GEOMICROBIOLOGICAL FIELD RESEARCH IN A SUBSURFACE ANALOGUE ENVIRONMENT FOR FUTURE PLANETARY CAVES MISSIONS. A. Z. Miller1,2, J. L. Gonzalez-Pimentel1,2, M. Maurer1, S. Stahl3, S. Castro-Wallace3, L. Bessone4, J. Martinez-Frias3, F. Sauro3, 1HERCULES Laboratory, University of Évora, Portugal (anamiller@uevora.pt), 2Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS-CSIC), Seville, Spain, 3European Astronaut Centre (EAC), European Space Agency (ESA), Cologne, Germany, 4JES TECH, Johnson Space Center (JSC), National Aeronautics and Space Administration (NASA), Houston, U.S.A., 5Human Health and Performance Directorate, JSC, NASA, Houston, U.S.A., 6Instituto de Geociencias, CSIC-Universidad Complutense de Madrid, Spain, 7BIGEA, University of Bologna, Italy (cescosauro@gmail.com).

Introduction: A rapid expansion of interest in the geomicrobiology and exploration of subsurface environments, such as caves, has recently emerged to better understand origins of life on Earth and on other planets. Volcanic caves have lately received particular attention as similar cavities have been reported on the Moon and Mars [1]. Hence, caves on Earth are planetary analogue environments suitable for the training of astronauts, both from the science prospective and for technological testing, for future exploration missions to other planets.

In this sense, since 2011, the European Space Agency (ESA) holds astronaut training courses in caves, including the CAVES (Cooperative Adventure for Valuing and Exercising human behaviour and performance Skills) and PANGAEA (Planetary ANalogue Geological and Astrobiological Exercise for Astronauts) programs.

Within the PANGAEA course, a geomicrobiology field training took place in 2017 in Lanzarote, Spain. This training involved the collection of microbiological samples in the Corona lava tube (one of the biggest volcanic caves known on Earth) and successive DNA-based analyses in the cave environment using fast and portable devices for DNA amplification and sequencing (Fig. 1).

Advanced on-site DNA sequencing: Samples of dark-colored microbial mats on the volcanic bedrock located in the twilight zone of the cave were collected by gently removing the microbial mat with sterile disposable scalpels and gathering it into sterile vials (Fig. 1A). Subsequently, DNA extraction was conducted using the DNeasy PowerSoil DNA extraction kit (Qiagen), followed by PCR amplification using the miniaturized and portable thermal cycler miniPCR (Amplysus), controlled from a laptop. Sequencing of the full-length 16S rRNA gene was conducted using the MinION (Oxford Nanopore Technologies - ONT) sequencer device attached to a laptop and using the operating software MinKNOW (ONT). Finally, taxonomic classification of generated reads was performed using the EPI2ME platform (ONT). These analyses took place in the module of the Lanzarote Geodynamic Laboratory (Fig. 1B,C) installed in the Corona lava tube, and currently managed by the Geopark of Lanzarote together with IGEOD (CSIC-UCM).

A total of 9149 reads were obtained from the MinION sequencing and more than 8300 reads with the full-length of the 16S rRNA gene (approx. 1500 base pairs) were analyzed for microbial diversity. Proteobacteria dominated in this cave ecosystem. Most of the DNA sequences (>35%) were affiliated to *Salinisphaera halophila*, a moderately halophilic bacterium belonging to the Gammaproteobacteria class. Of note is the presence of abundant deposits of gypsum, halite and clay minerals associated with the dark-colored microbial mats, as revealed by in-situ V-NIR spectroscopy and laboratory XRD analyses [2].

In-depth laboratory analyses: Replicate samples of the dark-colored microbial mats were analyzed under laboratory conditions for comparison purposes. The extracted DNA was analyzed with next-generation sequencing (NGS) of the bacterial V3 and V4 regions of the 16S rRNA gene with primers 338F and 806R (338F: 5′-GAGT'TTGATCCTGGCTCAG-3′ and 806R: 5′-CCGTAAAAAAAAAAACCTCAATAT-3′) using the Qiaseq V3+V4 kit (Qiagen) and MiSeq (Illumina) and MiSeq Reagent Kit v3 (Illumina).
of the 16S rRNA gene using the Illumina MiSeq platform by STAB Vida sequencing services (Portugal). Raw data was processed in Qiime2, which includes the DADA2 algorithm, for optimal quality control without clustering. Classification of the reads was based on the ARB-SILVA SSU database, and further analyzed and visualized using the online web-tool Calypso.

In addition, microbial mat samples were investigated by field emission scanning electron microscopy with energy dispersive X-ray spectroscopy (FESEM-EDS) using a FEI Teneo microscope equipped with an Ametek EDAX detector.

Phylogenetic analysis based on 16S rRNA gene sequences showed that the microbial mat samples are dominated by *Salinisphaera* sp., followed by *Halococcus*, a genus of extreme halophilic archaea. These results are in line with the in-situ DNA-based analysis and are consistent with the mineralogical composition of the rock substrate.

FESEM-EDS observations revealed abundant microbial cells, comprising short rods of approximately 1 µm long and 0.4-0.6 µm wide, and coccoid cells with less than 1 µm in diameter and septum formation (Fig. 2). Their size and morphology resemble those of *Salinisphaera halophile* [3], and *Halococcus* [4], respectively.

Figure 2. FESEM image of the dark-colored microbial mat sample depicting

**Conclusions:** Our field experiment revealed halophilic microorganisms associated with gypsum and halite, suggesting they are capable of adapting to this cave environment and to interact with secondary minerals. In addition, this study showed that subsurface environments in general and lava tubes from Lanzarote in particular offer one of the best possible terrestrial analogue sites to search for extremely specialized microbial life and to test new approaches to planetary science investigations, helping to understand the geological and potential microbial processes on Mars.


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