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**BIOGENICITY AND CHARACTERIZATION OF MOONMILK  
DEPOSITS IN THE GROTTA NERA, A LIMESTONE CAVE  
IN THE MAJELLA NATIONAL PARK (ABRUZZI, CENTRAL ITALY)**

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**Abstract**

Cave are extreme and specialised habitats for terrestrial life that sometime contain moonmilk, a microcrystalline cave speleothem with variable mineralogy, a high water content, a high porosity, and distinguishable macro- and micro-textures. About 95% of moonmilk deposits are carbonatic and its most common type is calcite moonmilk. The origin of this material is still discussed although the involvement of microbial activity has been suggested by a number of authors. Observation and hypotheses on the possible influence of unidentified calcifying bacteria for moonmilk speleothem formation in the Grotta Nera are reported for the first time. The Majella Massif hosts a complex karst system of more than one hundred caves; the accessible Grotta Nera is the most particular one. In spite of its name, the cave consists of two spectacular cream-colored chambers where there are many white unconsolidated and extraordinary speleothems of moonmilk to create a charming atmosphere. Moonmilk from the Grotta Nera was analyzed to determine the geochemistry, fabric and extent of biogenicity. X-ray diffraction analysis of the speleothems gave clear evidence for calcite. Using traditional cultivation techniques, we isolated an abundant heterotrophic microflora ( $4.5 \times 10^4$  cfu/g d.w.) associated with two moonmilk samples. Examination of Gram-stained smears taken from the twelve different colony types showed that the majority (79%) of the isolated strains were Gram negative. Single small rods and rod chains predominated. According to the literature, the relative abundances of each isolate with respect to the total cultivable bacterial microflora showed that the G- strains were the most abundant. No attempts were made to identify the various bacterial species present as our attention was directed towards the processes of carbonate deposition rather than taxonomy. In vitro culture experiments confirmed the capability of many of the bacterial isolates (93%) to precipitate calcium carbonate crystals in the laboratory at 15°C (the temperature of the cave), 22°C and 32°C. The corrosion behavior of the calcifying isolates was also studied showing that 50% of them solubilized calcium carbonate when grown on agar plates containing 2.5% CaCO<sub>3</sub>. The calcifying strains with the highest solubilization activity showed the lowest relative abundances. SEM showed that moonmilk speleothems in the Grotta Nera consist of a network of fiber calcite crystals and calcified or active microbial structures. These results allowed us to postulate the biogenic nature of the moonmilk speleothems hosted in the Grotta Nera and a low influence of the bacterial solubilization process.

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