

Human impact on *Actinobacteria* community revealed by *hsp65* marker as a complement to 16S rRNA by metabarcoding approach in natural limestone caves and the Lascaux Cave

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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 missing. We compared *Actinobacteria* communities among four French limestone caves (Mouflon, Reille, Rouffignac, and Lascaux) ranging from pristine to anthropized and within Lascaux Cave between marked (wall visual marks) and unmarked areas on walls in different rooms (Sas-1, Passage, Apse, and Diacalse). In addition to the 16S rRNA gene marker, 441-bp fragments of *hsp65* gene were used for the identification of *Actinobacteria* to the species level by Illumina-MiSeq analysis. The *hsp65* marker revealed higher resolution for species and higher richness (at 99% OTUs cutoff) than did 16S rRNA assessment which, however, identified more taxa at higher taxonomic ranks. *Actinobacteria* communities varied between Mouflon and Reille caves (both pristine), Rouffignac and Lascaux (both anthropized). Rouffignac displayed high diversity of *Nocardia*, pointing to human inputs, and Lascaux exhibited high *Mycobacterium* abundance, whereas *Gaiellales* were typical in pristine caves and the Diacalse (the least affected area of Lascaux Cave). Within Lascaux, *Pseudonocardiaceae* dominated on unmarked walls and *Streptomyetaceae* (especially *Streptomyces mirabilis*) on marked walls, raising questions on their possible role in the formation of visual marks. Our results show how the use of the *hsp65* marker, well beyond the resolution provided by 16S rRNA sequences, enabled for the first time to document species-level variations of the *Actinobacteria* community according to the extent of anthropogenic pressure. This approach proved effective when comparing different limestone caves or specific conditions within one cave.

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