TEMPORAL AND METAGENOMIC STUDIES OF AN EXTREME MARTIAN ANALOG ECOSYSTEM: POÁS VOLCANO, COSTA RICA. Justin Wang1, N. Dragone2, G. Avard3 and B. M. Hynek1,4 1Laboratory for Atmospheric and Space Physics, University of Colorado Boulder, USA, 2Dept. of Environmental and Evolutionary Biology, University of Colorado Boulder, 3OVSCORI, National University of Costa Rica, 4Department 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 Mars’ relict hydrothermal systems [1-4; 5, this conference]; it also hosts Laguna Caliente, an ultra acidic sulfurous crater lake. Of the environments on Mars that may have harbored life, hydrothermal acid-sulfate systems show promise in their ability to sustain life throughout much of Mars’ geologic history [1]. Laguna Caliente presents one of the most dynamic terrestrial environments with temperatures ranging from near-ambient to boiling, a pH range of -1 to 1.5, a wide range of chemicals and redox potential, and magmatic eruptions since 2017.

Microbiology field samples were taken in 2013 in order to assess the habitability in analogous martian acid-sulfate hydrothermal systems that were likely just as dynamic and challenging to life [6]. After a surprising result suggesting a monaculture in the *Acidiphilium* genus, field samples were taken again in 2017 and 2019 to study temporal changes in the microbial composition and to conduct metagenomic “shotgun” sequencing in order to understand how life survives in this extreme environment on Earth and to assess habitatbility on early 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]. The lake also possesses a variety of S and Fe redox chemistry. The large range of sulfur oxidation states in this lake comprises a complex sulfur cycle which could provide metabolic options for chemotrophic Archaea and Bacteria [7].

Poás Volcano Field Campaigns: Three field campaigns were conducted to study the microbiology of Laguna Caliente. Our sampling in November 2013 (temp. = 45°C, pH = 0.3) showed the lake microbiology community as a veritable monoculture in the *Acidiphilium* genus [6]. Sampling in March 2017 found little change in the microbial community even with 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 [6,8]. Sampling from our November 2019 field campaign marks the first-time microbial sampling of a newly-formed Laguna Caliente had been done, and analysis is 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.

Briefly, the V4 region of the 16S rRNA gene was amplified and then sequenced on an Illumina MiSeq platform at CU Boulder. Sequences were then demultiplexed, forward and reverse reads were merged, and quality-filtered with QIME and UPARSE. Counts of sequences matching clusters for each sample were made by mapping de-multiplexed sequences against *de novo* constructed databases. Using the RDP classifier with a threshold of 0.5, taxonomy was assigned to each taxon and sequences were aligned to known isolates using the BLASTn algorithm [9] and NCBI 16S rRNA gene database. These protocols were detailed in [6].

Metagenomic sequencing of both the 2013 and 2017 samples used the Nextera XT DNA Library Prep Kit and a NovaSEQ 6000 Sequencing System with paired-end 150 cycle 2x150bp format by the Genomics and Microarray Core at CU. Sequence results were processed and annotated using the SUPER-FOCUS pipeline, a homology based tool that uses a reduced SEED database to assign subsystem identity and function [10]. Taxonomic diversity was assessed using Metaxa2.

**Fig. 1.** The active Poás crater and Laguna Caliente during the November 2019 Field Campaign. Arrow indicates the biological sampling site.

Microbiology Results: From the November 2013 field campaign, ~98% of all 16S rRNA gene reads...
clustered in a single OTU within the *Acidiphilium* genus [6]. Results from the March 2017 field campaign showed little change in the microbial composition with the vast majority of the sequence reads clustered in the same single OTU within the *Acidiphilium* genus similar to the November 2013 sample. One difference between the two analyses was that a minor component of sequence reads in the March 2017 sample mapped to *Bacteroidetes*, which were likely washed in from surrounding soils.

The *Acidiphilium* species generally prefer pH from ~1.5-5.5 and temperatures ranging from ~17˚C-45˚C [11], 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 [12-14].

The analysis of the metagenomics sequencing was consistent with the 16S rRNA gene analysis showing that the sample is almost exclusively in the *Acidiphilium* genus. There was little change between the metagenome of the November 2013 and March 2017 samples; the most notable difference was that the November 2013 sample exhibited a higher abundance of genes that aided in its survival in more extreme conditions.

Analysis of the metagenome showed genes and adaptations consistent with both chemoheterotrophs and photoautotrophs allowing for both aerobic and anaerobic respiration. Sulfur oxidation is well-expressed, but there are also genes consistent with the utilization of complex sugar molecules, including the ethylmalonyl-CoA cycle, polyhydroxybutyrate cycle, and Entner-Doudoroff pathway. Gene regions associated with glycolysis, the pentose phosphate pathway, the citric acid cycle, and photosynthetic pathways like the Calvin cycle and photorespiration are also present. The bacterium utilizes many mechanisms to survive in Laguna Caliente that maintain a higher pH in the cell and export many toxic metals that are present in the lake, including a variety of membrane transport proteins.

**Discussion:** The analysis of the metagenome and 16S rRNA genes from both 2013 and 2017 samples suggested a veritable monoculture in the *Acidiphilium* genus, even with changing lake chemistry. As it was surprising to find a monoculture after the initial field campaign, it is also surprising to see that the monoculture remained, even at a brief period of more mild conditions. As Laguna Caliente is extremely dynamic with its fluctuating temperatures, changing pH, and frequent phreatic eruptions, it is likely that any other species not suitable for the more extreme conditions that were present in the November 2013 campaign would likely perish after a drastic environmental condition change. Eruptions, influx of heavy metals, and changing temperature or pH are common, making it possible that only this bacterium can withstand these changes.

Metagenomic analysis of the samples presents a complex process in how the bacterium survives in these conditions. Our initial analysis shows that this organism has many pathways in which it produces and stores energy and utilizes carbon, allowing for respiration in both aerobic and anaerobic conditions. This allows for survival through the range of dynamic conditions that Laguna Caliente exhibits. We propose that the organism also utilizes these many pathways to produce energy because it needs an immense amount of ATP in order to operate the many survival genes found in this bacterium. Survival genes were found including but not limited to genes that raise the pH inside the cell and genes that export toxic metals in Laguna Caliente out of the cell.

Further analysis of these mechanisms as well as the isolation of the single genome of the bacterium from the metagenome, which has been treated as a single genome due to the low biodiversity, is ongoing. Additionally, samples from the November 2019 campaign are being analyzed in order to characterize the microbial community at the incipient Laguna Caliente, which recently reformed as eruptive activity has waned.

Relict martian hydrothermal systems were likely as dynamic and at times as hostile to life as Laguna Caliente is; nevertheless, these environments might be the key to understanding if and how life on the Red Planet existed. Life on Mars would need to be able to adapt and adjust to live in varying conditions. We have shown through our bacterium how life on Earth is able to live in the most extreme and dynamic environments that exhibit martian-like conditions. Life in Laguna Caliente uses various pathways and methods to survive pushing the limits of life on Earth and perhaps also providing a gateway to life on Mars.

**References:**


