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Calcium carbonate of microbial origin in the Etruscan tombs of Tarquinia

A. Cirigliano[1], F. Mura[2], A. Quagliarello[3], R. Negri[4], and T. Rinaldi[4]

[1] Institute of Molecular Biology and Pathology, CNR, Rome, Italy
[2] Sapienza CNIS, Center for Nanotechnology Applied to Industry of La Sapienza, Rome, Italy
[3] Department of Comparative Biomedicine and Food Science, University of Padova, Italy
[4] Department of Biology and Biotechnology, Sapienza University of Rome, Italy e-mail: teresa.rinaldi@uniroma1.it

Abstract. The UNESCO World Heritage Etruscan necropolis of Tarquinia, (Viterbo, Italy), is the site of more than 200 painted hypogeal tombs (dated from the 7th to the 2nd century B.C.). In the Etruscan hypogeal tombs, we previously identified the presence of a thick secondary calcium carbonate nanocrystals deposit of biogenic origin (the moonmilk), usually found in karstic caves. The analysis of the bacterial communities of these deposits revealed that the rocks where the Etruscan hypogea were carved, the *macco*, contained the microbial community responsible for moonmilk formation. These communities harbor mainly mesophilic (non-extreme) bacteria, but cyanobacteria, such as the genus *Chroococcidiopsis* was found in abundance in the *Tomba delle Sculture* and *Tomba Bartoccini* that are kept in the dark. The moonmilk also hosts many bacterial species able to produce calcium carbonate in laboratory conditions. The study of the interactions between microbes and rock (e.g. moonmilk formation) and biomineralization can provide information with potential value for life detection.

Key words. Moonmilk – Calcium Carbonate – Microbial Community

1. Introduction

In the Etruscan Necropolis of Tarquinia, a $CaCO_3$ precipitate of biogenic origin (the moonmilk) is present on the walls and ceilings of the hypogeal tombs (Cirigliano et al. 2020; Mura et al. 2020). The moonmilk is produced by a bacterial community as a consequence of calcium ions detoxification process; in calcium rich environments, active calcite precipitation is induced by bacterial metabolic activities and the bacterial cell walls and biofilms can act as nucleation sites for carbonate precipitation (Cailleau et al. 2009). We previously observed that the presence of the moonmilk on tombs surfaces protects, rather than degrades, the ancient paintings on these surfaces (Tomassetti et al. 2017). In the Etruscan tombs, the deposition of biogenic needles of $CaCO_3$ is determined by bacteria hosted in a yellow calcarenite rock called macco, in which the tombs are excavated (Cirigliano et al. 2020), however the mechanism of their formation remains unknown and these calcium carbonate nanofibers could not be reproduced in laboratory conditions.

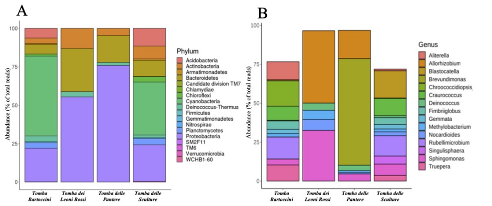

Fig. 1. Fig. 1. Barplot representing the microbial community from moonmilk samples of the Tomba Bartoccini, Tomba delle Sculture, Tomba delle Pantere, Tomba dei Leoni Rossi at the phylum (A) and genus levels (B).

The current study provides new insights in understanding the interactions between microbes and rock (moonmilk formation), between minerology and cells (fossilization) which could unravel biosignatures with potential value for life detection.

2. Results and Discussion

The microbial communities analyzed in this study were sampled from the moonmilk of the *Tomba Bartoccini*, *Tomba dei Leoni Rossi*, *Tomba delle Pantere* and *Tomba delle Sculture*. The most abundant bacterial phyla present in the moonmilk were *Acidobacteria*, *Actinobacteria*, *Cyanobacteria* and *Proteobacteria* (Fig. 1A) with a high similarity to the bacterial communities present in tombs located in close proximity (Cirigliano et al. 2020). Bacteria resistant to ionizing radiation were found in the moonmilk microbial communities: *Brevundimonas* is the second-most abundant genus in *Tomba delle Pantere*, that has survival ability under simulated Martian conditions and *Truepera* is the fifth-most abundant genus in *Tomba Bartoccini* and it is also present in *Tomba delle Sculture* (Fig. 1B). Of note, in the *Tomba Bartoccini* and *Tomba delle Sculture* the microbial community hosts a high percentage of *Xenococcaceae* (Cyanobacteria): 42% and 22,8% of total genomic reads respectively (Fig. 2B). The *Chroococcidiopsis* is the most-abundant Cyanobacteria in *Tomba Bartoccini* (11.56% of total genomic reads), and in *Tomba delle Sculture* (20.9% of total genomic reads): this genus has members able to grow in harsh environmental conditions, including both high and low temperatures, high doses of ionizing radiation (up to 15 kGy), and high salinity (Billi et al. 2000). While Cyanobacteria can adopt a heterotrophic lifestyle, there is a paucity of studies about heterotrophic nutrition of *Chroococcidiopsis* in complete darkness. Recently, *Chroococcidiopsis* and *Calothix* accounted for 80% of total genomic reads in deep subsurface rock samples (from 392 m to 613 m deep) in Spain (Puente-Sánchez et al. 2018)and in oxic subseafloor sediment millions of years old in a metabolically active form (Morono et al. 2020). *Calothrix* was identified in *Tomba Bartoccini*, *Tomba dei Leoni Rossi* and *Tomba delle Sculture* and it is able to switch to heterotrophic metabolism in complete darkness. Albeit at very low abundance, the following genera of *Cyanobacteria* were also found in the Etruscan tombs: *Acaryochloris*, *Leptolyngbya*, *Pleurocapsa*, *Phormidium*, *Scytonema* and *Toxopsis* (Cirigliano et al. 2020). It was recently shown that the Cyanobacteria producing chlorophyll d and f (*Leptolyngbya* and *Acaryochloris* spp.) were present limestone caves kept in complete darkness. These bac-

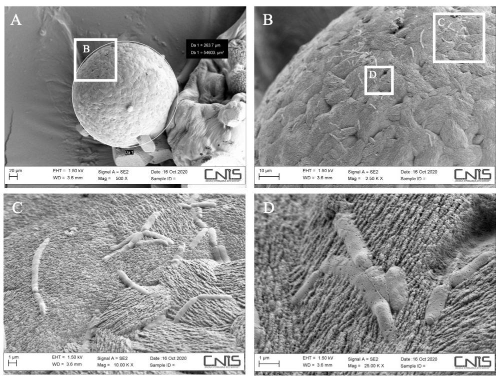

Fig. 2. The moonmilk microbial community hosts calcifying bacteria. (A) Scanning electron micrographs of a biocalcification induced by a bacterial strain; the calcite produced by the bacterial strain has a spherulite shape. Progressive magnifications (B to D) of the calcium carbonate shown in (A) revealed the presence of calcified bacteria on the surface (white arrows).

teria can photosynthesize using near-infrared radiation and chlorophyll d and f to generate energy (Behrendt, et al. 1998). The presence of Cyanobacteria in the *Tomba delle Sculture* and the *Tomba Bartoccini* must subsist heterotrophically because the moonmilk was formed in darkness in these hypogeal tombs. The metabolism of Cyanobacteria deserves further studies in these hypogeal environments, the presence of Cyanobacteria capable of utilizing near-infrared radiation in the tombs kept in darkness is needed to elucidate their potential contribution to moonmilk metabolism. We propose to consider these hypogea as environments that could provide information about how hypolithic bacteria are adapted to survive in total darkness. Many strains cultured from moonmilk were able to precipitate $CaCO_3$ on plates (Fig. 2), but the high amount of calcium carbonate they produce results in an entombment of the bacterial cells (Cirigliano et al. 2020). The biomineralization is often the first step of fossilization and produces structural and morphological features that can be preserved in fossil biominerals. The characterization of biomineralized microorganisms could offer new insights into how the biominerals are precipitated and what biological signatures can be potentially preserved in the geological record.

3. Materials and Methods

Sample collection, microscopy and analysis of 16S rRNA genes were described previously (Cirigliano et al. 2020).

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