

Biodeterioration of Roman tombs: The role of pigmented actinobacteria

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Etruscan and Roman necropolises are cemeteries with different types of burial practices, which are remarkable for their magnificent mural paintings. The conservation of these paintings is difficult, mainly derived from environmental factors and the opening of the tombs to public visits. One of the most complex phenomena observed on these sites is biodeterioration. The Circular Mausoleum tomb (Roman Necropolis of Carmona, Seville, Spain) is characterized by a heavy colonization of phototrophic microorganisms on the walls and ceiling. In addition, some areas near the ceiling exhibited an important number of violet stains of unknown origin. Previously, Agarossi (1994) found similar violet stains, attributed to streptomycetes, in two Etruscan tombs. However, no data on the species involved or the chemical structure of the pigments were reported. Here we show that the violet stains observed in the Circular Mausoleum are produced by a strain of the actinobacterium *Streptomyces* isolated from the mortar walls, and able to synthesize the same violet pigment in the laboratory.

Streptomyces parvus MC05 was identified after whole genome study by means of Next Generation Sequencing methods. Pairwise comparisons carried out for genomes of the type strain of *S. parvus* DSM40348T and the MC05 strain were performed using Jspecies service. While results observed for ANIb, ANIm and Tetra indexes indicated that both strains belong to the same species, the *S. parvus* MC05 strain differs from the DSM20348T type strain in genome size and secondary metabolites production. AntiSMASH analysis implemented for both strains showed that the *S. parvus* MC05, isolated from the tomb, presents the capacity of synthesize bioactive compounds which were absents in the type strain.

HPLC-MS of the culture extracts from *S. parvus* MC05 showed the production of three main granaticin derivatives (dihydrogranaticin A, granaticin A and granaticin B) in addition to minor products of other granaticin analogues. Granaticin pigments exhibited antibacterial activity, which justified the low number of clones of Gram positive bacteria found in the whole microbial community study. Gram negative bacteria were not affected (Dominguez-Moñino et al. 2017).

To conclude, the origin of the violet stains in the walls of the Circular Mausoleum is the presence of *S. parvus* MC05, a member of the complex microbial community thriving on the tomb. In growing periods (rainy season) the bacterium excretes the soluble violet granaticins, compounds with a benzoisochromanequinone structure, which diffuses to the mortar and surrounding substrata in wetting periods.

References

- Agarossi, G. 1994. In *Studi e Ricerche sulla Conservazione delle Opere d'Arte Dedicati alla Memoria di Marcello Paribeni*. F. Guidobaldi (ed.), CNRS, pp. 1–11.
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