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PERMAFROST MICROBIOLOGY

DISTRIBUTION OF MICROORGANIZMS IN FROZEN GROUND

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Various microbial forms have been discovered *in situ* in ice lenses within frozen loam by means of optical and electron microscopy. Migration of bacteria on freezing of fine-grained soil has been investigated in experiments. The viability of bacteria may be maintained by processes associated with ice segregation. Ice lenses in cryotic soils appear to be the best habitat for sustainable microbial life.

INTRODUCTION

The cryosphere (sphere of cold and ice) controls biodiversity, as well as the lithosphere, the atmosphere, the hydrosphere, and the biosphere. Besides the inhabited Earth, cryosphere exists on other planets as well: the still colder planets of Mars, Jupiter, Saturn, and Uranus store great amounts of ice and have almost fully glaciated satellites. Increasingly growing recent attention has been given to the role of ice in the origin of planets, star systems, and in the space as a whole. Ice had existed before the Solar System formed and before water appeared on the Earth. Ice is and will be present in the Universe whichever change the latter may run. Owing to its physical properties, ice is an ideal place for life to emerge, as it provides an incubator with minimum temperature gradients, a shelter from killing radiation, and protection from dangerous chemicals.

The original hypothesis of biota dormancy in the Earth's cryosphere has been undermined with ample evidence for the existence of viable microbial life in natural ice and in frozen ground of various genetic types [*Zvyagintsev et al., 1985, 1990; Abyzov, 1993; Gilichinsky et al., 1993; Vorobieva et al., 1997; Brushkov et al., 2006*]. The possibility for microorganisms to remain alive and metabolically active in perennially cold environments has been widely reported [*Friedmann, 1994; Gilichinsky and Wagener, 1995; Rivkina, 1998; Vishnivetskaya et al., 2000; Christner et al., 2003*].

Most psychrophilic (cryophilic) organisms, i.e., those capable of growth and reproduction in cold temperatures from −10 to +20 °C, are stable against the freezing stress. This strength can be of biotic or abiotic origin. The biotic factors are, for instance, special forms of cysts or spores bacteria can develop to adapt to harsh conditions, or special compounds (sugars and proteins) they can secret to survive in frozen sediment and in ice sheets. Unfrozen water in permafrost is an abiotic stability factor, which acts as both a cryoprotector and an environment for transport of nutrients and metabolism. There are many things that remain unclear but ever more data has been appearing on the wonderful viability of microbial organisms discovered in frozen ground [*Brushkov et al., 2009*].

It is surprising how bacteria can remain viable in pre-Holocene permafrost despite the known vulnerability of proteins and DNA to macromolecular damage. Of special interest in this respect is investigating the spatial and ecological links of microorganisms with other permafrost components, such as mineral matrix particles, ice lenses, unfrozen water, and air. Some evidence of these patterns was obtained while studying the cryotic microstructure [*Rogov, 2009*], but no special research has been undertaken so far.

MATERIALS AND METHODS

The distribution of bacteria in frozen ground was studied in samples collected from the well-known section of Mamontova Gora (Russian for *Mammoth Hill*) in Central Yakutia, on the left bank of the Aldan River, 325 km upstream of its inflow into the Lena. An outcrop in terrace III of the Aldan exposes alluvium that, since the earliest Quaternary, has filled the Lower Aldan basin lying along the junction between the Verkhoyansk Foredeep and the Siberian craton.

The lower section consists of Middle Pleistocene sand and loamy sand thinly interbedded with wet ice-cemented flood-plain loamy sand. Up the section there follows an ice complex with thick Late Pleistocene and Holocene ice wedges.

According to geological data, cooling and related freezing of the sediments began in the late Pliocene. Paleoclimate reconstructions for the area predict that temperatures in the Pleistocene were from −12 to −32 °C in winter and from +12 to +16 °C in summer seasons [*Bakulina and Spektor, 2000*]. The presence of ice wedges indicates that the permafrost did not thaw during the Holocene climate optimum. Thus, the ice lenses in the lower section must be of the same age as the sediments.

The river has strongly eroded its bank at the locality (more than 1 m of land washed out every year), which prevents the sediments from repeated freezing. Sampling was from the lower part of the outcrop over the water surface, 0.9–1.0 inward from the vertical wall. The ground microstructure was examined in 4–5 kg monolith blocks, and the samples for microbiological studies were about 50 g. Sampling was performed with metal instruments sterilized in ethanol and flame. The samples were sterile packed and transported in thermostatic containers with coolants at −5 °C, i.e., close to the natural conditions.

The sampled ground is laminated silt consisting of alternated thinner light-color and thicker dark-color layers, with thicknesses 0.5–1.0 mm, respectively. The cryostructure is made up of thin (0.05–0.10 mm) broken layers that inherit the sedimentary bedding combined with vertical, sometimes discontinuous, 0.1 mm lenses (Fig. 1, *a*). The ice layers consist of isometric or slightly elongate polygonal crystals from 10 × 15 to 30 × 80 μm.

The soil samples contained a cultivable bacterium capable of both aerobic and anaerobic growth. It belongs to the *Bacillus* genus but is more likely a new species most closely related to *Bacillus simplex* and *B. macroides* which are 96–97 % homological with 16S rRNA [*Brushkov et al., 2009*].

The bacterium is psychrotolerant and can be metabolically active at −5 °C. It is relatively large (1.0–1.5 × 3–6 μm) and rod-shaped; it forms chains in the culture and can develop round spores. It is immobile, haemolytic, and gram-positive. The bacteria shows catalase and oxydase activity, reduces nitrates, and can grow within a broad temperature range.

In the laboratory, the bacillus grew slowly at −5 °C (growth signature became evident after two or three months) in both frozen and cooled environments. Although growth upon an artificial substrate is possible at subzero temperatures, no visible colonies were observed on the frozen samples.

The samples were examined by means of optical and electron microscopy in specimens preconditioned in different ways. Frozen fresh chips were studied after vacuum drying; the most detailed information was from replicas of frozen samples. The method was developed by one of us [*Rogov, 2009*] to investigate the soil microstructure. Preliminary optical microscopy of the replicas revealed microorganisms in ice lenses within frozen ground. At the following stage, the replicas were scanned by a LEO 1450 VP raster electron microscope, using an in-built spectrometer for identifying the bacteria and their environment.

EXPERIMENTS AND RESULTS

The largest bacteria existing as round or ovoid cells from 5 to 10 μm were discovered in the middle of an ice layer, in groups of 5 to 8 individuals (Fig. 1, *b*); some cells grew at the ice-rock boundary. Other bacteria found in small ice lenses were rod-shaped with rounded ends, similar in their morphology to plated *Bacillus* (Fig. 1, *c*). Almost no colonies were observed, which may be evidence of very slow or no reproduction in frozen rocks. However, some images attested to the very possibility for such slow growth, about the ice flow rate. No signature of symbiosis was seen either. The organic origin of the cells was validated by spectrometry which showed salts (especially $CaCl_2$) in the environment. The distribution of salts and colloids as a reticulate coat around the cells became evident in vacuum-dried specimens. The coat had a complex composition dominated by calcium, iron, and silicium compounds (Fig. 1, *e*).

The presence of microbial life in frozen ground poses challenges for both microbiological and geocryological sciences. The results of the reported study furnish evidence for obvious links between the freezing, moisture transport, and ice formation processes and the distribution of ice-dwelling bacteria.

This hypothesis was checked in an experiment with a ground system of sterile kaoline made in a plastic cylinder, 50 mm in diameter and 250 mm high. Kaoline saturated with distilled water to a content of 40 % lay over 0.5–1.0 mm thick coarse sand fully impregnated with a solution containing the culture of *Bacillus cereus*. The system was exposed to one-dimensional freezing in a special device at the surface temperature −4 °C. After freezing, the upper 3 cm of kaoline developed a stratified cryostructure, with 3–4 mm thick ice lenses; the following 7.5 cm of the sample was massive; the lowermost layer (2.5 cm) remained thawed. Then the redistribution of bacteria was investigated, by plating without cultivation, in 250 μl ground samples from the upper and lower layers. As a result, we obtained 71 plate colonies in the former (frozen kaoline with segregated ice) and 25 colonies in the latter (massive kaoline). The presence

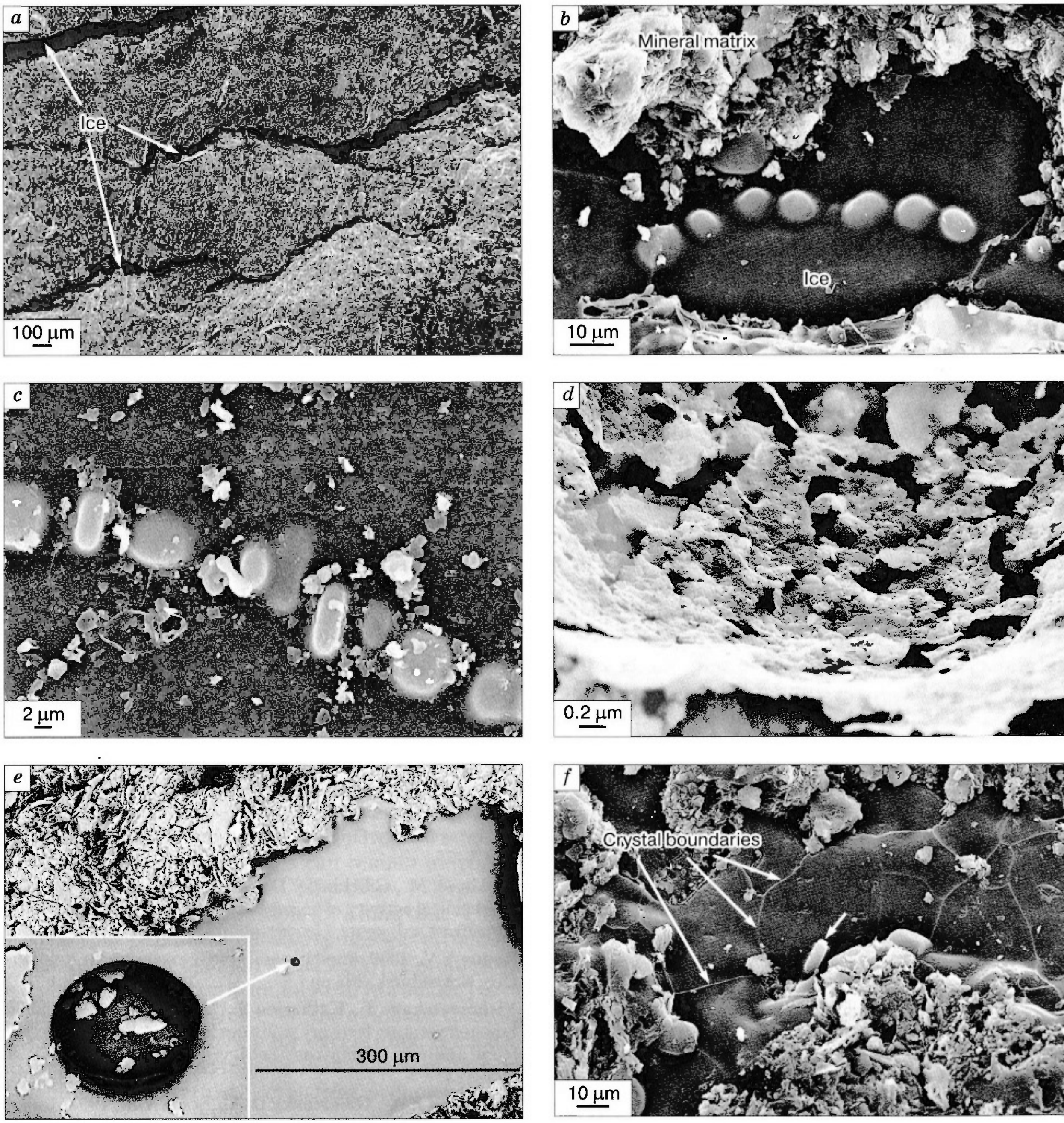

Fig. 1. Distribution of ice and bacteria in frozen ground.

a – segregated ice in loam; *b, c* – cells of various geometries in ice lenses; *d* – mineral coat around a cell; *e* – cells of *Bacillus cereus* in ice from kaoline (inset is magn. ×30); *f* – cells of *Bacillus* sp. in an ice lens.

of sporadic bacterial cells in segregated ice was confirmed microscopically (Fig. 1, *e*).

DISCUSSION

The experiment demonstrated the possibility for ice segregation and for migration of bacteria toward the freezing front in the course of moisture flow. The mechanism of this migration is not quite clear. Possibly, it occurs by cryogenic desiccation while moisture, together with growing ice crystals, moves along the cracks.

Another important issue concerns with the location of microbial cells relative to ice crystals. Microbial cells found in Taylor Glacier in Antarctica were located in small veins of water between individual ice crystals [*Doyle et al., 2008*] where dissolved nutrients and gases necessary to maintain metabolism may provide suitable life conditions. However, the cells we observed in ice lenses from frozen loam did not show such localization.

Natural ice as a microbial habitat is remarkable by being a multicomponent system with mobile solid surfaces that border upon fluid and gas inclusions. As the ice crystal surfaces become warmer, the liquid films thicken up and develop a branched capillary system on further heating. The total length and diameter of the capillaries depend on the amount of free and bound water and on its salinity that controls the eutectic temperature. The crystal morphology and cryotextures in ground ice also depend on the grain size and mineralogy of sediments. The life conditions for bacteria in the water capillary system appear extreme because of their instability in space (osmotic gradient) and time (diurnal and seasonal temperature gradients). Thus, the ice crystal interior with multiple gas-fluid and organic-mineral inclusions may be a better environment to sustain microbial life.

On the other hand, the ice genesis being definitely an ecology control, the patterns of microbe cells in natural ice may result either from snow-to-ice transformation or from freezing and icing in wet fine-grained sediments. The experimentally observed bacterial patterns (Fig. 1, *f*) indicate that microorganisms can participate in migration and segregation of ice because the capillary size and the structure defects (vertical and horizontal desiccation cracks) in freezing sediments are large enough to let the cells through.

Another agent that can further maintain bacterial life in frozen sediment is the adsorption of salt ions from pore fluid and formation of a saline water coat which impedes icing in cells and in their surroundings. Note that spectrometry shows $CaCl_2$ to be the common salt deposited on the cell periphery, this being the most active agent among the ice-borne salts to reduce the water freezing temperature. There may be also other survival factors. Namely, some cells were found out to keep their relation with the mineral substrate, which is implicit evidence of their viability.

CONCLUSIONS

1. Microorganisms in frozen ground show a distribution indicating their ability to migrate while rock is freezing and to localize in segregated ice.

2. Ground ice is apparently the best permafrost habitat for sustainable and comfortable life of diverse microbial forms.

3. Salt-bearing gas-fluid inclusions inside and between ice crystals provide suitable life conditions as they prevent cells and their ambient material from freezing.

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