

Diversity of cultivable heterotrophic bacteria from three Romanian karst caves

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Abstract

Karst caves are peculiar ecosystems accommodating specific biota that readily include microbial communities with key roles in the biogeochemical cycling of main elements and thus, in the functioning of trophic webs (Barton and Northup, 2007). In caves, macroscopic colonies of microorganisms are frequently encountered on the walls, ceilings, in aquatic sediments and on the surface of speleothems. To assess the cultivable microbial diversity in timely, accurately and cost-effective manner, the molecular (marker gene-based) identification of isolates is presently preferred as preceding detailed genotypic and phenotypic characterization. The revealing of microbial diversity in the cave ecosystem is crucial to the understanding of their ecological roles and, in addition, to the discovery of novel taxa and metabolic capabilities. The aim of this study was to isolate and evaluate the taxonomic diversity of bacterial strains from three Romanian karst caves, namely Cloșani, Ferice and Muierilor, located in different geographic areas. To achieve this goal, sample plating and isolation on non-selective, oligotrophic solid culture media and subsequent molecular identification of bacterial isolates have been employed. In total, 41 strains were isolated from soil sediments of the above-mentioned caves. These underwent genomic DNA extraction, 16S rRNA gene amplification, and Sanger sequencing. The retrieved 16S rRNA gene sequences were analyzed and a phylogenetic tree was constructed by bioinformatic tools. The isolates were assigned to *Proteobacteria* (37% of total number of isolates), followed by *Firmicutes* (36%), *Actinobacteria* (26%), and *Bacteroidetes* (2%). *Pseudomonas glareae* (Cloșani), *Aeromonas* sp. and *Polaromonas jejuensis* (Ferice) and *Streptomyces* sp. (Muierilor) were the most frequently recovered isolates. *Bacillus* sp. and *Paenibacillus* sp were retrieved in all caves, while *Flavobacterium* sp. was found only in Cloșani samples. Well-known as bioactive compound-producers, *Actinobacteria* members isolated from the floor sediments of the

explored cave soils were assigned to *Arthrobacter* sp. (Cloșani and Ferice), *Paeniglutamicibacter kerguelensis* (Ferice), and *Streptomyces* sp. (Muierilor). Our findings indicated that the diversity of cultivable bacteria varied among the studied caves, probably due to the geographic distances and slightly different physicochemical setting of each particular cave. However, further detailed investigations are needed to reveal the full picture of bacterial diversity and its roles in the tested cave ecosystems.

Keywords: karst cave; microbiota; phylogeny; 16S rRNA gene; cultivable diversity

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