

Comparison of Actinobacteria communities from human-impacted and pristine karst caves

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Abstract

Actinobacteria are important cave inhabitants, but knowledge of how anthropization and anthropization-related visual marks affect this community on cave walls is lacking. We compared Actinobacteria communities among four French limestone caves (Mouflon, Reille, Rouffignac, Lascaux) ranging from pristine to anthropized, and within Lascaux Cave between marked (wall visual marks) and unmarked areas in different rooms (Sas-1, Passage, Apse, Diacalse). In addition to the 16S rRNA gene marker, 441 bp fragments of the hsp65 gene were used and an hsp65-related taxonomic database was constructed for identification of Actinobacteria to the species level by Illumina-MiSeq analysis. The hsp65 marker revealed higher resolution for species and higher richness (99% OTU cutoff) versus 16S rRNA gene; however, more taxa were identified at higher taxonomic ranks. Actinobacteria communities varied between Mouflon and Reille caves (both pristine), and Rouffignac and Lascaux (both anthropized). Rouffignac displayed high diversity of *Nocardia*, suggesting human inputs, and Lascaux exhibited high *Mycobacterium* relative abundance, whereas *Gaiellales* were typical in pristine caves and the Diacalse (least affected area of Lascaux Cave). Within Lascaux, *Pseudonocardiaceae* dominated on unmarked walls and *Streptomycetaceae* (especially *Streptomyces mirabilis*) on marked walls, indicating a possible role in mark formation. A new taxonomic database (<https://zenodo.org/record/5576074>) was developed. Although not all Actinobacteria species were represented, the use of the hsp65 marker enabled species-level variations of the Actinobacteria community to be documented based on the extent of anthropogenic pressure. This approach proved effective when comparing different limestone caves or specific conditions within one cave.

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