms in permafrost, in common with other extremophiles (Ashcroft 2000), apparently have special mechanisms of repair of cell structures, necessary for their survival (Brouchkov and Williams 2002). A comparative study of structure and biochemical features of permafrost's micro-organisms to those of known soil micro-organisms could reveal this mechanism.

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