BIODIVERSITY OF MICROORGANISMS IN PERENNIAL ICE DEPOSITS FROM SCARISOARA ICE CAVE (ROMANIA). A. Rusu[1], A. Hillebrand[2], A.Persoiu[3], R. Filimon[1], E. Popa[1], B.P. Onac[3] and C. Purcarea[1], [1]Institute of Biology Bucharest, 296 Splaiul Independentei, Bucharest, Romania, cri48223@yahoo.com. [2]Institute of Speleology Emil Racovita, Bucharest, Romania. [3]Department of Geology, University of South Florida, Tampa, FL, USA.

Introduction: **:** Although the microbial diversity of cold environments was investigated from a large variety of exposed ice habitats, very little is known about microorganisms that live in ice deposits from caves, an isolated and light deprived cold environment that ensures advanced species' conservation. Since the discovery of liquid water and ice, and possible ice caves on Mars [1], ice caves biodiversity could bring insight into possible earlier environments on Earth and other planets, and on exobiology aspects [2], [3].

Scarisoara Ice Cave hosts the oldest (more than 1200 years old) and second largest underground perennial ice deposit in the world [4], [5]. The underground ice block from Little Reserve presents a clear regular horizontal stratification recently dated with stable isotopes [6], [7] where the profile of the ice block represents a chronological record of the climate patterns and biodiversity embedded in yearly formed ice deposits.

In this context, our study focuses on determining the microbial biodiversity and its chronological distribution in ice sediments from Scarisoara Cave.

Methods: The study comprises (1) ice sampling of various age and chemical composition, (2) microbial cultivation on different media and at differnt temperatures, (3) total DNA extraction from liquid cultures, (4) PCR amplification of bacterial 16S-rRNA genes and gene library construction, (5) ARDRA analysis, sequencing and phylogenetic analysis of bacterial 16S-rRNA genes.

Results: Samples were prelevated from recent ice sediments (sample 2010) and from the oldest exposed layer corresponding to 890 years old ice (sample 895) and 895 years old ice containing organic matter (sample 890). The ice was sampled from the ice wall of the Little Reserve under sterile conditions, at 10 cm depth.

Cultivation of ice-contained microorganisms from 2010, 890 and 895 ice samples on liquid and solid LB media, in the presence (LBG) and absence (LB) of 1% glucose, at 4°C and 15°C, revealed a different growth rate and number of colonies as a function of cultivation conditions. Accordingly, a higher number of colonies resulted at 15°C than that obtained at 4°C, independently from the ice age, suggesting the prevalence of moderate psychrophiles in this habitat.

Total DNA was extracted from ice originating microorganisms cultivated under various conditions, and the PCR amplification of both bacterial and archaeal 16S-rRNA genes using specific primers was tested. Only bacterial 16S-rRNA genes were amplified and used for construction of gene library in pGEM-T vector.

The 16S-rRNA gene library of the 890 sample cultivated in LB medium at 4°C contains 160 colonies. ARDRA analysis was carried out using digestion with *RsaI, HaeIII* and *HinfI* of the PCR amplified inserts, identifying various restriction patterns, and the selected clones were sequenced. In progress sequence identification and phylogenetic analysis of the bacterial species present in this 890 years old ice revealed some yet unidentified species, several species present in other cold habitats and others common to mesophilic environments.

These results represent preliminary steps in identifying the microbial biodiversity and its chronological distribution in ice sediments from Scarisoara cave, in view of defining climate-related biomarkers that could contribute to elaborating models for exobiology studies of ice-containing planets.

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