

MICROBIAL INHABITANTS AND BIOSIGNATURES OF VOLCANIC CAVES FROM MACARONESIA.

A. Z. Miller^{1,2}, J. L. Gonzalez-Pimentel^{2,3}, V. Palma², N. T. Jiménez-Morillo¹, S. Gutierrez-Patricio¹, B. Cubero¹, J. M. de la Rosa¹, A. T. Caldeira², J. A. González-Pérez¹ and F. Sauro^{4,5}. ¹Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS-CSIC), Seville, Spain (anamiller@irnas.csic.es); ²HERCULES Laboratory, University of Évora, Portugal; Andalusian Centre for Developmental Biology (CABD, UPO-CSIC-JA), University Pablo de Olavide, Seville, Spain. ⁴Planetary Caves Topical Team ESA, Miles Beyond Srl; ⁵Department of Earth Sciences and Environmental Geology, University of Bologna, Italy.

Introduction: Caves are exceptional habitats for highly specialized microorganisms that can interact with minerals, allowing us to better understand the role of microorganisms in biogeochemical cycles and biomineralization processes. These priceless subterranean resources can be found all over the world, but they are one of the least explored habitats on Earth. Yet, lava caves have recently received special attention due to the discovery of numerous volcanic cave entrances on the Moon and Mars [1]. Such subsurface geological settings, characterized by stable physicochemical conditions, may support microbial activity over geological timescales, resulting in the preservation of traces of life in the rock record, recognized as biosignatures. These subterranean environments and the microbial life within thus provide a unique opportunity to study extremely specialized microorganisms, novel metabolic strategies, and interactions with igneous rocks relevant to astrobiology and planetary sciences.

Methods: The diversity and function of microbial communities dwelling on speleothems from volcanic caves of Selvagens Islands (Madeira, Portugal), La Palma and Lanzarote (Canary Islands, Spain) have been investigated using portable cutting-edge DNA-based analysis, complemented by in-depth metagenomics and field emission scanning electron microscopy (FESEM), to understand which microorganisms grow in these extreme ecosystems, which functional properties they have, and their role in constructive and/or destructive mineral processes. In addition, advanced chromatography, and mass spectrometry techniques, such as GC/MS, Pyrolysis-GC/Q-TOF and Pyrolysis-compound specific isotope analysis (Py-CSIA) have been employed for an in-depth biogeochemical characterization of the siliceous speleothems and for the assessment of biosignatures preserved in the samples, as described in Miller et al. [2,3].

Results and Discussion: Bacterial communities showed heterogeneity in composition among lava tubes, with the *Actinomycetota*, *Bacillota* and *Bacteroidota* phyla as the most abundant, exceeding 50% for most of the sampling sites. Using FESEM, we described the morphology of microbial cells and their

interactions with the mineral substrate. FESEM images showed abundant actinobacteria-like cells and other morphotypes, resembling those reported by Riquelme et al. [4] in lava tubes from USA, Canada, Portugal and Spain, and in Etna lava tubes [5]. Observations conducted on microbial mat samples from a lava tube in Lanzarote revealed the presence of Ca-rich spheroids closely associated with filamentous cells of *Crossiella* sp.. The functional profile of microbial communities predicted by PICRUSt showed the presence of urease enzyme, that has been identified as responsible for the microbial precipitation of CaCO₃ via urea hydrolysis [6], suggesting that this genus could promote speleothem formation. Similar biogenic-like CaCO₃ microspheres were reported in colored microbial mats from Kipuka Kanohina lava cave in Hawaii, USA [4].

The presence of short-chain *n*-alkanes (C_{<20}) and methylated fatty acids identified in the organic fraction of the siliceous speleothems are recognized as biosignatures of microbial origin (membrane lipids and/or microbial metabolites). High molecular weight lipid compounds (e.g., sterols and long-chain *n*-alkanes) are indicative of vegetation biomarkers derived from lixiviation processes of the soil overlying caves.

Acknowledgments: This study has been funded by the Spanish Ministry of Science and Innovation from the Spanish State Agency through the TUBOLAN (PID2019 108672RJ-I00) and HIRES-SOM (TED2021-130683B-C22) projects. The Portuguese Foundation for Science and Technology (FCT) and the Regional Government of Andalusia (Spain) are also acknowledged for funding the MICROCENO (PTDC/CTA-AMB/0608/2020) and MICROLAVA (PROYEXCEL_00185) projects, respectively. The Spanish National Research Council (CSIC) is acknowledged for funding the intramural project PIE_20214AT021. A.Z.M and N.T.J.M. thank the Ramón y Cajal research contracts (RYC2019-026885-I and RYC2021-031253-I, respectively).

References: [1] Sauro F. et al. (2020) *Earth Sci. Rev.* 209, 103288. [2] Miller A. Z. et al. (2020) *Sci. Total Environ.* 698, 134321. [3] Miller A. Z. et al. (2022) *IScience*, 25, 104556. [4] Riquelme C. et al. (2015) *Front. Microbiol.* 6, 1342. [5] Nicolosi et al.

(2023) *Microb. Ecol.* doi:10.1007/s00248-023-02181-2.
[6] Kosznik-Kwaśnicka K. et al. (2022) *Microorganisms* 10, 222.