



Exploring possible Mars-like microbial life in a lava tube from Lanzarote: preliminary results of in-situ DNA-based analysis as part of the PANGAEA-X Test Campaign

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The discovery of a highly diverse subsurface biosphere revolutionized biology and changed how we think about the origins of life on Earth and possibly on other planets. Lava tubes are particularly attracting interest due to their importance as astrobiological targets on Earth and Mars. A whole collection of microbial life and biosignatures preserved in volcanic rocks can be found in lava tubes. Understanding and identifying these microorganisms and their biosignatures may help us to recognize structures that could indicate the presence of life on other planets characterized by similar volcanic features. Within the PANGAEA-X project (Planetary ANalogue Geological and Astrobiological Exercise for Astronauts) organized by the European Space Agency (ESA), we have conducted a microbiological campaign in the Corona lava tube in Lanzarote (Spain). Lanzarote represents the easternmost island of the Canary archipelago located in the Atlantic Ocean. Because of the arid climate and volcanic landscape it is considered an excellent analogue of the Moon and Mars where it is possible to test new approaches to planetary science investigations, helping to understand the geological and potential microbial processes on Mars. The Corona lava tube is considered one of the biggest lava tubes by volume known on Earth, being formed during an effusive eruption of the Corona Volcano occurred $21,000 \pm 6,500$ years ago. With a total length of 8 km, tube sections of over 30 meters in diameter and a series of collapses aligned along the tube development, this cave represents one of the most promising analogue to candidate lava tubes detected on Mars.

The objectives of this experiment comprised: 1) Recognition of microbial colonization patterns on cave walls and sampling of microbial mats; 2) In-situ 16S rRNA gene analysis to identify microbial communities and avoid cross-contamination. For this purpose, DNA extraction, PCR amplification and full-length 16S amplicon sequencing using the miniaturised and portable thermal cycler miniPCR (Ampliyus) and DNA sequencer MinION of Oxford Nanopore Technologies (ONT) were performed in the lava tube, and 3) Geochemical and mineralogical characterization of secondary mineral deposits associated with microbial mats.

The 16S Nanopore sequencing of coloured microbial mats revealed that Proteobacteria dominated in this cave ecosystem. Of note was the presence of moderate bacterial halophiles within the Gammaproteobacteria class. In-situ V-NIR spectroscopy and laboratory XRD analyses revealed the presence of abundant deposits of gypsum, halite and clay minerals associated to some specific microbial mats.

Our data indicated that the Corona lava tube hosts a highly specialized subsurface biosphere dominated by microorganisms able to interact with minerals. This study showed that in-situ DNA analysis of subsurface microbial mats and secondary mineral deposits in the lava tube environment offers a challenging opportunity for geomicrobiological and astrobiological research.

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