**ROMANIAN ACADEMY** Project PN-II-ID-PCE-2011-3-0742

**INSTITUTE OF BIOLOGY BUCHAREST** Contract no.159 / 28.10.2011

[**Biodiversity and chronological distribution of microorganisms in perennial ice deposits from Scarisoara Ice Cave (Romania)**](http://www.ibiol.ro/proiecte/PNII/Scarisoara/index.html)

**Scientific report**

**(2014)**

Investigation of the structural and functional diversity of microbial communities from the ice block of Scarisoara Ice Cave encompassed the previously established objectives and activities for this phase, as follows:

1. **STUDY OF THE DIVERSITY OF MICROORGANISMS FROM SCARISOARA ICE BLOCK USING MOLECULAR AND MICROBIOLOGICAL APPROACHES**
2. **Samples were collected from the ice deposits of Scarisoara ice block aged up to 2000 years BP**. Both vertical and horizontal drilling were used for obtaining ice samples of 400-900 years old from the Little Reserve and an ice core up to 2000 years old from the Great Hall area of the cave. Ice sampling was carried out under sterile conditions.
3. **Microbial communities contained in ice samples were analyzed by flow cytometry and epifluorescence microscopy.** Cell content of ice samples was measured using a Gallios (Beckman Coulter) flow cytometer, indicating a total cell density of 105-106 cells/mL of ice, with a viable cell content of 60-80%. The presence of phototrophic microorganisms in all analyzed ice strata was revealed by natural fluorescence microscopy and chlorophyll A and B measurements of melted ice.
4. **Nucleotide sequence of SSU rRNA genes were determined** from the DGGE amplicons of bacteria, cyanobacteria and fungi from recent ice (1 year old exposed to sun and indirect light), 400 and 900 years old ice with a high and low organic content, and cultures in R2B and BG11 media at 4°C and 15°C. DNA sequences were processed with CodonCode Aligner (www.codoncode.com) and BioEdit ([Hall,](http://www.mbio.ncsu.edu/BioEdit/bioedit.html) 2007) and further analyzed with BLAST-NCBI (Altschul *et al*., 1997). 33 bacterial OTUs were determined and deposited in GenBank, indicating closest match with other cold environments strains. 48 cyanobacterial and 82 fungal amplicons were sequenced and currently analyzed.
5. **Phylogenetic analysis.** Phylogenetic trees of ice contained bacterial 16S rRNA were constructed using ClustalW (Larkin *et al*., 2007), Gblocks 0.91b (Castresana, 2000; Talavera, *et al*, 2007), and PhyML 3.0 aLRT (Guidon *et al*., 2003and visualized with TreeDyn 198.3 (Chevenet *et al*., 2006). The identified species were grouped in four different clusters belonging to Gammaproteobacteria, Bacteroidetes, Firmicutes, and Actinobacteria phylotypes.
6. **COMPARATIVE ANALYSIS OF MICROBIAL DIVERSITY FROM SCARISOARA ICE SEDIMENTS**
7. **Geochemical analysis of ice samples** were carried out for determining the total carbon (TC) and nitrogen (TN) content as well as total organic carbon content (TOC). The microbial functional diversity of heterotrophic microorganisms from 5 ice samples cultivated 4°C and 15°C expressed as diversity index (H) and richness (R) parameters determined by using the BIOLOG EcoPlates system was analyzed in correlation with the chemical content TN and TOC using PCA PRISMTC (<http://www.prismtc.co.uk/>) software. The resulted distribution showed a strong correlation (> 0,878) of the diversity parameters with the chemical content of the ice substrate.
8. **Statistical analysis of microbial diversity from different locations of the cave ice block** was analyzed by PCA. The PCA plot resulted indicated a statistical clustering of diversity parameters of recent ice microbiota exposed to light and 900 years old ice, while sun-exposed ice and 400 years old ice microbial communities form distinct groups of high microbial diversity, independently of growth temperatures. The relative abundance of bacterial phyla characterizing 5 ice contained microbiota showed the dominance of different phyla in all analyzed ice sediments, dependent of the age, light exposure, and organic content of the ice sample, and presence of Actinobacteria only in recent light exposed ice.

**III. IDENTIFICATION OF NEW CRYOPHILIC MICROBIAL STRAINS**

1. **Isolation and characterization of new microbial strains from Scarisoara ice block.** Microbial communities contained in cave ice from 7 ice samples up to 2000 years old were cultivated on LB and R2A media specific for environmental heterotrophic microorganisms’ growth. After cultivation for 15 days at 15°C or 30 days at 4°C, the colonies formed were counted and stored at -20°C in glycerol stocks. The morphology and color (yellow, grey, orange, red, pink, white) of the dominant type of colonies grown at both 4°C and 15°C were analyzed, showing a large diversity range and dependence with the ice age, organic content, light exposure and cultivation temperature. The cultured strains were isolated and purified by serial passages and cultivation under the same conditions. Species identification of the cultured bacteria and fungi contained in Scarisoara ice block sediment was performed by PCR amplification of 16S rRNA (bacteria) and 18S rRNA (fungi) using specific primers and amplicon sequencing. Analysis of the resulted nucleotide sequences (Macrogen) is currently underway.

**REFERENCES**

1. Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W & Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* **25**: 3389-3402.
2. Garland JL, Mills AL (1991) Classification and characterization of heterotrophic microbial communities on the basis of patterns of community level sole-carbon-source utilization. *Appl Environ Microbiol* **57**: 2351-2359.
3. Hall T (2007) BioEdit: Biological sequence alignment editor for Win95/98/NT/2K/XP. Carlsbad, CA: Ibis Biosciences. [*http://www.mbio.ncsu.edu/BioEdit/bioedit.html*](http://www.mbio.ncsu.edu/BioEdit/bioedit.html)
4. [Miteva](http://www.ncbi.nlm.nih.gov/pubmed/?term=Miteva%20VI%5Bauth%5D) VI, PP & [Brenchley](http://www.ncbi.nlm.nih.gov/pubmed/?term=Brenchley%20JE%5Bauth%5D) JE (2004) Phylogenetic and Physiological Diversity of Microorganisms Isolated from a Deep Greenland Glacier Ice Core. *Appl Environ Microbiol* **70**: 202–213.
5. Perşoiu A, Pazdur A, 2010, *The* *Cryosphere Discuss* **4**: 1909–1929