

LACK OF MICROBIAL DIVERSITY IN AN EXTREME MARS ANALOG SETTING: POÁS VOLCANO, COSTA RICA. Brian M. Hynek¹⁻², K. L. Rogers³, M. Antonovich¹, G. Avaró⁴ and G. E. Alvarado⁵, ¹Laboratory for Atmospheric and Space Physics, University of Colorado Boulder, USA, ²Department of Geological Sciences, University of Colorado Boulder, ³Earth and Environmental Sciences, Rensselaer Polytechnic Institute, ⁴OVSICORI, National University of Costa Rica, ⁵Centro de Investigaciones Geológicas, Red Sismológica Nacional, Universidad de Costa Rica. Contact: hynek@lasp.colorado.edu

Introduction: The Poás volcano in Costa Rica has been studied as a Mars geochemical analog environment, since both the style of hydrothermal alteration present and the alteration mineralogy are consistent with Mars' relict hydrothermal systems [1-4]. The site hosts an active volcano, with high temperature fumaroles (up to 980°C) and an ultra acidic sulfurous lake. This lake, Laguna Caliente, is one of the most dynamic environments on Earth with frequent phreatic eruptions, temperatures ranging from near-ambient to almost boiling, a pH range of -1 to 1.5, and a wide range of chemistries and redox potential. Martian acid-sulfate hydrothermal systems were likely similarly dynamic and equally challenging to life. Thus, Laguna Caliente represents a setting to assess the habitability of life in similar environments on early Mars and also explore the limits of life on Earth.

Poás Volcano and Laguna Caliente: The Poás volcano is a basaltic andesite stratovolcano centered at 10°11' 49.36"N, 84°13' 47.19"W in the Central Cordillera of Costa Rica. Poás has been active throughout the Holocene with ongoing eruption at the time of this writing. Even in times of quiescence, phreatic to phreatomagmatic eruptions are common and include mostly 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 [1-4]. The lake also possesses a variety of S and Fe redox chemistry.



Fig. 1. The active Poás crater and Laguna Caliente. Arrow represents the biological sampling site.

November 2013 Field Campaign: During our sampling Laguna Caliente exhibited a pH of 0.29 and temperature of 45°C. The lake fluids showed very strong enrichment in the major cations (in decreasing abundance: Al³⁺, Fe(T), Ca²⁺, Na⁺, Mg²⁺, K⁺, and Si²⁺), with Al³⁺ at >110,000 ppm and Si²⁺ at >6,000 ppm. SO₄²⁻ and Cl⁻ were the major anions, at >57,000 ppm and >14,000 ppm, respectively. In addition to sulfate (SO₄²⁻), dissolved sulfite (SO₃²⁻) and thiosulfate (S₂O₃²⁻) were also detected. Together with the elemental sulfur which makes up the lake bottom, this large range of sulfur oxidation states represents a complex sulfur cycle that could provide a myriad of metabolic options for the wide variety of chemotrophic Archaea and Bacteria known to take advantage of sulfur redox chemistry in natural waters [5].

Methods: Briefly, lake fluid along with entrained sediment was collected aseptically in duplicate near the shore in sterile 50 mL Falcon tubes and immediately frozen. DNA was extracted using the MoBio PowerMax Soil kit following manufacturer's instructions. DNA from multiple extractions of the duplicate sample was pooled and concentrated with Amicon Ultra-4 spin filters. For sequence-based analyses of 16S rRNA genes, we used the approaches described previously [6]. The V4 region of the 16S rRNA gene was PCR-amplified using barcoded primers. Products from duplicate PCR reactions for each sample, as well as "no template" and "DNA extraction" negative controls, were pooled, cleaned, and normalized using the ThermoFisher Scientific SequelPrep Normalization Plate kit, and sequenced on an Illumina MiSeq platform using v2 500-cycle paired-end kits in the Fierer Lab at CU Boulder.

Sequence reads were processed as previously described [7]. Briefly, sequences were de-multiplexed, forward and reverse reads were merged, and quality-filtered with QIIME and UPARSE. A database of ≥97% similar sequence clusters was constructed in USEARCH (Version 8; [8]). For the Laguna Caliente lake water and sediment sample, there was a total of 20,239 reads that passed quality filtering. De-multiplexed sequences were mapped against the *de novo* constructed databases to generate counts of sequences matching clusters (i.e. taxa) for each sample. Taxonomy was assigned to each taxon using the RDP classifier with a threshold of 0.5, trained on the

Greengenes database (for prokaryotes; V. 13_8; [9]), and sequences were aligned to known isolates using the BLASTn algorithm [10] and NCBI 16SrRNA database.

Microbiology Results: Taxonomy and distribution of the microbial population in the Laguna Caliente sample are shown in Table 1. Remarkably, across all of the Laguna Caliente 16S rRNA gene reads, ~98% of all of the sequence reads clustered in a single OTU within the genus *Acidiphilium*. The remaining OTUs, each representing <0.5% of the sequenced amplicons, typically identified within the α -Proteobacteria. The partial sequencing of the V4 region of the 16S rDNA resulted in only 230 base pairs to use for comparison to existing genomic databases, thus a species level identification is not possible. However, comparing the sequence of the dominant OTU (OTU 63) to known isolates using the BLASTn algorithm and the 16S isolate database showed that this sequence was most closely related (100% ID and 100% query coverage) to several species of *Acidiphilium*, including *A. angustum*, *A. rubrum*, and *A. acidophilum*.

Members of the *Acidiphilium* genus are aerobic acidophilic bacteria, with several obligate heterotrophs and at least one facultative chemoautotroph [e.g., 11–12]. Most *Acidiphilium* species prefer pH from ~1.5–5.5 and temperatures ranging from ~17°C–45°C [13]; conditions less extreme than Laguna Caliente. Several *Acidiphilium* species, including, *A. rubrum*, *A. cryptum*, and *A. acidophilum*, are known to oxidize elemental sulfur (and often other forms of reduced sulfur) for growth [e.g. 14]. Additionally, a number of the members of genus *Acidophilium* are able to reduce Fe(III) [e.g. 15].

Discussion: One of the most surprising aspects of our investigation was the remarkable, and nearly unprecedented, low diversity found in Laguna Caliente. With 98% of the amplicons falling within a single OTU, we argue that Laguna Caliente hosted a veritable monoculture (that is, an ecosystem with very limited taxonomic diversity), at least at the time of sampling. Such occurrences of low diversity in natural ecosystem are quite rare. Laguna Caliente is one of the most extreme habitats on our planet, and may well represent

the edge of the habitable range, likely due to the extremely low pH, coupled to fluctuating temperatures and volcanic dynamics, as well as very high concentrations of dissolved ions.

Mars likely did not have long lived clement conditions or high enough solar influx to develop photosynthesis. It is also likely that there were not abundant organic carbon sources, especially given the highly oxidizing atmosphere through most of its time. Thus, chemolithautotrophic organisms might have been some of the first to appear on Mars (and Earth) and had the ability to be sustained.

Given the chemical make-up of Mars (Fe and S enrichments) and the prevalence of early hydrothermal systems, deeply rooted, sulfur- (and Fe) oxidizing Bacteria and Archaea are possibly the most likely organisms that would have originated and evolved on Mars. Microbes like the one species in Laguna Caliente are perhaps the type of organism we should search for in future astrobiological missions to Mars.

Our full methods and results will appear in Hynek et al., *Astrobiology*, August 2018 print date; with earlier Open Access online.

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Table 1. Distribution and taxonomy of OTUs obtained from Laguna Caliente. Only OTUs with > 0.1% of the total reads are included.

OTU ID	# Quality Controlled Reads	% of Total Reads	Taxonomy (family/genus)	BLAST Alignment Results			
				Type Strain of Top Match	Accession #	Sequence ID	Sequence Coverage
OTU 63	19781	97.73	Acetobacteraceae/ Acidiphilium	Acidiphilium angustum strain KLB	NR 025850	100	100
OTU 2111	96	0.47	Acetobacteraceae/ Acidiphilium	Acidiphilium multivorum strain AIU301	NR 074327	95.65	100
OTU 214	67	0.33	Acidobacteraceae/NA	Granulicella sapmiensis strain S6CTX5A	NR 118023	98.81	100
OTU 5708	58	0.29	Acetobacteraceae/ Acidiphilium	Acidiphilium angustum strain KLB	NR 02580	96.84	100
OTU 2941	57	0.28	Acidomicrobiaceae/NA	Ferrimicrobium acidiphilum strain T23	NR 041798	90.12	99