LINKING BIOLOGICAL AND GEOCHEMICAL DATA FROM ICELANDIC LAVA TUBES: INSIGHTS FOR UPCOMING MISSIONS IN THE SEARCH FOR EXTANT OR EXTINCT LIFE ON MARS

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Introduction: The search for extraterrestrial life in our solar system has been pursued most extensively on our close neighbor, Mars. Early Mars may have been just as hospitable to life as early Earth, with surface liquid water and a mild climate capable of sustaining microbial life [1]. Sampling on the Martian surface has thus far not resulted in the detection of any traces of life, which is unsurprising given the instruments used and the areas sampled, where ionizing radiation has most likely degraded any signatures of potential past life (see [2] for a review). However, it is possible that microbial life migrated to the subsurface as the surface radiative environment became increasingly harsher.

Subterranean environments on Mars would have provided shelter from ionizing surface radiation, a relatively stable internal temperature, and potential entrapment of volatiles, such as water, that may have sustained life. If life was able to flourish in such environments, it or traces of it should still exist in the form of kerogens, permineralized polymers [3], mineral structures or fossils [4], or other geochemical signatures, since the preservation of biosignatures in cave minerals may be stable for many millions of years [5].

Lava tubes form from basaltic flows, typical of pahoehoe eruptions from low lying shield volcanoes. The High Resolution Imagining Experiment (HiRISE), a camera on board the Mars Reconnaissance Orbiter (MRO) launched in 2005, has shown features indicative of lava tube entrances on Mars. The Elysium Mons shield volcano is postulated to have formed in the more habitable time period of Mars, whereas the Tharsis Montes region is potentially too young and was formed after the loss of the magnetic field [6]. This range in the age of lava flows creates a debate on where potential microbial fossils (or life) may lie, but the existence of these lava tubes nonetheless presents a possibility for habitable environments.

Lava caves on Earth are mineralogically similar to caves on Mars, as are the energy sources for life inside, namely redox gradients of iron, sulfur and manganese for example. The chemoautotrophs that feed off of these gradients, and subsequent heterotrophs that feed off of the organic carbon they produce, together form biofilms and microbial mats of incredible complexity. As a consequence, microbes in caves alter their host rocks chemically and induce the dissolution and precipitation of speleothems, either passively, where microbial cells act as nucleation sites, or actively, where bacterially produced enzymes control mineralization [7]. Many speleothems are also produced abiotically. It is in differentiating between the biotically- and abiotically-formed speleothems wherein the art of biosignature detection in caves lies.

Lava caves on Earth provide analogue sites where these processes can be studied and the art of biosignature detection developed. To differentiate between abiotic and biotic speleothems, it is necessary to put them in context, i.e. to understand the chemical environment in which they formed. For biotically-formed speleothems, it is also necessary to understand the biological agent which facilitated its formation. To this end, both geological and biological information, as well as the interface between the two, need to be acquired.

The expeditions of the Planetary Analogues and Lava Tube Expedition (PELE) team involve the collection of interdisciplinary sample sets in order to characterize the interaction between the biological activity and its mineral substrate in Icelandic lava caves, with the goal of identifying possible biosignatures which might be expected to be preserved in deep time.

Here we report some of the findings from several Icelandic lava caves located at the Laki and Óðáðahraun lava fields in south-eastern and northern Iceland, respectively. The caves were chosen for their relatively oligotrophic nature, making them good candidates as analogues to Martian caves.

Methods: Lava caves are very isolated, fragile and potentially dangerous environments, and as such their exploration requires much care, planning and special equipment. The PELE team consistently works with local speleological societies during our sampling expeditions. The Icelandic Speleological Society (ISS) was indispensable to this work, with in-depth knowledge of the terrain, and by doing its best to keep these caves in their pristine states by protecting them from vandalism and human contamination.

Sampling strategy. Sampling spots were chosen based on visual appreciation of suspected biomass and nutrient sources. Several expeditions with the same core team honed the sampling strategy which follows: each area sampled is first approached by the biologists, who under sterile conditions collect microbial mat samples.

After they finish, a handheld X-ray fluorescence spectrometer (XRF) is used on the rock below the sampled mats to record the elemental composition. This is then followed by geological sampling, which consists of chipping away a small amount of surface rock with a geological hammer.

Analytical techniques. DNA is extracted from biological samples using a Phenol/Chloroform protocol and analyzed with 16S rRNA gene sequencing on the Illumina MiSeq platform. Geological samples are characterized by means of X-ray diffraction (XRD) and thin section optical microscopy. The organic fraction is extracted from these samples for lipidomics analysis. Additionally both biological and geological samples are analyzed with Raman spectroscopy and scanning electron microscopy (SEM) for supplementary compositional and morphological information.

Results and Discussion: A high diversity of geochemical and microbiological features were detected in each cave. Differences in microbial populations were found to exist between the northern and southern caves, though some species were found in all caves.

As reported in other lava caves around the world, Proteobacteria were ubiquitous in the investigated caves but varied in abundances of 20–90% depending on the sampling site and cave. This differs from previously reported comparison studies between Hawai'ian and Azorean lava caves, where *Actinobacteria* were more abundant in the former and *Acidobacteria* in the latter [8]. Thirty three bacterial phyla and more than two hundred genera were found across the samples. *In situ* XRF data indicates a high diversity of substrates, with significant concentrations of iron, copper, calcium and silica, and traces of cobalt, nickel and titanium. Interesting genera were identified capable of feeding off of these substrates. Several genera have been found to be fairly ubiquitous, e.g. *Methylobacterium*, *Cupriavidus* or *Lactobacillus,* whereas some were only found in limited sample sites within caves or in distinct geographical areas. For example, *Dechlorobacter* and *Leptolinea* were only found in the northern region of the Oðáðahraun lava field. The former is potentially interesting as some species are known to reduce perchlorate salts, which are of high relevance for a Martian electron acceptor. Correlations between elemental abundances derived from the XRF and the presence and relative abundances of bacterial genera have yet to be established.

Archaeal and fungal communities, understudied in cave environments, will be further investigated as some of the bacterial genera found, such as *Chitinophaga,* are known endosymbionts of fungi.

Raman microspectroscopy data (acquired with a 532 nm excitation wavelength as that of the Raman Laser Spectrometer instrument on-board the ESA/Roscosmos ExoMars2020 rover) revealed the presence of carotenoid molecules which are invaluable targets as potentially detectable biosignatures on Mars. The mineral natrojarosite, usually formed in highly oxidizing conditions and detected on Mars by Spirit and Opportunity, was also interestingly found in several samples.

Conclusion: By linking biological and geochemical data, we aim to characterize the metabolisms of primary and secondary bacterial colonizers of one of the only initially sterile environments found on Earth, and to describe how they alter their host rocks. Further investigation of secondary mineral deposits with Raman, XRD and SEM will hopefully elucidate whether these are of biological origin, and their potential as biosignatures.

Figure 1: Copper-rich precipitate in Icelandic lava tube.

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