MICROBIAL COMMUNITY STRUCTURE, METABOLISM AND FUNCTION IN BASALTIC CAVES AND LAVA TUBES

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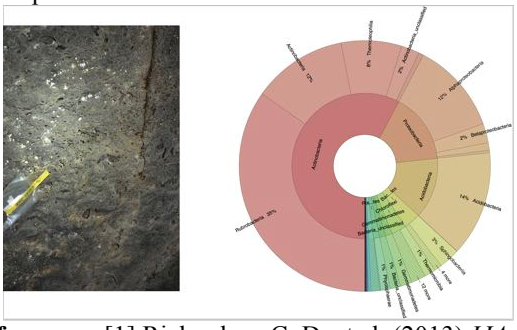
Background: Subsurface environments provide stable conditions and shield microbial life from radiation and temperature fluctuations, allowing life to evolve and persist in otherwise harsh conditions. Importantly, the relative stability in environmental parameters of subsurface habitats also permit the preservation of evidence of past and current biological activity. Consequently, lava tubes and basaltic caves observed on Mars may potentially be significant targets for study while searching for signs of martian life. Volcanic environments on Earth, such as the iron-rich basalts found at Craters of the Moon (COTM) in Idaho (analogs to martian basalts in terms of composition), can be used as analogs to understand microbial metabolism, community composition and biosignature preservation (secondary mineral assemblages, for example).

Secondary sulfate and carbonate mineral deposits are found in lava tubes and caves of all sizes in the Blue Dragon flow within COTM [1]. The deposits occur in small cavities on cave walls and ceilings, as well as larger localized mounds on the cave floor [1]. Abiotic origins for these secondary deposits have been proposed, however, peaks observed by FTICR-MS indicated association of biogenic organic compounds with secondary sulfate deposits, linking these minerals, directly or indirectly, to biological activity [2]. These observations, together with sulfur isotope fractionation results, led to the hypothesis that biological activity contributes to secondary mineral deposit formation [2]. Specifically, [2] proposed that microbes may mediate oxidation of pyrite, one of the most abundant metal sulfides in basalt, to sulfate through multiple intermediates and subsequent precipitation [2].

Oxidation of sulfidic compounds is an important energy source for sulfur oxidizing bacterial species and could help support a microbial community. However, it is not known whether organisms capable of sulfide oxidation are present in proximity to the deposits or what other microbes, and metabolisms, are found in the minerals. We use next generation sequencing to identify microbial organisms associated with secondary mineral deposits, delineate physiologically active members of the community and describe their potential metabolic roles.

Methods: Samples collected for nucleic extraction were either stored in a nucleic acid preservation buffer or placed in a LN-primed cryoshipper immediately following collection for transport to the lab, where they were stored at -80°C until processing. Extracted nucleic acids were prepared for sequencing on an Illumina MiSeq, and the resulting sequence data were subject to taxonomic and functional analysis. Microbial community structure was investigated using 16S rRNA gene amplicon sequencing, and community metabolic potential, genetic content and identity of microbial groups associated with sulfur, carbon and metal metabolism were assessed using metagenomics. Microbial abundance and identity of active community members is currently underway using qPCR and cDNA amplicon sequencing targeting the 16S rRNA gene.

Results and Discussion: Our initial taxonomic analysis has revealed that the microbial community associated with sulfate deposits in small cavities along the wall of a cave as described in [1,2] is diverse, encompassing 22 bacterial phyla. These phyla consisted of aerobic, microaerophilic and anaerobic organisms with potential roles in metal oxidation as well as S and N cycling. *Actinobacteria* was the most abundant phylum, representing 58% of the community, and included the extremophilic lineages *Rubrobacteria* and *Thermoleophilia*. The Class *Rubrobacteria* contains thermophilic, radiation and desiccation-resistant species [3]; *Rubrobacteria* species have also been detected in sulfidic metal tailings, where they were associated with soluble sulfate [4]. Three million pairs of high quality paired-end metagenomics reads were generated for this deposit. Functional analyses to probe possible Rubrobacteria roles in sulfur metabolism in basalt cave secondary minerals are underway. *Proteobacteria*, primarily alpha-*proteobacteria* affiliated with *Sphingomonadales*, *Acidobacteria*, *Bacteroidetes*, and *Chloroflexi* were the other major phyla. Analysis is ongoing, but these results open a window into the microbial ecology and biogeochemistry of secondary mineral deposits in basalt caves, and establish the site as a terrestrial analogue for modeling the biotic potential of subsurface environments on Mars.



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