
Introduction: Due to Mars surface environmental conditions [1] (oxidative stress, high UV radiation levels, etc.) there are very few chances for life on the surface of the red planet. Since location of water ice on the subsurface on Mars by Thermal Emission Spectrometer onboard Mars Odyssey spacecraft [2] and from High Energy Neutron Detector [3] was reported, ecosystems underneath the surface are of special interest from an astrobiological point of view. These locations are shielding habitats again UV radiation [4]. Chemolithotrophic microorganisms could take advantage from inorganic chemicals potential energy for its biomass synthesis. Best understanding of permafrost ecosystems on Earth is needed. Permafrost on earth is located at circumpolar latitudes. Of special interest is permafrost on volcanic areas due to the similarities with Mars geology [5].

Future space missions will be focus on looking for life on the subsurface of Mars. New techniques and methodologies for studying such systems need to be developed.

Imuruk lake campaign: We located an interesting volcanic area with associated permanent permafrost in the region of Imuruk lake (Alaska). An exploration campaign was developed during July 2005 in order to study the geology and microbiology of the area. Imuruk lake is located at 65.6°N, 163°W. This region is a volcanic area located at the Bering Land Bridge National Preserve (fig. 1).

Three main objectives were considered during the expedition: 1) microbial diversity analysis, with special interest on deeper part of the column because of the old age of this permanent permafrost. Preservation pattern of biosignatures in cold environment is of extraordinaire astrobiological interest. 2) Development of instrumentation for automated remote life detection systems and 3) Cold ecosystem understanding for detecting and mapping of permafrost niches. These objectives were achieved by using geophysical sounding techniques. Once permafrost was located, we drilled on the area and sampled different levels of the rock cores. Different studies were developed “in situ”, and several representative depths samples were transported for laboratory analysis also.

Figure 1: Bering Land Bridge National Preserve situation. Imuruk lake is located on a volcanic area.

The 2005 campaign was located in the eastern part of the lake, near Nimrod Hill. Previous geology studies [6] have been reported. The area is characterized by volcanic formations with basaltic composition. Some basalt lava flows are present. Over the lava structures there is two covers form different composition: the first is a windblow silt layer and the second is a peat cover at the top. Some intermediate terraces were located around the hill with sedimentary material.

Geophysical studies. Permafrost depth was located by geophysical techniques (electric tomography sounding). A Syscal KID Swich-24 equipment was used. Thirteen parallel lines from the lake, over the hill slope were carried out (Prieto et al. this conference for better explanation). Each tomographic line was 48 meters long, using 2 meters as electrodes separation. The space between lines was 15 meters.

Temperature recording during core sampling indicated a permafrost depth of around 30 cm, but tomographic data indicated that permafrost began at a mean depth of 0.50 meter from the surface. After tomographic date interpretation a place for drilling was choose.
**Stratigraphic column.** A portable drilling system was used for stratigraphic and sampling studies. Cardi E-400 fuel powered system was adapted for core retrieve. The dimension of the pits was 0.5 long and 50 mm diameter. Pits could joint each other for a maximum depth core of 5 m. Microbiological studies were developed over tomographic line 11 core. Maximum depth on this drill was 3.6 m.

**Microbial diversity studies.** Several core depths were choose for sampling in order to microbiological studies accomplishment. Three different methodologies for microbial population analysis were used: 1) Media inoculation for microbial enrichment. After media incubation, microbial populations were identified by 16S rDNA amplification, cloning and sequencing. 2) DNA isolation from soil for 16S rRNA genes amplification. The sequences of these genes were compared to the 16s rRNA sequences from known species. 3) Fluorescence “in situ” hybridization with specific DNA probes. Samples were “in situ” fixed with formaldehyde and maintained during 2 hours at low temperatures. After 2 h. samples were filtered and washed with PBS buffer. Filters were dried and maintained at low temperature for later lab hybridization techniques application. Figure 2 shows a universal stained microbial preparation of a sample from tomographic line 11 obtained at 2 m deep.

Direct bacterial counting by light microscopy was used in order to density quantification. Figure 3 shows the population gradient (cells/gr of soil) with depth.

Several media for microbial enrichment were used including organic aerobic media for heterotrophic bacteria, minimal media for anaerobic bacteria with different energy sources (methanol, volatile fatty acids, amino acid mix, etc.). Microbial growth has been obtained in all of them.

**Figure 3:** Population density (cells per gram of soil) gradient in the borehole from tomographic line 11.

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