

# Lack of Microbial Diversity in an Extreme Mars Analog Setting: Poás Volcano, Costa Rica

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## Abstract

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. The site hosts an active volcano, with high-temperature fumaroles (up to 980°C) and an ultra-acidic 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. The microbiology existing within Laguna Caliente was characterized for the first time, with sampling taking place in November, 2013. The diversity of the microbial community was surveyed via extraction of environmental DNA from fluid and sediment samples followed by Illumina sequencing of the 16S rRNA gene. The microbial diversity was limited to a single species of the bacterial genus *Acidiphilium*. This organism likely gets its energy from oxidation of reduced sulfur in the lake, including elemental sulfur. Given Mars' propensity for sulfur and acid-sulfate environments, this type of organism is of significant interest to the search for past or present life on the Red Planet. Key Words: Mars astrobiology—Acid-sulfate hydrothermal systems—Extremophiles—Acidic—High temperature—*Acidiphilium* bacteria. Astrobiology 18, 923–933.

## 1. Introduction and Motivation

**D**UE TO THE PREVALENCE of both liquid water and volcanic activity early in Mars' history (*e.g.*, Phillips *et al.*, 2001; Hynek *et al.*, 2010), it has been suggested that large-scale hydrothermal alteration was a common process resulting in extensive deposits of acid-sulfate alteration minerals (*e.g.*, Bibring *et al.*, 2005; Solomon *et al.*, 2005; Hynek *et al.*, 2013). Indeed, Mars shows evidence of rampant volcanism and magmatism during the Noachian epoch (>3.7 Ga) (*e.g.*, Phillips *et al.*, 2001; Carr and Head, 2010; Robbins *et al.*, 2011), and this time period also exhibited extensive interaction of the crust with abundant surface waters that formed the valley networks (*e.g.*, Hynek *et al.*, 2010). Given the lack of plate tectonics on Mars, individual volcanoes had intermittent

summit eruptions spanning nearly the entire 4 billion years of preserved geological history (Robbins *et al.*, 2011). At these locales, condensation of volcanic vapors and hot springs would have provided localized warm and moist, albeit acidic, habitats for life even while cold and dry conditions persisted globally. Similarly, at least 78 large impact basins ( $\geq 150$  km diameter) formed prior to  $\sim 3.5$  Ga (Robbins *et al.*, 2013), and the larger ones likely hosted hydrothermal systems for up to 10 million years (Abramov and Kring, 2005). Given that widespread surface waters were episodic and limited in time, volcanic and impact systems may represent the most habitable niches for life on Mars through time and should be primary targets for astrobiological exploration.

Widespread relict hydrothermal systems have been identified on Mars' surface based on the observed mineralogy

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and geomorphology data from orbiters and landers. The Spirit rover in Gusev crater detected sulfate and silica accumulations on the surface that suggest acid-sulfate alteration in fumarolic and/or hot spring environments next to a putative volcanic vent known as Home Plate (e.g., Squyres *et al.*, 2007, 2008; Yen *et al.*, 2008; Schmidt *et al.*, 2009; Ruff *et al.*, 2011). The large rift valley named Valles Marineris also shows mineral suites that include sulfates, hydrated silica, and phyllosilicates from putative regional-scale acid-sulfate hydrothermal alteration (Gendrin *et al.*, 2005; Chojnacki and Hynke, 2008; Weitz *et al.*, 2011; Thollot *et al.*, 2012; Marcucci *et al.*, 2013). Numerous volcanic edifices show morphological and mineralogical signs of hydrothermal activity. It has been hypothesized that radial valley networks on volcanic constructs formed via hydrothermal processes (Gulick, 1998, 2001; Tanaka *et al.*, 1998; Dohm and Tanaka, 1999; Hynke *et al.*, 2010; El Maarry *et al.*, 2012). Although most martian volcanoes are currently covered in dust, preventing orbital observations of elemental and mineralogical composition, Skok *et al.* (2010) identified numerous hydrated silica deposits on the flanks of a volcanic cone in the Nili Patera caldera that they attributed to hydrothermal alteration. Finally, acidic hydrothermal alteration of basalt has been proposed as one possible explanation for the extensive sulfate deposits at Meridiani Planum (McCollom and Hynke, 2005, 2006).

Hydrothermal environments provide sources of heat, energy, and water for life (e.g., Walter and Des Marais, 1993; Schulze-Makuch *et al.*, 2007; Hays *et al.*, 2017) and may represent a unique habitable niche on Mars, past or present (MEPAG, 2015), as well as on early Earth. For example, it has been repeatedly hypothesized that the last universal common ancestor (LUCA) of life on Earth was a thermophile or hyperthermophile (e.g., Nisbet and Sleep, 2001; Di Giulio, 2003; Gaucher *et al.*, 2008), likely capitalizing on iron-sulfur redox chemistry within hydrothermal settings (e.g., Martin and Russell, 2003; Wächtershäuser, 2006; Martin *et al.*, 2008; Weiss *et al.*, 2016). It is conceivable that Mars experienced a similar history, making terrestrial hydrothermal systems with similar water-rock interactions ideal analogs to investigate Mars' habitability.

Inherent to water-rock reactions in hydrothermal systems are chemical disequilibria among redox-sensitive species, including those that are common in microbial metabolisms (e.g.,  $\text{H}_2\text{S}$ ,  $\text{S}^0$ ,  $\text{SO}_4^{2-}$ , Fe(II), Fe(III),  $\text{CO}_2$ , CO,  $\text{CH}_4$ , and  $\text{H}_2$ ). In fact, most extant thermophiles are chemolithotrophs, exploiting these disequilibria to drive their metabolisms (e.g., Woese, 1987; Wächtershäuser, 2006). In many hydrothermal systems on subaerial volcanoes, hot and acidic fluids arise either from interaction of magmatic gases with meteoric-derived waters or local steam condensates with abundant  $\text{SO}_2$ ,  $\text{SO}_3$ ,  $\text{H}_2\text{S}$ , and HCl. Moreover, when these systems host microbial communities, active mineralization of hydrothermal deposits such as silica sinter can encase and permineralize microbes to preserve a suite of textures, biofabrics, microfossils, and organic compounds (e.g., Hays *et al.*, 2017). However, the dynamic nature of these environments often leads to a heterogeneous distribution of biosignatures that are vulnerable to oxidizing conditions that limit preservation of organic material.

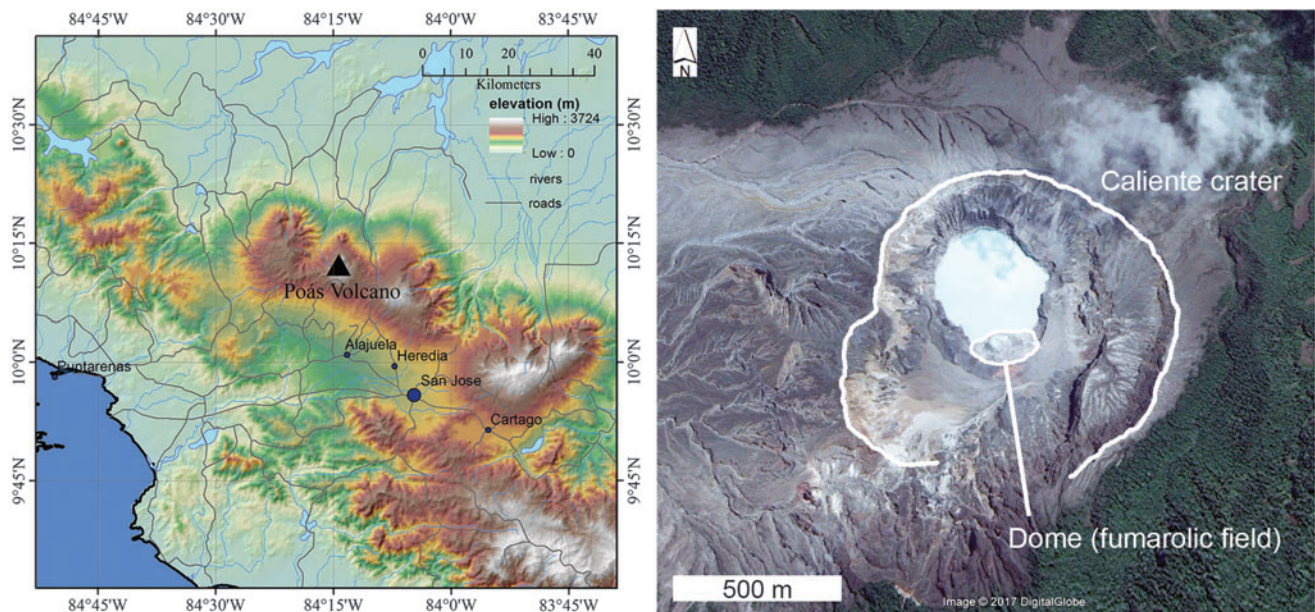
Many of these acidic and sulfate-rich systems on Earth host thriving microbial ecosystems (e.g., Hedlund *et al.*, 2016; Johnson and Aguilera, 2016). For example, Río Tinto,

Spain, often studied as a Mars analog, is an acidic (mean pH value 2.3) Fe-S system with a high concentration of heavy metals and high level of microbial diversity (e.g., Amils *et al.*, 2007). While the microbial community is dominated by eukaryotes, both sulfur- and iron-oxidizing bacteria—such as *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans*, *Leptospirillum* spp., *Acidiphilium* spp.—were also present (e.g., González-Toril *et al.*, 2003; Amils *et al.*, 2007). In general, sulfur-dependent organisms span a range of environments and include thermophiles, hyperthermophiles, acidophiles, and neutrophiles. Furthermore, S-based metabolisms are ubiquitous throughout the tree of life and are quite diverse, taking advantage of the wide range of sulfur redox states ( $-2$  in  $\text{H}_2\text{S}$  to  $+6$  in  $\text{SO}_4^{2-}$ ) (Amend *et al.*, 2004). Sulfur-dependent microbes thriving in acidic hydrothermal systems must be adapted to both high temperatures, which can denature proteins and nucleic acids (e.g., Rothschild and Mancinelli, 2001), as well as low pH, which is often neutralized in the cytoplasm by highly efficient proton pumps (e.g., Johnson and Aguilera, 2016). Among the S-dependent microbes commonly found in acidic hydrothermal environments on Earth are members of *Acidianus*, *Acidithiobacillus*, and *Sulfolobus* (e.g., Johnson and Aguilera, 2016). Despite the apparently harsh conditions, modern acid-sulfate hydrothermal systems on Earth host a wide variety of microbial communities adapted to these conditions, suggesting that similar relict martian systems could have also provided habitable niches across the planet throughout its history.

With this in mind, understanding the habitability at Mars' hydrothermal acid-sulfate systems is paramount to understanding the Red Planet's astrobiological potential through time. One commonly used method for assessing Mars' habitability is the study of Mars analog environments on Earth that replicate the chemistries, environmental conditions, and mineralogies inferred for the martian examples. One Mars-relevant analog representing hydrothermal acid-sulfate alteration in a volcanic setting is Poás volcano, Costa Rica. The active summit crater hosts fumarole fields and a warm to hot lake called Laguna Caliente. Poás volcano has previously been used as a model of planetary volcanism either to compare it with the scarps around the Olympus Mons volcano (Borgia *et al.*, 1990) or to compare its sulfur-rich eruptions with those of Jupiter's moon Io (Oppenheimer and Stevenson, 1989; Oppenheimer, 1992). This volcanic crater has also been studied as a Mars geochemical analog, based on the similarities between alteration minerals and the inferred geochemical pathways of hydrothermal alteration at Poás and on Mars (Hynke *et al.*, 2014; Black *et al.*, 2015, 2016; Rodríguez and van Bergen, 2015, 2017; Black and Hynke, 2017). However, the microbiology of the lake system had never been detailed. It is also an end-member extreme environment (in terms of ultra-acidity and widely varying temperature and chemistry; see Section 2) that allows us to test the limits of life. Here, we present results of a microbial community profile within Poás' Laguna Caliente from a field excursion in November 2013 and discuss the implications for similar settings on ancient Mars.

## 2. Poás Volcano and Laguna Caliente

The Poás volcano is a structurally complex, basaltic andesite stratovolcano (Prosser and Carr, 1987) centered at



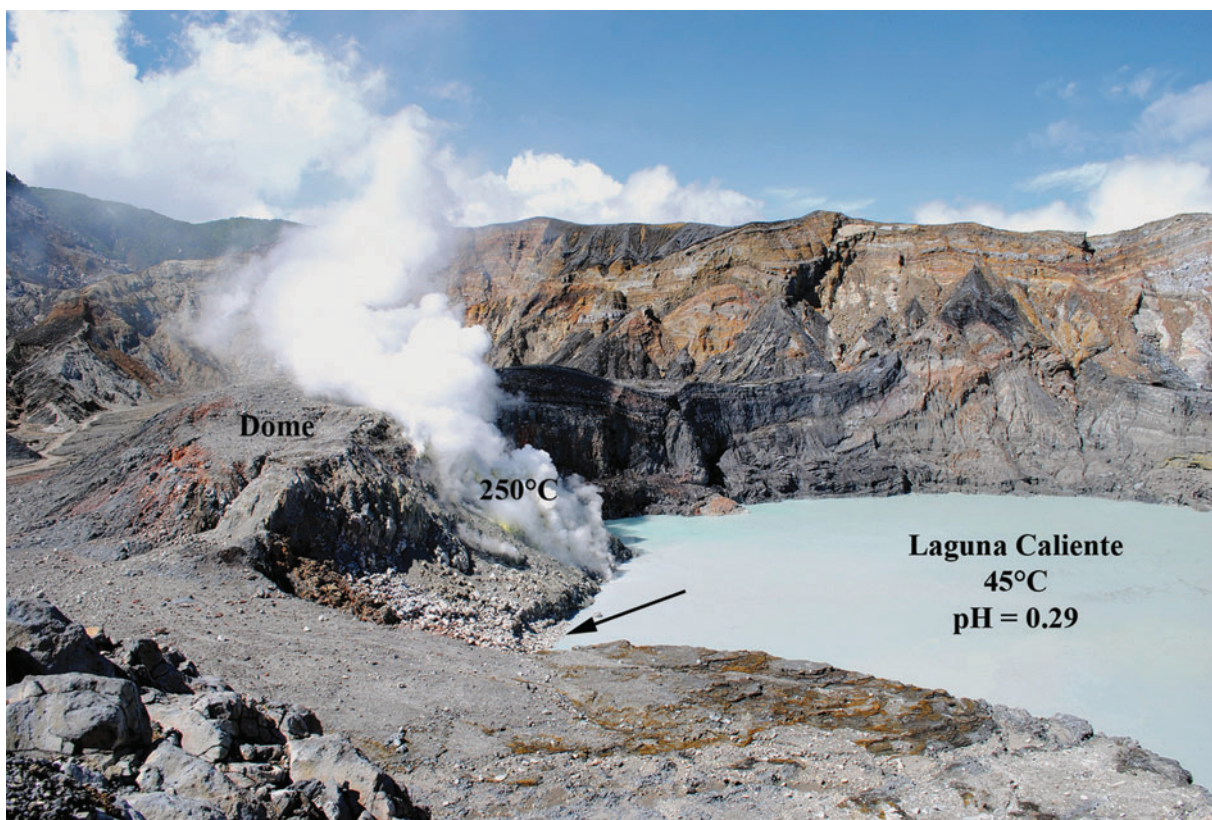
**FIG. 1.** (left) Location map of central Costa Rica and Poás volcano. (right) Google Earth image of Laguna Caliente and surrounding features of interest. Note the lack of vegetation to the west of the crater due to volcanic emissions and dominant easterly winds.

10°11'49.36"N, 84°13'47.19"W in the Central Cordillera of Costa Rica (Fig. 1). The elevation of the active crater rim at the tourist lookout is 2575 meters above sea level (m.a.s.l.), but the general crater rim is at about 2450 m.a.s.l., with the active crater lake 130 m below. Unaltered Poás andesitic basalts collected in this study from the 1953–1955 magmatic activity are primarily composed of plagioclase, with minor orthopyroxene and olivine, and are compositionally similar to Thermal Emission Spectrometer (TES) measurements of the martian surface (McSween *et al.*, 2009; Black and Hynek, 2017). The most recent eruptive period at Poás has occurred throughout the Holocene (Prosser and Carr, 1987), and the volcano has been active through historical times with significant eruptions in 1834, 1910, 1953–1955, and 2017 (ongoing at the time of this writing). These involved vulcanian and strombolian activity, phreatic to phreatomagmatic eruptions, and occasional lava flows (Prosser and Carr, 1987; de Moor *et al.*, 2016). The phreatic to phreatomagmatic eruptions are common even during times of otherwise quiescence and include mostly unpredictable geyserlike expulsions from the crater lake, although recent efforts for prediction have improved (de Moor *et al.*, 2016). The interval between these events can be hours to years and can send ash, lake mud, and elemental sulfur hundreds of meters into the air. The last phreatic eruption prior to our November 20, 2013, sampling of the crater occurred on June 3, 2013, and included a SO<sub>2</sub>-rich gas plume that rose 1 km above the crater (Global Volcanism Project, 2013). The ongoing magmatic eruptive phase began in April 2017 and has been of styles very similar to surtseyan, vulcanian, and strombolian eruptions.

Laguna Caliente is an extremely dynamic, ultra-acidic, ambient to hot lake in the active Poás crater (Fig. 2). A shallow magma body (~500 m depth) (Rymer and Brown, 1989; Rymer *et al.*, 2000) and associated rising magma dendrites and magmatic gases interact with meteoric and

lake water at very shallow depths (Rymer *et al.*, 2000), leading to the frequent phreatic activity. Lake chemistry is highly variable and controlled by both input and dissolution/hydrolysis of magmatic volatiles and partial to wholesale dissolution of rock, enriching the lake in rock-forming elements (Rowe *et al.*, 1992). Volcanic gases (HCl and H<sub>2</sub>S and SO<sub>2</sub>, which hydrolyze to H<sub>2</sub>SO<sub>4</sub>) contribute to the extreme acidity of the lake with pH through time ranging from -1 to 1.5 (Table 1). Times of higher lake temperature and lower pH lead to increased contribution from HCl and SO<sub>2</sub> relative to H<sub>2</sub>S (Rowe *et al.*, 1992). Fluctuations in lake water temperature measured over decades range from ~19°C to 96°C, and total dissolved solids spanned a few to hundreds of grams per kilogram (Table 1) (*e.g.*, Rowe *et al.*, 1992). Higher temperatures and acidity also correlate with higher dissolved ion concentrations in the lake fluid (Table 1). The color of the lake ranges from bluish green, when ferrous iron concentration is higher in the lake waters due to increased injection of H<sub>2</sub>S, to yellow, when more SO<sub>2</sub> is added and/or elemental sulfur is actively entrained in lake convection cells. Additionally, the lake volume changes drastically due to meteoric fluctuations and, more importantly, volcanic activity. The lake has had an estimated maximum volume of >1 km<sup>3</sup> but was also entirely drained in 1953 and 1989 (Oppenheimer and Stevenson, 1989; Rowe *et al.*, 1992; Rymer *et al.*, 2000). Images from July 14, 2017, show that the lake has once again disappeared. At the lake bottom exists a molten elemental sulfur deposit up to a few meters thick (*e.g.*, Oppenheimer and Stevenson, 1989; Rowe, 1994); this is the source of the sulfur roiling in the lake's convective cells. Some of this elemental sulfur often floats as a froth on the lake surface (Fig. 3).

Fumaroles have been active in discrete locales within the active crater throughout historical times. Since the 1953–1955 eruptions, fumarole activity has been concentrated mostly along the dome area, extending down to the lake shore and underneath the fluids. The dome was formed during the



**FIG. 2.** The active Poás crater and Laguna Caliente. Arrow represents the biological sampling site.

1953 eruption when the lake had entirely drained (Rymer *et al.*, 2000), and the majority of the cone has since been lost into the current crater lake during subsequent eruptions. The fumarole temperatures and gas composition have varied drastically through time in the dome area and rose to 900°C in 1981, implying that magma was just a few meters below the surface (Stevenson, 1993). Such an episode repeated in 2011. In April 2017, the dome area was entirely obliterated by ongoing eruptions.

These fumaroles have provided alteration minerals that are akin to a variety of relict hydrothermal sites on Mars (Hynek *et al.*, 2014; Black *et al.*, 2015, 2016; Rodríguez and van Bergen, 2015, 2017; Black and Hynek, 2017). The basaltic andesites alter rapidly in the high-temperature, ultra-acidic outgassing at fumaroles. The plagioclase minerals dissolve first in the parent rocks and combine with Fe from the orthopyroxenes and olivines to produce an

abundance of natroalunite ( $\text{AlNa}_3(\text{SO}_4)_2(\text{OH})_6$ ) and Fe-rich natroalunite, where  $\text{Fe}^{3+}$  replaces the  $\text{Al}^{3+}$  in the mineral formula. This mineral is a common alteration product in Mars analog experiments (McCullom *et al.*, 2013; Marcucci and Hynek, 2014), in hydrothermal Mars analog field sites in Nicaragua and Iceland (Hynek *et al.*, 2013, 2014; Marcucci *et al.*, 2013; Black and Hynek, 2017), and in corollary geochemical models (McCullom *et al.*, 2013; Marcucci and Hynek, 2014). McCullom *et al.* (2014) showed that jarosite and Fe-bearing natroalunite are indistinguishable in Mössbauer spectroscopy and that the jarosite detections on Mars by the Opportunity rover may in fact be Fe-bearing alunite. Jarosite is also a relatively common alteration product at Poás, as is gypsum, and these materials are both found in putative relict hydrothermal deposits on Mars (*e.g.*, Squyres *et al.*, 2008; Thollot *et al.*, 2012; Marcucci *et al.*, 2013). Hydrated silica is another common alteration mineral that is

**TABLE 1.** LAGUNA CALIENTE FLUID SAMPLE DATA FROM HISTORICAL RECORDS AND THIS STUDY

	Temp. (°C)	Density (g/cc)	pH	[Na <sup>+</sup> ]	[K <sup>+</sup> ]	[Ca <sup>2+</sup> ]	[Mg <sup>2+</sup> ]	[Fe]T	[Al <sup>3+</sup> ]	[Br <sup>-</sup> ]	[F <sup>-</sup> ]	[Cl <sup>-</sup> ]	[SO <sub>4</sub> <sup>2-</sup> ]
Summary of 39 Laguna Caliente fluid samples collected from 1980 to 1990 (Rowe <i>et al.</i> , 1992)													
Min.	38	1.04	-0.87	430	160	0	500	780	1,170	0	970	16,500	36,900
Max.	90	1.31	0.26	3,840	2,620	4,800	3,650	11,500	18,300	1,820	25,400	129,000	286,000
Avg.	67	1.13	-0.29	1,610	1,009	384	1,521	3,428	5,752	241	5,098	41,701	114,669
Fluid data from Laguna Caliente collected November 20, 2013, for this study													
	45	not measured	0.29	26,401	14,644	28,330	18,384	59,350	110,888	23	569	14,381	57,230*

All concentrations are in mg/kg.

\*Some of the sulfate reported in this sample is sulfite and also thiosulfate.



**FIG. 3.** Clumps of elemental sulfur floating on Laguna Caliente observed during the March 2017 field season. The longest clump is  $\sim 6$  cm in length.

widespread at Mars hydrothermal analog sites (*e.g.*, Hynek *et al.*, 2011, 2013, 2014) and on inferred similar paleoenvironments on Mars (*e.g.*, Skok *et al.*, 2010; Ehlmann *et al.*, 2011; Ruff *et al.*, 2011; Marcucci *et al.*, 2013). The high-temperature silica phase of tridymite is also widespread around Poás fumaroles and was recently detected in Gale crater by the Curiosity rover (Morris *et al.*, 2016). Dissolved Fe at Poás leads to abundant hematite and occasional goethite, which are common products across relict hydrothermal systems on Mars, such as within Gusev crater (*e.g.*, Morris *et al.*, 2005; Ming *et al.*, 2008). Elemental sulfur is ubiquitous around the active fumaroles, forming large tabular crystals and occasionally solidified flow ribbons of previously molten sulfur. Collectively, the suite of alteration minerals at Poás crater and identified across Mars at relict hydrothermal sites shows strong correlation, indicating the high temperature and highly acidic geochemical processes occurring at Poás are quite relevant to understanding past hydrothermal processes on Mars.

### 3. Laguna Caliente and Fumarole Characteristics during the November 20, 2013, Campaign

During the 2013 sampling expedition, a broad area of the dome adjacent to Laguna Caliente was active with fumaroles (Fig. 2). The highest measured temperature was  $\sim 250^{\circ}\text{C}$ , and fumarole condensates had a pH of  $-1$ . The

fumarole field extended from near the top of the dome down to and under the lake. HCl was present in the fumarolic gas along with the dominant  $\text{SO}_2$ . Alteration minerals were collected and analyzed with XRD, XRF, VNIR spectroscopy, and Raman spectroscopy (Hynek *et al.*, 2014; Black *et al.*, 2016; Black and Hynek 2017), and general results are presented in Section 2. The lake muds at the location of biological sampling were analyzed with XRD, and mineralogy consisted predominately of elemental sulfur, moderate amounts of cristobalite, and minor amounts of alunite and tridymite.

The pH of the lake was 0.29, and the temperature was  $45^{\circ}\text{C}$ , relatively similar to long-term averages (Table 1). Lake fluids were collected in sterile Falcon tubes and then filtered through  $0.2\ \mu\text{m}$  Sterivex filters and stored in the dark until analysis by ICP-MS and ICP-OES for cations, anions, and 62 additional element concentrations (Table 1). The highly acidic Poás lake waters showed very strong enrichment in the major cations (in decreasing abundance:  $\text{Al}^{3+}$ ,  $\text{Fe}(\text{T})$ ,  $\text{Ca}^{2+}$ ,  $\text{Na}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{K}^+$ , and  $\text{Si}^{2+}$ ), with  $\text{Al}^{3+}$  at  $>110,000$  ppm and  $\text{Si}^{2+}$  at  $>6,000$  ppm.  $\text{SO}_4^{2-}$  and  $\text{Cl}^-$  were the major anions, at  $>57,000$  ppm and  $>14,000$  ppm, respectively. In addition to sulfate ( $\text{SO}_4^{2-}$ ), dissolved sulfite ( $\text{SO}_3^{2-}$ ) and thiosulfate ( $\text{S}_2\text{O}_3^{2-}$ ) were also detected. Together with the elemental sulfur often seen floating in the lake, 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 (e.g., Friedrich, 1997; Amend *et al.*, 2004). Note that in our 2013 field campaign, no elemental sulfur was present on the lake surface, and thus it was not sampled directly, although on a recent trip in March 2017, abundant sulfur was observed floating on the lake's surface. The elemental sulfur is present as hollow spheres, up to a millimeter in size (allowing flotation), and XRD analysis shows  $S^0$  is the only mineral present. Microbiology analyses of these sulfur clumps is ongoing.

#### 4. Microbiology Methods

Lake fluid along with entrained sediment was collected aseptically in duplicate near the shore in sterile 50 mL Falcon tubes and immediately stored on water ice. These samples were frozen to  $-4^{\circ}\text{C}$  within hours and transferred to a  $-80^{\circ}\text{C}$  freezer within 4 days. DNA was extracted by 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 (Millipore, Bellerica, MA). For sequence-based analyses of 16S rRNA genes, we used the approaches described previously (Carini *et al.*, 2016). The V4 region of the 16S rRNA gene was PCR-amplified with barcoded primers (515F, 5'-GTGCCAGCMGCCGCGGTAA-3' and 806R, 5'-GGACTACVSGGGTATCTAAT-3'; Caporaso *et al.*, 2010). Products from duplicate PCR reactions for each sample, as well as "no template" and "DNA extraction" negative controls, were pooled, cleaned, and normalized with the ThermoFisher Scientific SequelPrep Normalization Plate kit, and sequenced on an Illumina MiSeq platform by using v2 500-cycle paired-end kits in the Fierer Lab at CU Boulder.

Sequence reads were processed as previously described (Leff *et al.*, 2015). Briefly, sequences were de-multiplexed, forward and reverse reads were merged, and quality-filtered with QIIME (Caporaso *et al.*, 2010) and UPARSE (Edgar, 2013). A database of  $\geq 97\%$  similar sequence clusters was constructed in USEARCH (Version 8; Edgar, 2010) by merging paired end reads, using a "maxee" value of 0.5 when quality filtering sequences, de-replicating identical sequences, removing singleton sequences, clustering sequences after singleton removal, and filtering out cluster

representative sequences that were not  $\geq 75\%$  similar to any sequence in the Greengenes database (for prokaryotes; Version 13\_8) (McDonald *et al.*, 2011). 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 by using the RDP classifier with a threshold of 0.5 (Wang *et al.*, 2007), trained on the Greengenes database, and sequences were aligned to known isolates with the BLASTn algorithm (Altschul *et al.*, 1990) on the NCBI 16S rRNA database.

#### 5. Microbiology Results

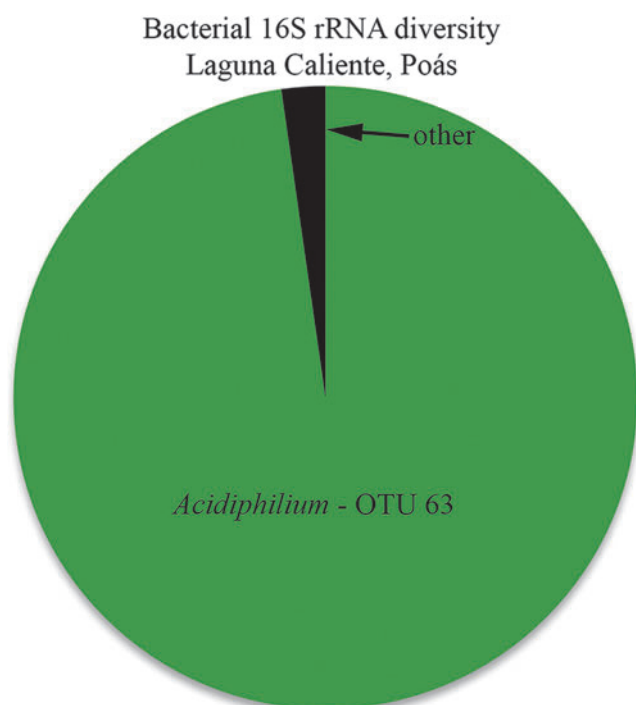
Taxonomy and distribution of the microbial population in the Laguna Caliente sample are shown in Table 2 and Fig. 4. Remarkably, across all the Laguna Caliente 16S rRNA gene reads,  $\sim 98\%$  of all the sequence reads clustered in a single operational taxonomic unit (OTU) within the genus *Acidiphilium* (Fig. 4 and Supplementary Material, available at <http://online.liebertpub.com/suppl/doi/10.1089/ast.2017.1719>). The remaining OTUs each represented  $< 0.5\%$  of the sequenced amplicons and typically identified within the  $\alpha$ -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* (formerly *Thiobacillus acidophilus* [Guay and Silver, 1975; Hiraishi *et al.*, 1998]) (see Table 2 and Supplementary Material).

Generally, members of the *Acidiphilium* genus are aerobic acidophilic bacteria, with several obligate heterotrophs and at least one facultative chemoautotroph (Harrison, 1981; Wichlacz *et al.*, 1986; Dopson and Johnson, 2012). Most *Acidiphilium* species prefer pH from  $\sim 1.5$  to 5.5 and temperatures ranging from  $\sim 17^{\circ}\text{C}$  to  $45^{\circ}\text{C}$  (Schippers, 2007), and members of this genus have been identified in and isolated from acid mine drainage (including the Mars analog

TABLE 2. DISTRIBUTION AND TAXONOMY OF OTUS OBTAINED FROM LAGUNA CALIENTE

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	19,781	97.73	Acetobacteraceae/ <i>Acidiphilium</i>	<i>Acidiphilium angustum</i> strain KLB	NR 025850	100	100
OTU 2111	96	0.47	Acetobacteraceae/ <i>Acidiphilium</i>	<i>Acidiphilium multivorum</i> strain AIU301	NR 074327	95.65	100
OTU 214	67	0.33	Acidobacteraceae/NA	<i>Granulicella sapmiensis</i> strain S6CTX5A	NR 118023	98.81	100
OTU 5708	58	0.29	Acetobacteraceae/ <i>Acidiphilium</i>	<i>Acidiphilium angustum</i> strain KLB	NR 02580	96.84	100
OTU 2941	57	0.28	Acidomicrobiaceae/NA	<i>Ferrimicrobium acidiphilum</i> strain T23	NR 041798	90.12	99

Only OTUs with  $> 0.1\%$  of the total reads are included.



**FIG. 4.** Bacterial diversity at Laguna Caliente, Poás crater, Costa Rica. See text for details.

Río Tinto, Spain: González-Toril *et al.*, 2003; Amils *et al.*, 2007), with a limited number from hydrothermal environments and coal mine drainage (Harrison, 1981; Wichlacz *et al.*, 1986; Lane *et al.*, 1992; Kishimoto *et al.*, 1995; Martin-Uriz *et al.*, 2011). 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 (Rohwerder and Sand, 2003; Rohwerder *et al.*, 2003; Ghosh and Dam, 2009), and a number of isolates from this genus contain the *soxXY-ZABCD* gene cluster, used in the well-known *sox* (sulfur-oxidation) metabolic pathway for bacteria (Liu *et al.*, 2016). Additionally, a number of the members of genus *Acidiphilium* are able to reduce Fe(III) (*e.g.*, Lane *et al.*, 1992; Küsel *et al.*, 1999; Johnson *et al.*, 2017).

## 6. Discussion and Implications for Mars

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 (*i.e.*, 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. Even Iron Mountain, California, which hosts acid mine drainage with pH as low as  $-1.5$ , contains much more diverse microbial communities that include all three domains of life (*e.g.*, Edwards *et al.*, 1999, 2000). 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 moderate but fluctuating temperatures and volcanic dy-

namics, as well as very high concentrations of dissolved ions. Yet in our broad survey of Costa Rican hot springs as Mars analogs, we found one other monoculture at Borinquen Hot Springs source pool located on the side of the Rincón de la Vieja volcano (see Supplementary Material). Here, more moderate pH conditions,  $\text{pH}=4.8$ , coupled with a much higher temperature,  $\sim 92^\circ\text{C}$ , contribute to the limited habitability of this system. Thus, as shown by others (*e.g.*, Rothschild and Mancinelli, 2001; Hedlund *et al.*, 2016), temperature can be another important environmental variable in acid-sulfate hydrothermal systems. The microbial diversity was assessed at Borinquen following the methods described above. At this site, a hyperthermophilic archaeon was the sole representative in the thermal pool and was classified with the genus *Pyrobaculum* (see Supplementary Material). Members of this genus grow either chemolithoautotrophically by sulfur reduction or organotrophically by sulfur respiration.

Results of our taxonomic analysis strongly suggest that the species hosted in Laguna Caliente is a member of the genus *Acidiphilium*, which contains several isolates that exploit sulfur redox in their metabolism. The thermal conditions of the lake fall within the known tolerance of *Acidiphilium* species ( $\sim 17\text{--}45^\circ\text{C}$ ); however, the reported pH tolerance of this genus ( $\sim 1.5\text{--}6.5$ ) (*e.g.*, Schippers, 2007) is higher than that measured at the time of sampling (0.29) or in historical records (Table 1). Thus, it remains possible that Laguna Caliente at Poás volcano hosts a novel species of *Acidiphilium* that has a lower pH tolerance than other isolates within this genus. Nonetheless, the most closely related species within this genus can be used to infer potential metabolisms of the microbial population within the lake. Two of the most closely related species, *A. acidophilum* and *A. rubrum*, oxidize elemental sulfur and/or thiosulfate (*e.g.*, Rohwerder and Sand, 2007), a metabolic strategy that is consistent with the conditions at Laguna Caliente, where there is an abundance of  $\text{S}^0$  and other reduced sulfur compounds (sulfite and thiosulfate) within the lake waters. The deepest phylogenetic branches of both Bacteria and Archaea include sulfur-based chemolithoautotrophic extremophiles (Woese, 1987; Fuchs *et al.*, 1996). Russell and Hall (1997) used these data to argue that sulfur chemolithotrophy might have been the earliest self-sustaining metabolism. Others have argued that anaerobic iron oxidation might have supported Earth's earliest organisms (*e.g.*, Russell and Hall, 1997; Martin *et al.*, 2008). An iron-based metabolism is also consistent with our results, as some members of *Acidiphilium*, and many other acidophiles, are known to exploit Fe-based metabolisms (Lane *et al.*, 1992; Küsel *et al.*, 1999).

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, chemolithoautotrophic organisms might have been some of the first to appear on Mars (and Earth) and had the ability to be sustained. Given the abundance of Fe and S on the martian surface, and the preponderance of evidence supporting acid-sulfate alteration in volcanic hydrothermal systems, microbes like the one species in Laguna Caliente are perhaps the type of organism we should search for in future astrobiological missions to Mars.

The Poás crater lake environment is immensely dynamic, ultra-acidic, and exhibits rapid changes in water volume, chemistry, and temperature (from near ambient to near boiling). Given the challenges for sustained, habitable environments on Mars through time, it is this level of diversity we might expect. From the abundance of jarosite and other sulfates found on the surface, combined with desiccation/freezing to concentrate salts and widespread volcanic activity, it is hypothesized that many of the habitable niches at Mars' surface through time were of low pH, moderate to high temperature, and highly dynamic. The mineralogy and geochemical processes occurring at Poás volcano are quite similar to Mars (as seen in the resultant alteration mineralogy: Hynek *et al.*, 2014; Black *et al.*, 2015, 2016; Rodríguez and van Bergen, 2015, 2017; Black and Hynek, 2017). The geochemical parameters and environmental conditions are also similar to many of the hydrothermal environments inferred for Mars, which, as argued above, may represent the most habitable niches through time on Mars. Given the chemical makeup of Mars (Fe and S enrichments) and the prevalence of early hydrothermal systems, deeply rooted, sulfur- (and Fe-) oxidizing bacteria and archaea are perhaps the most likely organisms that would have originated and evolved on Mars. The characteristics of these microbes deserve further scrutiny, which will aid in the search for life on the Red Planet.

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#### Disclosures

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**Abbreviation Used**

OTU = operational taxonomic unit