



## Microbial biodiversity of Yamal Peninsula overcooled water brines within permafrost

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Microorganisms are capable to survive from tens to millions years in permafrost, ice and water brines (cryopegs). Water brines (overcooled water systems of marine origin - cryopegs) were found in permafrost at the depth from 5 to 120 meters from sodium-chloride water brines with the constant temperature of  $-4^{\circ}\text{C}$  and mineralization of 31-70 g/l. The formation of cryopeg lenses is determined by the Quaternary history as well as geological, physical and geographic conditions.

Three isolated lenses different age of water brines were exposed by boreholes on the Bovanenkovo gas condensate deposit, the largest deposit in the Yamal Peninsula: 1) Holocene (depth 5m, mineralization - 58 g/l), 2) Holocene (depth 7,5m, mineralization - 70 g/l, 3) late Pleistocene (depth 120m, mineralization - 31 g/l). Yamal cryopegs are characterized by permanently subzero temperatures, high salinity, and the absence of external influence during geological time. Kara Sea water sample was used as a control for comparative study. The brines samples were stored in a refrigerator at  $-4^{\circ}\text{C}$ .

This study describes the biodiversity of the indigenous aerobic halophilic microbial community in the Yamal cryopegs. The number of bacteria was determined by direct counting of cells stained with DAPI on agarose-coated slides. The stained cells were counted using an Axiostar (Zeiss) fluorescence microscope with excitation and emission filters. Diversity and enumeration of viable aerobic halophilic bacteria were obtained by direct plating on standard nutrient medium R2A and  $\frac{1}{2}$  TSB with NaCl 100, 150, 200, 250, 300 g/l. from enrichment culture, at 4 and  $20^{\circ}\text{C}$ .

About 78 pure cultures were isolated from Yamal cryopegs samples. Phenotypic tests to determine the key taxonomical characteristics of bacteria: Gram's staining, the presence of oxidase, catalase, nitratoreductase, lipase and lecithinase and glucose fermentation were determined by standard methods. Cells of strains were mostly represented by gram-positive non-motile cocci  $1-1.5 \mu\text{m}$  and coccobacilli  $1-1.5 \times 2-3 \mu\text{m}$ .

As was shown by phylogenetic analysis the majority of isolated aerobic halophilic bacteria were closely related to members of classes *Proteobacteria*, *Actinobacteria* and family *Bacillaceae*.

From the astrobiological perspective, water brines provide the only opportunity for liquid water within the Martian subsurface permafrost. Survival of microorganisms in cryopegs under such harsh environmental condition (a low-temperature, high salinity) on a geological time scale gives us the possibility to predict same saline ecosystems on Mars and use cryopegs as a terrestrial model to study adaptation strategies of biological objects to subzero temperatures at aquatic environment.