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Thermoplasmatales and sulfur-oxidizing bacteria dominate the microbial community at the surface water of a CO₂-rich hydrothermal spring located in Tenorio Volcano National Park, Costa Rica

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Abstract

Here we report the chemical and microbial characterization of the surface water of a CO₂-rich hydrothermal vent known in Costa Rica as Borbollones, located at Tenorio Volcano National Park. The Borbollones showed a temperature surrounding 60 °C, a pH of 2.4 and the gas released has a composition of ~97% CO₂, ~0.07% H₂S, ~2.3% N₂ and ~0.12% CH₄. Other chemical species such as sulfate and iron were found at high levels with respect to typical fresh water bodies. Analysis by 16S rRNA gene metabarcoding revealed that in Borbollones predominates an archaeon from the order Thermoplasmatales and one bacterium from the genus *Sulfurimonas*. Other sulfur- (genera *Thiomonas*, *Acidithiobacillus*, *Sulfuriferula*, and *Sulfuricurvum*) and iron-oxidizing bacteria (genera *Sideroxydans*, *Gallionella*, and *Ferrovum*) were identified. Our results show that CO₂-influenced surface water of Borbollones contains microorganisms that are usually found in acid rock drainage environments or sulfur-rich hydrothermal vents. To our knowledge, this is the first microbiological characterization of a CO₂-dominated hydrothermal spring from Central America and expands our understanding of those extreme ecosystems.

Keywords Borbollones · Wet mofette · CO₂-rich thermal · Archaea · Sulfur-oxidizing bacteria · Tenorio Volcano National Park

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Introduction

The analysis of microbial communities in extreme environments aims to understand how organisms have adapted to environmental conditions unfavorable for life. One of these extreme environments is the hydrothermal springs: fissures

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in the surface of the Earth's crust through which gases emerge. These sites are located in areas with volcanic activity and their waters are usually rich in dissolved minerals. Costa Rica is a country with a large volcanic influence. Its territory is traversed by four mountain ranges, with most volcanoes in the Guanacaste and central volcanic ranges (Castellón et al. 2013, Alvarado Induni 2011). In the complex basaltic-andesitic volcanic massif of Tenorio (volcanic mountain range of Guanacaste) multiple environmental niches of volcanic origin such as thermal waters, acidic rivers or streams rich in sulfur compounds and silicates can be found (Castellón et al. 2013). One of those niches is located in the tourist visitation area of the Tenorio Volcano National Park, popularly known as the Borbollones or Hervideros (see Fig. 1 and the supplementary video S1) (Alvarado Induni 2011). The Borbollones shows a continuous release of gas specifically in the area of the Rio Roble (Roble river) and a geochemical survey conducted in 2011 (Capecchiacci et al. 2015) indicates that the gas is composed of 90–95% vol/vol CO₂, 0.04–0.08% vol/vol H₂S, 0.037–0.039% vol/vol CH₄ and 1.85–2.32% vol/vol N₂.

Worldwide, several ecosystems have been reported in which CO₂ is naturally released at high levels. At these extreme sites termed mofettes (Šibanc et al. 2014), CO₂ migrates from the lithosphere to the surface water (wet mofettes) or the soil (dry mofettes) (Kämpf et al. 2013; Krauze et al. 2017). In addition to the high levels of CO₂ these environments are characterized by the presence of other gases such as H₂S, H₂, and CH₄, similar to what was found in the Borbollones (Oppermann et al. 2010; Beulig

et al. 2015; Frerichs et al. 2013). From a microbiological point of view, the vast majority of studies have been conducted in dry mofettes (Oppermann et al. 2010; Frerichs et al. 2013; Sáenz de Miera et al. 2014; Beulig et al. 2015) whereas only few studies are available on wet mofettes (Krauze et al. 2017). These studies have indicated the presence of diverse microbial communities mainly composed of anaerobic, microaerophilic, acidophilic, and chemolithotrophic bacteria. For example, Oppermann et al. (2010) described CO₂-utilizing methanogenic archaea, Geobacteraceae and sulfate-reducing bacteria in a dry mofette in Lateral Caldera in the Vulsinian volcanic district in central Italy and Beulig et al. (2015) reported a microbial community dominated by methanogens and acidobacteria in a wetland mofette in the Czech Republic. Analysis of the microbial composition along a CO₂ gradient in a dry mofette located in Campo de Calatrava, Spain revealed that the relative abundance of members of the phylum Chloroflexi (genera *Thermogemmatispora*, *Ktedonobacter*, and *Thermomicrobium*) increased with increasing CO₂ flux, whereas the relative abundance of members of phyla Acidobacteria, Verrucomicrobia and Gemmatimonadetes decreased (Sáenz de Miera et al. 2014). Also, Frerichs et al. (2013) reported on changes in the microbial community as a function of the spatial distribution along the CO₂ vent in a pastured field in Germany where Geobacteraceae decreased and sulfate-reducing prokaryotes increased in relative abundance in the vent centre. Interestingly, Krauze et al. (2017) described the microbial communities in surface and subsurface water in a wet mofette in the Czech Republic. The authors concluded

Fig. 1 The wet mofette known as Borbollones at Tenorio Volcano National Park, Costa Rica. Borbollones is located in Tenorio Volcano National Park (Guanacaste Mountain Range), on the Rio Roble, beside the touristic path of the park. In the mofette the release of gas is observed and a strong odour of sulfur compounds is perceptible



that microbial communities at the surface water from mofettes of central Europe are in large proportions similar to the deep biosphere of geysers and marine thermal vents, such as black smokers, that is, environments rich in microorganisms involved in the sulfur metabolism.

So far, all the microbiological studies on mofettes have been conducted in Europe or North America. In the current study we investigated in detail the chemistry and microbiological composition of the surface water of the Costa Rican mofette known as Borbollones. Our results show that the surface water of this wet mofette has a chemical and microbial composition similar to acid rock drainage (ARD) environments or sulfur-rich hydrothermal vents, where microorganisms belonging to the sulfur cycle are predominant. These results constitute the first report of microbiota of mofettes in Costa Rica and Central America and contribute to our knowledge on microbial communities inhabiting ecosystems with extraordinary levels of CO₂.

Materials and methods

Sampling and field measurements

All necessary permits for sampling waters were obtained from the National System of Conservation Areas (SINAC) of the Ministry of Environment and Energy (MINAEC) of Costa Rica (Resolution No. 097-2014-ACAT). For chemical analysis, samples of water were collected on October 2015, February 2017 and January 2018, directly in the bubbling zone (N10.70585 W84.99415; video S1 in Supplementary Information). The temperature and pH were measured in the field with a dissolved oxygen meter Model 50B (Yellow Springs Instrument Company Inc, Ohio, USA). Gas samples were collected with a submerged inverted funnel connected to an evacuated glass flask (250 mL, “Giggenbach bottle”) containing NaOH solution (50 mL, 4 M), following the methods of Giggenbach and Goguel (1989). For chemical analysis of cations and anions, surface water samples were collected in clean glass bottles, chilled over ice and stored at 4 °C until analysis. For analysis of microbial communities, three samples (1 L each) of surface water were collected in 2015 January directly at the bubbling zone, into clean and sterile glass bottles and processed within less than 24 h.

Chemical analysis

Gas samples were analyzed at OVSICORI-UNA following the methods outlined by de Moor et al. (2013). Head space gases (H₂, He, Ar, O₂, N₂, CH₄, CO) were analyzed on a gas chromatograph (Agilent 7890) equipped with two molecular-sieve columns (30 m, HP). Samples were introduced via a vacuum line with Ar or H₂ as carrier gases.

Gases were measured with a thermal-conductivity detector (TCD) on the Ar side of the GC, and via a second TCD and a flame-ionization detector (FID; configured in series with the TCD) on the H₂ side of the system. The liquid portion of the sample (i.e., components dissolved in NaOH solution) was analyzed for CO₂ content with an alkalinity titration, and for total sulfur (all H₂S) after oxidation to sulfate [using H₂O₂, followed by 10× dilution and filtration using cartridges (Dionex OnGuard IHH) to remove hydroxyl groups] on an ion chromatograph (Dionex) at OVSICORI-UNA.

Total DNA isolation, construction of 16S rRNA gene libraries and Illumina sequencing

Water samples were passed through a vacuum filtration system under sterile conditions using a membrane filter (pore size 0.22 µm; Millipore, GV CAT No GVWP04700). To prevent rupture, another filter membrane (pore size 0.45 µm; Phenex, Nylon Part No AF0-0504) was placed below. The upper filter was collected and stored at –80 °C until processing. The DNA was extracted from aseptically cut pieces of the filter with a DNA isolation kit (PowerSoil[®], MoBio, Carlsbad, CA, USA). Cell lysis was accomplished in two steps of 30 s bead beating (FastPrep-24, MP Biomedicals, Santa Ana, CA, USA) at 5.5 m s⁻¹. For the construction of microbial 16S rRNA amplicon libraries, the V5–V6 hyper-variable regions were PCR-amplified from a pool of DNA extracted from the 3 samples with universal primers 807F and 1050R (Bohorquez et al. 2012). The barcoding of the DNA amplicons and the addition of Illumina adaptors were conducted with PCR as described previously (Camarinha-Silva et al. 2014; Burbach et al. 2016). The PCR-generated amplicon libraries were subjected to 250nt paired-end sequencing on an Illumina MiSeq (San Diego, CA, USA).

Bioinformatic and phylogenetic analysis of 16S rRNA gene(s) amplicon data

Raw MiSeq sequences were quality-filtered (moira.py script with default parameters and the-paired flag; Puente-Sánchez et al. 2016). Filtered sequences were subsequently analyzed (mothur version 1.31.2; Schloss et al. 2009), as recommended by Kozich et al. (2013). Briefly, the sequences were aligned to a combination of silva.archaea and silva.bacteria databases (Quast et al. 2013), screened for chimeras (UCHIME; Edgar et al. 2011) and clustered at 97% similarity with an average-neighbour algorithm. The most abundant taxa (i.e., OTU with relative abundance > 0.05% of total reads sampled) was retrieved and classified against the Ribosomal Database Project (RDP) reference using the Classify tool (version 4.3.3; Wang et al. 2007). The results from this initial classification were individually verified and curated manually using the RDP Seqmatch tool.

Additionally, a phylogenetic analysis of the two Thermoplasmatales OTUs was carried out as follows. The 16S rRNA gene sequences from validly described type strains and isolates belonging to phylum Euryarchaeota, as well as some uncultured representatives closely related to our OTU candidates were retrieved using the RDP SeqMatch tool and by BLASTn against the curated 16S ribosomal RNA sequence database of NCBI. The sequences were aligned by means of the SINA web-based tool (Pruesse et al. 2012) and the alignments were used for the reconstruction of the phylogenetic tree with MEGA7 software (Kumar et al. 2016) and the maximum-likelihood method based on the general time-reversible model. In total, 100 bootstrap replications were calculated to ensure the robustness of the results.

PCR amplification of *mcrA*. The *mcrA* gene was amplified using the degenerated primer pair ML-F (5'-GGTGGT GTMGGATTCACACARTAYGCWACAGC-3') and ML-R (5'-TTCATTGCRTAGTTWGGRTAGTT-3') (Luton et al. 2002). Each PCR mixture (50 μ L) contained the reaction buffer (GoTaq colorless buffer, 10 μ L, 5 \times), DNA polymerase (GoTaq, 0.25 μ L, 5 U/mL), deoxynucleoside triphosphate mixture (1 μ L, 10 mM), primers ML-F and ML-R (1 μ L each, 1 μ M), and DNA sample (3 μ L, 7 ng/ μ L). Due to the degenerate nature of primers, the polymerase chain reaction (PCR) was performed on a Mastercycler ep Gradient S thermal cycler (Eppendorf, Hamburg, Germany) using a slow ramp in temperature (0.1 $^{\circ}$ C s $^{-1}$) between the annealing and extension cycles, as described by (Luton et al. 2002). As a positive control, we prepared a mixture of DNA isolated from methanogenic archaea (including *Methanobrevibacter smithii*, *Methanocella arvoryzae*, *Methanococcus vannielii*, *Methanomicrobium mobile*, *Methanopyrus kandleri*, *Methanosaeta concilii*, *Methanomassiliicoccus luminyensis*, *Methanothermobacter marburgensis* and *Methanococcoides burtonii*). A negative control was prepared with all components of the PCR reaction except the DNA sample. PCR products were analyzed on 2% agarose gels using standard protocols.

Results and discussion

Physico-chemical analysis of the Borbollones hydrothermal vent

The Borbollones hydrothermal vent has a temperature surrounding 60 $^{\circ}$ C and a pH of 2.4 (Table 1). This temperature is within the range reported for CO $_2$ -rich springs (Pauwels et al. 1997; Giammanco et al. 2007). The Borbollones presents a lower pH than those reported in similar environments in which values between 3.5 and 4.7 are characteristic (Beaubien et al. 2008; Krauze et al. 2017). The analysis of the released gas reveals a composition of almost exclusively CO $_2$ (97% vol/vol) with smaller levels of H $_2$ S (0.07% vol/vol), N $_2$ (2.3% vol/vol) and CH $_4$ (0.12% vol/vol). Despite the small levels of H $_2$ S, its presence at the site is evident and readily identifiable as its odour is perceptible from several metres around the mofette. The composition of the gas obtained here is similar to that reported for samples taken in 2011 which indicates a highly stable chemical composition (Capecchiacci et al. 2015). The chemical analysis of filtered samples (Table 1) revealed the presence of sulfate, chloride, and iron at concentrations much higher than those typical of freshwater rivers. The sulfate level was particularly high (0.25 g/L), and characteristic of ARD environments (He et al. 2007; Sánchez-Andrea et al. 2012; Jones et al. 2015; Arce-Rodríguez et al. 2017). Sulfate in these environments is produced by the biotic or abiotic oxidation of sulfur compounds (e.g., pyrite or H $_2$ S). The high sulfate levels hence indicate that the low pH of Borbollones is mainly a product of the oxidation of H $_2$ S or sulfides to sulfate. Furthermore, considering that the product of the CO $_2$ dissolution is carbonic acid (a weak acid), it is very reasonable to speculate that its contribution to the acidification of the environment is minimal with respect to that generated by sulfur oxidation.

In summary, the physico-chemical data confirm that Borbollones is a wet mofette. The high sulfate concentrations, the presence of iron minerals and the acidity indicate that

Table 1 Physical properties and chemical composition of the wet mofette Borbollones

Property/component	Value/concentration	Property/element/ion (mg/L)	Property/component
Temperature/ $^{\circ}$ C	60.4 \pm 0.1	Flouride	2.70 \pm 0.06
pH	2.4 \pm 0.1	Chloride	175.4 \pm 0.2
CO $_2$ (% vol/vol)	96.9 \pm 0.5	Bromide	ND
H $_2$ S (% vol/vol)	0.07 \pm 0.02	Sulfate	253 \pm 10
CH $_4$ (% vol/vol)	0.12 \pm 0.02	Nitrate	4.99 \pm 0.01
N $_2$ (% vol/vol)	2.3 \pm 0.4	Iron	2.86 \pm 0.37
O $_2$ (% vol/vol)	0.54 \pm 0.08	Calcium	75 \pm 19
H $_2$ (% vol/vol)	0.0026 \pm 0.0015	Sodium	19.2 \pm 0.9
Ar (% vol/vol)	0.05 \pm 0.01	Potassium	3 \pm 1

biochemical processes similar to those in the ARD environments or sulfur-rich hydrothermal vents are present in the Borbollones. The resulting chemistry in this type of environments is usually the product of the metabolic activity of microorganisms (Arce-Rodríguez et al. 2017). From the 16S rRNA gene (s) data and the literature, it is possible to identify the microbial community of the Borbollones as well as infer some of the metabolic activities that explain the chemical composition of the ecosystem. Next, based on 16S rRNA gene(s) data, the microbiota of the Borbollones as well as its putative metabolism will be discussed.

Analysis of microbial communities in the surface waters of Borbollones

A total of 110,424 read pairs were generated and subjected to pair merging and quality filtering. The remaining 76,769 high-quality sequences were clustered into 128 OTUs belonging to thirteen phyla from both bacteria and archaea were identified (Fig. 2a and Supplementary Table S1). The largest number of sequence reads (72.05%) was assigned to the phylum Proteobacteria and in particular members of the Betaproteobacteria class (34.13%) were highly abundant, followed by Epsilon- (20.95%) and Gammaproteobacteria (13.24%). A significant portion of the total sequence reads belonged to two phylotypes from phylum Euryarchaeota (21.24%) of order Thermoplasmatales (unknown family).

The remaining bacterial and archaeal phyla were present only in relatively low amounts (> 1.50% each, see supplementary table S1). The classification by families (Fig. 2b) reveals that the most abundant was the Helicobacteraceae (~ 19.46%), followed by families Acidithiobacillaceae (~ 8.03%), Burkholderiales *incertae sedis* (~ 7.59%), Gallionellaceae (~ 4.58%), Comamonadaceae (~ 3.15%), Sulfuricellaceae (~ 3.08%) and Desulfovibrionaceae (~ 1.10%). The detected microbial community is formed almost exclusively by aerobic, microaerophilic or facultatively anaerobic microorganisms.

The detection of members of the archaea domain with the present method is known as a previous report of primers used in this work for the amplification of V5–V6 regions of 16S rRNA gene was able to amplify > 97% of the total archaeal sequences from the RDP database (version of 2008; Bohorquez et al. 2012). Specifically, we found two Thermoplasmatales sequence types, of which one (OTU RCBor_001) constitutes 17.50% of the total sequence reads. The second archaeal OTU (OTU RCBor_005) represents 3.74% of the microbial population (Supplementary Table S1). Members of the order Thermoplasmatales are described as facultatively anaerobic, thermoacidophilic, autotrophic or heterotrophic organisms (Huber and Stetter 2006). Members of this kind of microorganisms are also considered as extreme thermoacidophiles that generally grow at optimum temperatures of > 60 °C and at pH < 4 (Auernik

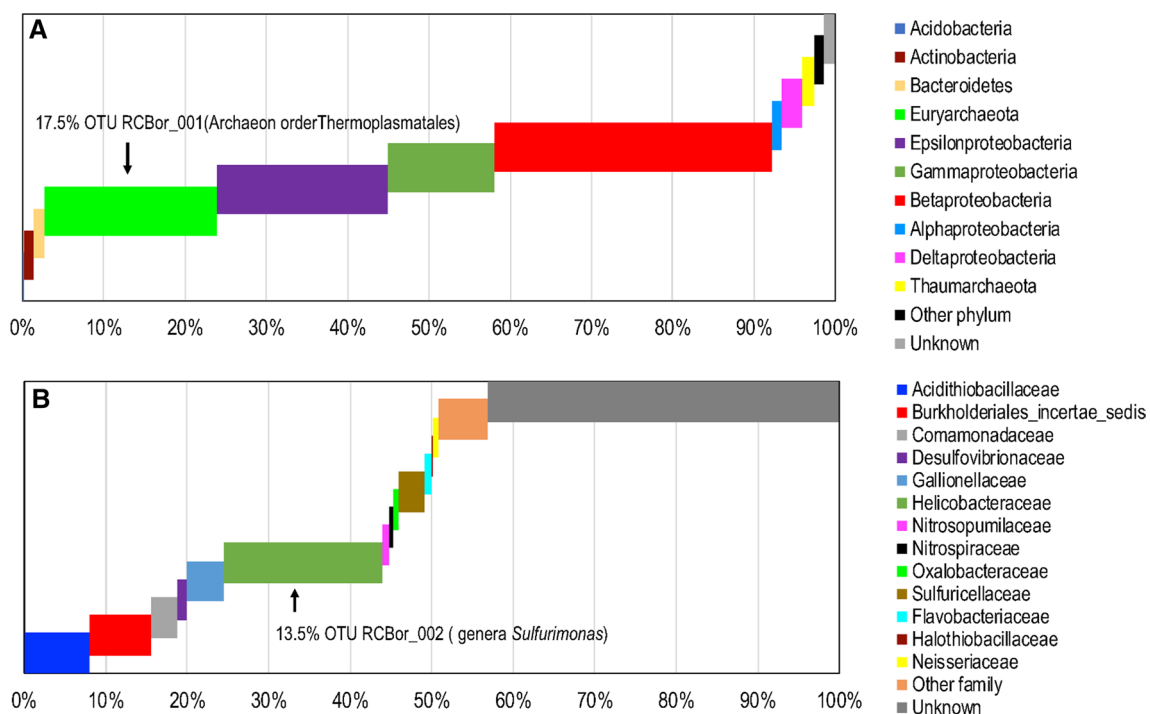


Fig. 2 Taxonomic composition at phylum and family level of the microbial community of the wet mofette Borbollones. The relative abundance of bacteria and archaea is shown (a) at the phylum and (b) at the family level

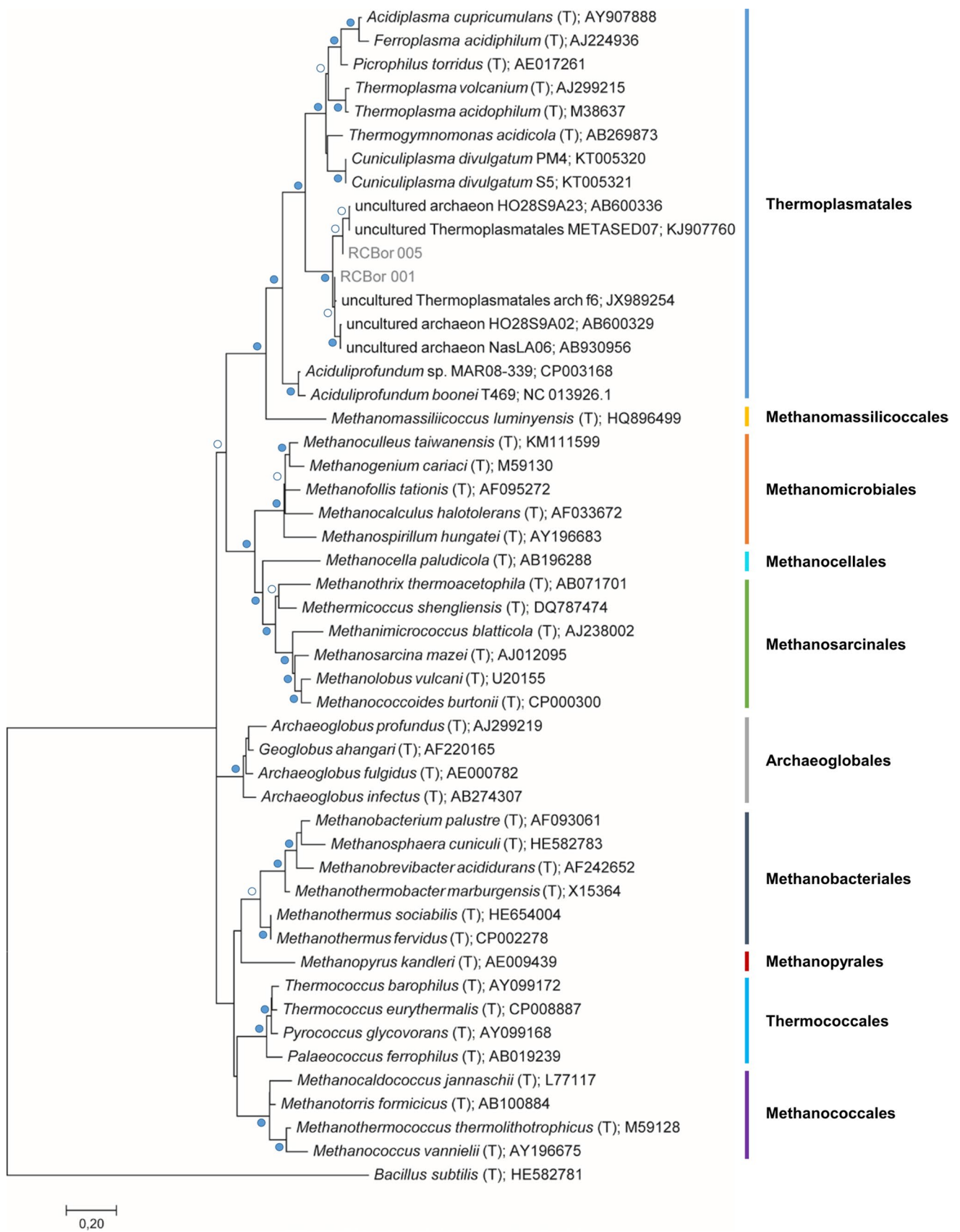


Fig. 3 Phylogenetic tree showing relationships of identified Thermoplasmatales (OTU RCBor_001 and OTU RCBor_005) and closely related members of the Euryarchaeota phylum. The tree was reconstructed using the maximum-likelihood method based on the general time-reversible model as described in Methods. Solid circles (filled circle) indicate bootstrap values ≥ 90 ; open circles (open circle) denote bootstrap values ≥ 70

et al. 2008), conditions which match perfectly with the niche observed in Borbollones (Table 1). The presence of Thermoplasmatales was previously reported in another CO_2 -rich environment by Oppermann et al. (2010). Other species of the order Thermoplasmatales have been isolated from hydrothermal springs (DeLong 1992; Yasuda et al. 1995; Takai and Horikoshi 1999) and soils or sediments within solfataric fields (Seegerer et al. 1988). The archaeal OTUs identified in Borbollones (RCBor_001 and RCBor_005) are phylogenetically related to a group of non-cultivated Thermoplasmatales (see Fig. 3) with the closest isolates being *Thermoplasma acidophilum* (Yasuda et al. 1995), *Thermoplasma volcanium* (Seegerer et al. 1988), *Thermogymnomonas acidicola* (Itoh et al. 2007), *Cuniplasma divulgatum* (Golyshina et al. 2016), and *Picrophilus torridus* (Schleper et al. 1996; Serour and Antranikian 2002). All these strains have been obtained from solfataric hydrothermal areas or acidic streamers containing sulfidic deposits, i.e., sulfur-rich environments which may indicate that the Thermoplasmatales identified in Borbollones could be involved in sulfur metabolism. Both oxidation and reduction processes of sulfur compounds have been described in archaea. Specifically, anaerobic elemental sulfur reduction has been reported in microorganisms from Thermoplasmatales order (Barton et al. 2014).

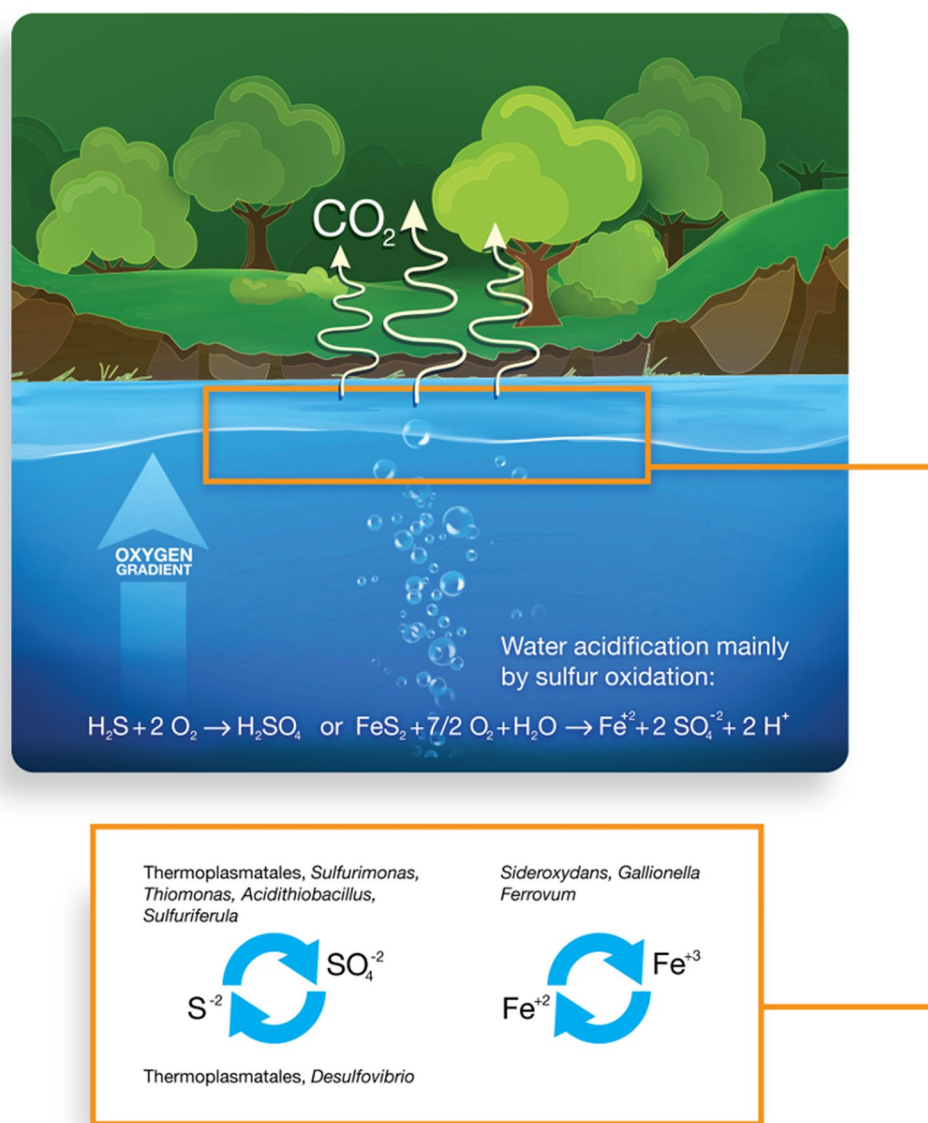
As shown in Fig. 3 the archaea identified in this study are only distantly related to methanogenic archaea. In accordance with the absence of sequences of methanogenic archaea in our samples, no amplification of the *mcrA* gene was obtained from DNA isolated from Borbollones samples (Fig. S1). This gene codifies for the α -subunit of methyl coenzyme M reductase, which has been established as a molecular marker for methanogenic archaea (Paul et al. 2012; Jiang et al. 2011) This contrasts most other studies on microbial communities in CO_2 -rich environments where methanogenic archaea could usually be identified (Beulig et al. 2015; Oppermann et al. 2010). However, the samples analyzed here originate from surface water where oxic conditions predominate. The chemical analysis of Borbollones (i.e., presence of CO_2 and methane) indicates that at anoxic zones of the mofette (i.e., in the maximum depth or the sub-soil) methanogens might in fact be present.

In addition to the identified Thermoplasmatales, the microbial composition of Borbollones showed a community with microorganisms usually found in ARD environments or sulfur-rich hydrothermal vents such as sulfur-, sulfide-, thio-sulfate- and iron-oxidizing bacteria. As mentioned above,

the most abundant family was Helicobacteraceae (~19.46%), in which is found the second most abundant microorganism in Borbollones, a bacterium of genus *Sulfurimonas* (~13.54%, OTU RCBor_002; see Fig. 2B and Supplementary Table S1). *Sulfurimonas* is a bacterial genus known for reducing nitrate and oxidizing sulfur and to date consists of four species (Labrenz et al. 2013). This genus is extremely versatile and is considered one of the most important organisms in the sulfur cycle. Microorganisms of this genus can be aerobic (Inagaki et al. 2003) aerotolerant, or facultatively anaerobic (Labrenz et al. 2013). The ecological niches of *Sulfurimonas* vary from deep-sea hydrothermal vents (Zhou et al. 2009; Akerman et al. 2013) to transition zones oxic/anoxic with sulfidic environments (Sievert et al. 2008). For *Sulfurimonas* species, optimal growth occurs chemolithoautotrophically with sulfide, S^0 , thiosulfate and H_2 as electron donors, and with nitrate, nitrite, and O_2 as electron acceptors, using CO_2 as a carbon source (Labrenz et al. 2013). As seen in Table 1, the chemical composition of Borbollones has all the necessary nutrients for an adequate growth of *Sulfurimonas* species (i.e., CO_2 , sulfide, nitrate, hydrogen, etc.) so its presence in high proportions in this hydrothermal vent is reasonable. Specifically, the taxonomic breadth of OTU RCBor_002 indicates that it is closely affiliated with *S. denitrificans* (Sievert et al. 2008), which was isolated for the first time from the Dutch Wadden Sea (Timmer-ten Hoor 1975). After that report, the bacterium has been isolated from several hydrothermal and marine habitats (Reysenbach et al. 2000; Huber et al. 2003; Zhang et al. 2009). Consistent to our results, Krauze et al. (2017) reported *Sulfurimonas* and *Sulfuricurvum* species as the main microorganisms in a CO_2 -dominated wet mofette in the Czech Republic. Other sulfur-oxidizing bacteria of genera *Acidithiobacillus* (~9.14%), *Thiomonas* (~6.69%), *Sulfuriferula* (~3.08%), *Sulfuricurvum* (~2.47%) and *Sulfurovum* (~1.06%) were identified. In addition to sulfur-oxidizing bacteria, a small portion of sulfur-reducing bacteria were found in the surface waters of Borbollones. Sulfate-reducing bacteria are strict anaerobes, so the small percentage of bacteria in the family Desulfovibrionaceae (~1.10%) possibly originates in the anoxic zone of Borbollones. Sulfate-reducing bacteria obtain energy by coupling the oxidation of organic compounds or H_2 to the reduction of sulfate, generating hydrogen sulfide (Camacho 2009). Taking together these results, in the Borbollones hydrothermal vent, microorganisms were identified with the metabolic activities necessary to complete the sulfur cycle: sulfur-oxidizing activity is carried out by Proteobacteria (*Sulfurimonas*, *Sulfuricurvum*) and sulfur-reducing activity could be carried out by thermophilic archaea (i.e., Thermoplasmatales) and *Desulfovibrio* bacteria (Fig. 4).

On the other hand, within genus *Acidithiobacillus* (family Acidithiobacillaceae) is found to be the third most abundant OTU in Borbollones (RCBor_003; ~5.56%),

Fig. 4 Cartoon of Borbollones representing the main transformations and microorganisms involved. The surface waters of the Borbollones are acidic, with high temperature, rich in iron and sulfate. The bubbling gas is almost exclusively made of CO₂. The microbiota of Borbollones is rich in species involved in sulfur and iron metabolism



an *Acidithiobacillus ferrooxidans*-like bacterium (Valdés et al. 2008). This chemolithotrophic bacterium is also capable of oxidizing sulfide to sulfate, coupling this reaction to iron (III) (under anaerobic conditions) or oxygen reduction (under aerobic conditions) (Suzuki et al. 1990; Pronk et al. 1992). The low pH of the Borbollones mainly obtained by sulfur oxidation, also favors the presence of this acidophilic bacterium. We consider that *A. ferrooxidans* is a key bacterium in the Borbollones ecosystem as it participates in both iron and sulfur metabolism, which is consistent with the chemistry of the wet mofette. In addition to Thermoplasmatales and sulfur-oxidizing bacteria, we identified the presence of iron-oxidizing (genera *Sideroxydans*, *Gallionella*, and *Ferrovum*), ammonia and nitrite-oxidizing bacteria (e.g., genus *Nitrospira*). Bacteria belonging to these genera are autotrophic iron oxidizers, which catalyse the oxidation of iron(II); their presence is

reasonable considering the levels of iron (~2.86 mg/L) measured in the Borbollones.

Conclusion

The hydrothermal vent known as Borbollones, located at Volcano Tenorio National Park, releases gas with ~97% vol/vol CO₂, and its waters present a chemical composition similar to those found in ARD environments, i.e., low pH (2.4) and large levels of sulfate. 16S rRNA gene(s) analysis reveals a microbial community dominated by aerobic, microaerophilic or facultatively anaerobic microorganisms which is reasonable considering the sample corresponds to surface water. The most abundant species in the Borbollones corresponds to a non-methanogenic archaeon of order Thermoplasmatales. Based on its phylogeny, we hypothesize

that this archaeon participates in the sulfur cycle in this hydrothermal vent. The chemical composition is also consistent with the notion that reduced sulfur compounds (e.g., H₂S) are transformed to sulfate by sulfur-oxidizing bacteria belonging to genera *Sulfurimonas*, *Acidithiobacillus*, *Thiomonas*, *Sulfuriferula*, *Sulfuricurvum*, and *Sulfurovum*. These sulfur-oxidizing microorganisms could use CO₂ as a carbon source as well as other organic compounds. In addition, iron-oxidizing bacteria (genera *Sideroxydans*, *Gallionella*, and *Ferrovum*) were identified. The results obtained in this study suggest that the microbial community of the surface water in Borbollones is dominated by species involved in sulfur cycling and correspond to a mixture of a low proportion of bacteria which originate from the deep subsurface (e.g., *Desulfovibrio*) and a high proportion of species from the surface (most of the identified microorganisms) (Fig. 4). The continuous vertical flow of CO₂ and the movement that is generated in the water interconnect the microbial communities in the oxic and anoxic zone. We did not have evidence for the presence of methanogenic microorganisms, which can be explained by the microaerophilic or aerobic environment where the samples were taken (surface water). This report provides the first description of the physico-chemical and microbiological composition of a Costa Rican and Central American wet mofette. These findings increase our knowledge of microbial communities that thrive in these extreme environments.

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Author contributions AA-R, FP-S, MC conceived and designed the experiments. AA-R, RA, MM-C, MdM performed the experiments. AA-R, FP-S, MC analyzed the data. DHP, MC contributed reagents or materials or analysis tools. AA-R, FP-S, DHP, MC wrote the paper. All authors reviewed and approved the final version of the manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest.

Ethical approval This study does not describe any experimental work related to human.

References

- Akerman NH, Butterfield DA, Huber JA (2013) Phylogenetic diversity and functional gene patterns of sulfur-oxidizing subsurface Epsilonproteobacteria in diffuse hydrothermal vent fluids. *Front Microbiol* 4:1–14. <https://doi.org/10.3389/fmicb.2013.00185>
- Alvarado Induni G (2011) Los volcanes de Costa Rica: geología, historia, riqueza natural y su gente. Editorial Universidad Estatal a Distancia, San José, p 386
- Arce-Rodríguez A, Puente-Sánchez F, Avendaño R et al (2017) Pristine but metal-rich Río Sucio (Dirty River) is dominated by *Gallionella* and other iron-sulfur oxidizing microbes. *Extremophiles* 21:235–243. <https://doi.org/10.1007/s00792-016-0898-7>
- Auernik KS, Cooper CR, Kelly RM (2008) life in hot acid: pathway analyses in extremely thermoacidophilic archaea. *Curr Opin Biotechnol* 19:445–453. <https://doi.org/10.1016/j.copbio.2008.08.001>
- Barton LL, Fardeau ML, Fauque GD (2014) Hydrogen sulfide: a toxic gas produced by dissimilatory sulfate and sulfur reduction and consumed by microbial oxidation. *Met Ions Life Sci* 14:237–277. https://doi.org/10.1007/978-94-017-9269-1_10
- Beaubien S, Ciotoli G, Coombs P et al (2008) The impact of a naturally occurring CO₂ gas vent on the shallow ecosystem and soil chemistry of a Mediterranean pasture (Latera, Italy). *Int J Greenh Gas Con* 2:373–387. <https://doi.org/10.1016/j.ijggc.2008.03.005>
- Beulig F, Heuer VB, Akob DM et al (2015) Carbon flow from volcanic CO₂ into soil microbial communities of a wetland mofette. *ISME J* 9:746–759. <https://doi.org/10.1038/ismej.2014.148>
- Bohorquez LC, Delgado-Serrano L, López G et al (2012) In-depth characterization via complementing culture-independent approaches of the microbial community in an acidic hot spring of the Colombian Andes. *Microb Ecol* 63:103–115. <https://doi.org/10.1007/s00248-011-9943-3>
- Burbach K, Seifert J, Pieper DH, Camarinha-Silva A (2016) Evaluation of DNA extraction kits and phylogenetic diversity of the porcine gastrointestinal tract based on Illumina sequencing of two hypervariable regions. *Microbiolopen* 5:70–82. <https://doi.org/10.1002/mbo3.312>
- Camacho A (2009) Sulfur bacteria. *Encyclopedia of Inland waters*. Academic Press, Cambridge, pp 261–278. <https://doi.org/10.1016/b978-012370626-3.00128-9>
- Camarinha-Silva A, Jáuregui R, Chaves-Moreno D et al (2014) Comparing the anterior nare bacterial community of two discrete human populations using Illumina amplicon sequencing. *Environ Microbiol* 16:2939–2952. <https://doi.org/10.1111/1462-2920.12362>
- Capecchiacci F, Tassi F, Liegler A, Fentress S, Deering C, Vaselli O, Martínez M, Taylor-Castillo W (2015) Geochemistry of water and gas discharges from the Tenorio volcanic system (Costa Rica). Book of abstracts Conference: Il Pianeta Dinamico: sviluppi e prospettive a 100 anni da Wegener. Firenze 2-4 Settembre 2015
- Castellón E, Martínez M, Madrigal-Carballo S et al (2013) Scattering of light by colloidal aluminosilicate particles produces the unusual sky-blue color of Río Celeste (Tenorio Volcano Complex, Costa Rica). *PLoS One* 8:e75165. <https://doi.org/10.1371/journal.pone.0075165>
- de Moor JM, Fischer TP, Sharp ZD, Hilton DR, Barry PH, Mangasini F, Ramirez C (2013) Gas chemistry and nitrogen isotope compositions of cold mantle gases from Rungwe Volcanic Province, southern Tanzania. *Chem Geol* 339:30–42. <https://doi.org/10.1016/j.chemgeo.2012.08.004>
- DeLong EF (1992) Archaea in coastal marine environments. *Proc Natl Acad Sci USA* 89:5685–5689. <https://doi.org/10.1073/pnas.89.12.5685>

- Edgar RC, Haas BJ, Clemente JC et al (2011) UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* 27:2194–2200. <https://doi.org/10.1093/bioinformatics/btr381>
- Frerichs J, Oppermann BI, Gwosdz S et al (2013) Microbial community changes at a terrestrial volcanic CO₂ vent induced by soil acidification and anaerobic microhabitats within the soil column. *FEMS Microbiol Ecol* 84:60–74. <https://doi.org/10.1111/1574-6941.12040>
- Giammanco S, Parello F, Gambardella B et al (2007) Focused and diffuse effluxes of CO₂ from mud volcanoes and mofettes south of Mt. Etna (Italy). *J Volcanol Geotherm Res* 165:46–63. <https://doi.org/10.1016/j.jvolgeores.2007.04.010>
- Giggenbach WF, Gougel R (1989) Method for the collection and analysis of geothermal and volcanic water and gas samples. Chem Div Report No 2387, New Zealand DSIR
- Golyshina OV, Lünsdorf H, Kublanov IV et al (2016) The novel extremely acidophilic, cell-wall-deficient archaeon *Cuniculiplasma divulgatum* gen. nov., sp. nov. represents a new family, *Cuniculiplasmataceae* fam. nov., of the order *Thermoplasmatales*. *Int J Syst Evol Microbiol* 66:332–340. <https://doi.org/10.1099/ijsem.0.000725>
- He Z, Xiao S, Xie X et al (2007) Molecular diversity of microbial community in acid mine drainages of Yunfu sulfide mine. *Extremophiles* 11:305–314. <https://doi.org/10.1007/s00792-006-0044-z>
- Huber H, Stetter KO (2006) Thermoplasmatales. In: Dworkin M, Falkow S, Rosenberg E, Schleifer K-H, Stackebrandt E (eds) *The prokaryotes*. Springer, New York, pp 101–112
- Huber JA, Butter DA, Baross JA (2003) Bacterial diversity in a subsea floor habitat following a deep-sea volcanic eruption. *FEMS Microbiol Ecol* 43:393–409. <https://doi.org/10.1111/j.1574-6941.2003.tb01080.x>
- Inagaki F, Takai K, Kobayashi H et al (2003) *Sulfurimonas autotrophica* gen. nov., sp. nov., a novel sulfur-oxidizing ϵ -proteobacterium isolated from hydrothermal sediments in the Mid-Okinawa Trough. *Int J Syst Evol Microbiol* 53:1801–1805. <https://doi.org/10.1099/ijms.0.02682-0>
- Itoh T, Yoshikawa N, Takashina T (2007) *Thermogymnomonas acidicola* gen. nov., sp. nov., a novel thermoacidophilic, cell wall-less archaeon in order *Thermoplasmatales*, isolated from a solfataric soil in Hakone, Japan. *Int J Syst Evol Microbiol* 57:2557–2561. <https://doi.org/10.1099/ijms.0.65203-0>
- Jiang L, Zheng Y, Chen J et al (2011) Stratification of archaeal communities in shallow sediments of the Pearl River Estuary, Southern China. *Antonie Van Leeuwenhoek* 99:739–751. <https://doi.org/10.1007/s10482-011-9548-3>
- Jones DS, Kohl C, Grettenberger C et al (2015) Geochemical niches of iron-oxidizing acidophiles in acidic coal mine drainage. *Appl Environ Microbiol* 81:1242–1250. <https://doi.org/10.1128/AEM.02919-14>
- Kämpf H, Bräuer K, Schumann J et al (2013) CO₂ discharge in an active, non-volcanic continental rift area (Czech Republic): characterisation ($\delta^{13}\text{C}$, $^3\text{He}/^4\text{He}$) and quantification of diffuse and vent CO₂ emissions. *Chem Geol* 339:71–83. <https://doi.org/10.1016/j.chemgeo.2012.08.005>
- Kozich JJ, Westcott SL, Baxter NT et al (2013) Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the miseq illumina sequencing platform. *Appl Environ Microbiol* 79:5112–5120. <https://doi.org/10.1128/AEM.01043-13>
- Krauze P, Kämpf H, Horn F et al (2017) Microbiological and geochemical survey of CO₂-dominated mofette and mineral waters of the Cheb Basin, Czech Republic. *Front Microbiol* 8:2446. <https://doi.org/10.3389/fmicb.2017.02446>
- Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol Biol Evol* 33:1870–1874. <https://doi.org/10.1093/molbev/msw054>
- Labrenz M, Grote J, Mammitzsch K et al (2013) *Sulfurimonas gotlandica* sp. nov., a chemoautotrophic and psychrotolerant epsilonproteobacterium isolated from a pelagic redoxcline, and an emended description of the genus *Sulfurimonas*. *Int J Syst Evol Microbiol* 63:4141–4148. <https://doi.org/10.1099/ijms.0.048827-0>
- Luton PE, Wayne JM, Sharp RJ, Riley PW (2002) The *mcrA* gene as an alternative to 16S rRNA in the phylogenetic analysis of methanogen populations in landfill. *Microbiology* 148:3521–3530. <https://doi.org/10.1099/00221287-148-11-3521>
- Oppermann BI, Michaelis W, Blumenberg M et al (2010) Soil microbial community changes as a result of long-term exposure to a natural CO₂ vent. *Geochim Cosmochim Acta* 74:2697–2716. <https://doi.org/10.1016/j.gca.2010.02.006>
- Paul K, Nonoh JO, Mikulski L, Brune A (2012) “Methanoplasmatales” Thermoplasmatales-related archaea in termite guts and other environments, are the seventh order of methanogens. *Appl Environ Microbiol* 78:8245–8253. <https://doi.org/10.1128/AEM.02193-12>
- Pauwels H, Fouillac C, Goff F, Vuataz FD (1997) The isotopic and chemical composition of CO₂-rich thermal waters in the mont-dore region (Massif-Central, France). *Appl Geochemistry* 12:411–427. [https://doi.org/10.1016/S0883-2927\(97\)00010-3](https://doi.org/10.1016/S0883-2927(97)00010-3)
- Pronk JT, De Bruyn JC, Bos P, Kuenen JG (1992) Anaerobic growth of *Thiobacillus ferrooxidans*. *Appl Environ Microbiol* 58:2227–2230
- Pruesse E, Peplies J, Glöckner FO (2012) SINA: accurate high-throughput multiple sequence alignment of ribosomal RNA genes. *Bioinformatics* 28:1823–1829. <https://doi.org/10.1093/bioinformatics/bts252>
- Puente-Sánchez F, Aguirre J, Parro V (2016) A novel conceptual approach to read-filtering in high-throughput amplicon sequencing studies. *Nucleic Acids Res* 44:e40. <https://doi.org/10.1093/nar/gkv1113>
- Quast C, Pruesse E, Yilmaz P et al (2013) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res* 41:D590–D596. <https://doi.org/10.1093/nar/gks1219>
- Reysenbach A, Longnecker K, Kirshtein J (2000) Novel bacterial and archaeal lineages from an in situ growth chamber deployed at a mid-atlantic ridge hydrothermal vent. *Appl Environ Microbiol* 66:3798–3806. <https://doi.org/10.1128/AEM.66.9.3798-3806.2000>
- Sáenz de Miera LE, Arroyo P, de Luis Calabuig E et al (2014) High-throughput sequencing of 16S RNA genes of soil bacterial communities from a naturally occurring CO₂ gas vent. *Int J Greenh Gas Control* 29:176–184. <https://doi.org/10.1016/j.ijggc.2014.08.014>
- Sánchez-Andrea I, Rojas-Ojeda P, Amils R, Sanz JL (2012) Screening of anaerobic activities in sediments of an acidic environment: tinto River. *Extremophiles* 16:829–839. <https://doi.org/10.1007/s00792-012-0478-4>
- Schleper C, Pühler G, Klenk P, Zillig W (1996) *Picrophilus oshimae* and *Picrophilus tomidus* fam. nov., gen. nov., sp. nov., Two species of hyperacidophilic, thermophilic, heterotrophic, aerobic archaea. *Int J Syst Evol Microbiol* 46:814–816. <https://doi.org/10.1099/00207713-46-3-814>
- Schloss PD, Westcott SL, Ryabin T et al (2009) Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl Environ Microbiol* 75:7537–7541. <https://doi.org/10.1128/AEM.01541-09>
- Segerer A, Langworthy TA, Stetter KO (1988) *Thermoplasma acidophilum* and *Thermoplasma volcanium* sp. nov. from solfatara fields. *Syst Appl Microbiol* 10:161–171. [https://doi.org/10.1016/S0723-2020\(88\)80031-6](https://doi.org/10.1016/S0723-2020(88)80031-6)
- Serour E, Antranikian G (2002) Novel thermoactive glucoamylases from the thermoacidophilic archaea *Thermoplasma acidophilum*, *Picrophilus torridus* and *Picrophilus oshimae*. *Antonie Van*

- Leeuwenhoek 81:73–83. <https://doi.org/10.1023/A:1020525525490>
- Šibanc N, Dumbrell AJ, Mandić-Mulec I, Maček I (2014) Impacts of naturally elevated soil CO₂ concentrations on communities of soil archaea and bacteria. *Soil Biol Biochem* 68:348–356. <https://doi.org/10.1016/j.soilbio.2013.10.018>
- Sievert SM, Scott KM, Klotz MG et al (2008) Genome of the epsilon-proteobacterial chemolithoautotroph *Sulfurimonas denitrificans*. *Appl Environ Microbiol* 74:1145–1156. <https://doi.org/10.1128/AEM.01844-07>
- Suzuki I, Takeuchi TL, Yuthasastrakosol TD, Oh JK (1990) Ferrous iron and sulfur oxidation and ferric iron reduction activities of *Thiobacillus ferrooxidans* are affected by growth on ferrous iron, sulfur, or a sulfide ore. *Appl Environ Microbiol* 56:1620–1626
- Takai K, Horikoshi K (1999) Genetic diversity of archaea in deep-sea hydrothermal vent environments. *Genetics* 152:1285–1297. [https://doi.org/10.1016/s0723-2020\(87\)80053-x](https://doi.org/10.1016/s0723-2020(87)80053-x)
- Timmer-ten Hoor A (1975) A new type of thiosulphate oxidizing, nitrate reducing microorganism: *Thiomicrospira denitrificans* sp. nov. *Netherlands J Sea Res* 9:344–350. [https://doi.org/10.1016/0077-7579\(75\)90008-3](https://doi.org/10.1016/0077-7579(75)90008-3)
- Valdés J, Pedroso I, Quatrini R et al (2008) *Acidithiobacillus ferrooxidans* metabolism: from genome sequence to industrial applications. *BMC Genomics* 9:597. <https://doi.org/10.1186/1471-2164-9-597>
- Wang Q, Garrity GM, Tiedje JM, Cole JR (2007) Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl Environ Microbiol* 73:5261–5267. <https://doi.org/10.1128/AEM.00062-07>
- Yasuda M, Oyaizu H, Yamagishi A, Oshima T (1995) Morphological variation of new *Thermoplasma acidophilum* isolates from Japanese hot springs. *Appl Environ Microbiol* 61:3482–3485
- Zhang M, Zhang T, Shao MF, Fang HHP (2009) Autotrophic denitrification in nitrate-induced marine sediment remediation and *Sulfurimonas denitrificans*-like bacteria. *Chemosphere* 76:677–682. <https://doi.org/10.1016/j.chemosphere.2009.03.066>
- Zhou H, Li J, Peng X et al (2009) Microbial diversity of a sulfide black smoker in main endeavour hydrothermal vent field, Juan de Fuca Ridge. *J Microbiol* 47:235–247. <https://doi.org/10.1007/s12275-008-0311-z>