MICROBIAL SURVIVAL IN AN EXTREME MARTIAN ANALOG ECOSYSTEM: POÁS VOLCANO, COSTA RICA. Justin Wang[1], N. Dragone[2], G. Avard[3] and B. M. Hynek[1,4] [1]Laboratory for Atmospheric and Space Physics, University of Colorado Boulder, USA, [2]Dept. of Environmental and Evolutionary Biology, University of Colorado Boulder, [3] OVSICORI, National University of Costa Rica, [4] Department of Geological Sciences, University of Colorado Boulder. justin.wang@colorado.edu

Introduction: The active Poás volcano in Costa Rica has been studied as a Mars analog due to its mineralogical consistency with relict hydrothermal systems [1-5]; it also hosts Laguna Caliente, an ultra acidic sulfurous crater lake. Of potential habitable environments on Mars, hydrothermal acid-sulfate systems show promise in their ability to sustain suitable conditions throughout much of Mars' geologic history [1].

Field samples were collected from Laguna Caliente in 2013, 2017, and 2019 to assess the habitability of Martian acid-sulfate hydrothermal systems that were likely just as dynamic and challenging to life. Metagenomic 'shotgun' sequencing was conducted on a set of samples to assess how organisms adapted to the conditions of Laguna Caliente and to predict how life could survive in similar extreme environments on Mars.

Poás Volcano and Laguna Caliente: The Poás volcano is a basaltic andesite stratovolcano in the Central Cordillera of Costa Rica. Poás has been active throughout the Holocene; phreatic eruptions are common even in times of quiescence, and they mostly consist of unpredictable geyser-like expulsions from the crater lake. Acid-sulfate alteration of the parent rocks leads to a variety of sulfates, Fe-oxides, and clays akin to those found on Mars in relict hydrothermal settings [1-5]. Laguna Caliente is a dynamic terrestrial environment with temperatures ranging from near-ambient to boiling, a pH range of -1 to 1.5, a wide range of chemistries and redox potential, regular episodes of phreatic-to-phreatomagmatic eruptions up to October 2019, and magmatic eruptions April to November 2017.

Poás Volcano Field Campaigns: Three field campaigns were conducted to study the microbiology of Laguna Caliente. Sampling of lake fluid in November 2013 (temp.=45˚C, pH=0.3) showed the lake to host a veritable monoculture in the *Acidiphilium* genus [6]. Sampling in March 2017 of mud and floating sulfur clump samples found greater diversity in microbial community, attributed to sample bias, in more habitable conditions (temp.= 35 ˚C, pH=1.5).

In April 2017, a small magmatic eruptive period drained and likely sterilized the crater for the first time since 1989 [6, 7]. Sampling from the November 2019 field campaign (temp.= 55˚C, pH=0.1) marks the first-time microbial sampling of a newly formed Laguna Caliente had been done, with analysis ongoing.

Methods: Lake fluid and sediment samples were collected aseptically and immediately frozen. DNA was extracted using the MoBio PowerMax DNA Isolation Kit, and DNA from multiple extractions of the same samples were pooled and concentrated.

Fig. 1. A phreatic eruption in Poás Volcano in June 2014.

Briefly, the V4 region of the 16S rRNA gene was amplified and then sequenced on an Illumina MiSeq platform at CU Boulder. 16S rRNA gene sequences were processed using the DADA2 pipeline [8], quality filtered and clustered into amplicon sequence variants (ASVs), and compared to reference sequences from the 16S rRNA bacterial and archaeal SILVA database [9].

Metagenomic sequencing of the 2013 and 2017 samples was performed by the Genomics and Microarray Core at CU using the Nextera XT DNA Library Prep Kit and a NovaSEQ 6000 Sequencing System with paired-end 150 cycle 2x150bp chemistry. The SqueezeMeta metagenomic pipeline was used to process sequence results [10]. Gene annotation was performed by comparing reads to the KEGG Pathway database [11] using Diamond [12]. Taxonomic diversity was assessed using Metaxa2 [13].

Microbiology Results: ~92% of 16S rRNA gene reads of lake fluid samples from the November 2013 field campaign were identified as being from the genus *Acidiphilium*. Results from the March 2017 field campaign also show *Acidiphilium* in large proportions; however, the samples analyzed are from mud and sulfur clumps which expectedly yielded greater biodiversity, with *Bacteroidetes* making up a significant portion of the community.

Taxa of the *Acidiphilium* genus have been shown to grow in temperatures ranging from ~17˚C-45˚C with optimal pH ~3 [14]; conditions less extreme than that of Laguna Caliente. All members of the *Acidiphilium* genus are aerobic acidophilic bacteria, and these members have exhibited a wide range of environmental adaptations. Members of the *Acidiphilium* genus have included obligate heterotrophs, facultative chemotrophs, and autotrophs [15-17].

The annotated metagenome from the 2013 sample shows genes consistent with both chemoheterotrophs and photoautotrophs allowing for both aerobic and anaerobic respiration. Genes that are part of pathways that perform simple sugar metabolism, such as glycolysis and the citric acid cycle, are well-expressed as are genes consistent with more complex cycling of these sugars, including the Entner-Doudoroff pathway, glyoxylate cycle, ethylmalonyl-CoA cycle, and the polyhydroxybutyrate (phb) cycle. Photosynthetic genes like the Calvin cycle and photorespiration are also present.

The sulfur oxidation (sox) system, arsenite oxidase (aox) and Cyc2 are also present allowing for oxidation of thiosulfate, arsenite, and Fe(II), respectively, which both detoxifies these metals and allows bacteria to yield energy. Acid-resistance genes, heat shock operons, multidrug efflux pumps, and exporters of Cu(I), Co(II), Ni(II), Zn(II), and Cd(II) are also present.

The annotated metagenome from the 2017 samples, which exhibited greater biodiversity, contained similar genes. Notably, photosynthetic genes are absent in both mud and sulfur samples, and Cyc2 is absent in the sulfur sample from the 2017 field campaign.

Discussion: Analysis of the metagenome and 16S rRNA genes from both 2013 and 2017 samples suggests the *Acidiphilium* genus is dominating the microbial environment, even with changing lake chemistry. As it was surprising to find life in Laguna Caliente in 2013, it was equally as surprising that life was found here four years later, despite phreatic and phreatomagmatic eruptions which have the potential to sterilize the lake.

Another surprising result was that there are many ways for these organisms to produce energy despite there being an abundant energy source, sulfur, in the environment when utilizing the sox system. We provide three potential theories for these intriguing findings.

Our first theory is that Laguna Caliente is a carbon limiting environment (cf Mars), and thus these various pathways are adaptations to survive for carbon starvation. Our second theory is that because many survival genes rely on primary or secondary transport to function, a high energy yield requiring multiple pathways is always required for survival in this lake.

Our third, and most eccentric, theory calls to question how, regardless of the adaptations present, any microorganism could survive in this environment. We consider that microbes are transported to the periphery of Laguna Caliente via water-level fluctuations. Then, an eruption event occurs, effectively sterilizing this lake. Life on the periphery survives by utilizing complex carbon cycling and survival genes, such as the phb cycle, and is then transported back to the lake via groundwater fluctuations or rainfall. This cycle is then repeated over time conferring long-duration survival in a volcanic environment. This theory answers the outstanding questions from this study: 'Why is complex sugar cycling highly expressed in this sample?' and 'How could microbes survive these eruptions?'

Re-analysis of the 2013 and 2017 samples are currently underway with the goal of narrowing the scope of this study to better answer these central questions. Additionally, analysis of the samples from the November 2019 campaign, after the 2017 major eruption events, is currently underway.

Relict Martian hydrothermal systems were likely as dynamic and at times as hostile to life as Laguna Caliente is, yet these environments might be the key to understanding if and how life on Mars existed. Life would need to be able to adapt and adjust to live in varying conditions, like life in Laguna Caliente.

As discussed in [5], Mars analog acid-sulfate hydrothermal systems in our survey have ranged in diversity as a response to both environmental dynamics and physical extremes. Laguna Caliente marks an end member of our survey by exhibiting extreme temperature, pH, and ion concentrations that all drastically fluctuate.

This study sought to find how a sample of low biodiversity could survive in a highly dynamic environment, which is a probable constraint in a Martian setting. We have shown how life on Earth is able to live in the most extreme and dynamic Mars-like environments. It is probable that these mechanisms, complex carbon cycling and energy metabolism, is essential to survival in this type of environment. Continuing studies will better assess this hypothesis to both determine the extremes of life on Earth and provide insights on speculative Martian microbiology that will aid in the search for life on the Red Planet.

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