Metagenomic and Metabolic Analyses of Poly-Extreme Microbiome from an Active Crater Volcano Lake

Betsy Anaid Peña-Ocaña
Tecnológico Nacional de México / IT de Tuxtla Gutiérrez
Javier Tamames
Centro Nacional de Biotecnología, CSIC
Luis Eduardo Servín-Garcidueñas
Universidad Nacional Autonoma de Mexico
Elena González-Toril
Centro de Astrobiología, CAB (INTA-CSIC)
Cesar Iván Ovando-Ovando
Tecnológico Nacional de México / IT de Tuxtla Gutiérrez
Wilbert Gutiérrez-Sarmiento
Tecnológico Nacional de México / IT de Tuxtla Gutiérrez
Ricardo Jasso-Chávez
Departamento de Bioquímica, Instituto Nacional de Cardiologia
Víctor Manuel Ruíz-Valdiviezo (bioqvic@hotmail.com)
Tecnológico Nacional de México / Instituto Tecnológico de Tuxtla Gutiérrez https://orcid.org/0000-0003-0572-8845

Research

Keywords: poly-extreme conditions, anaerobic metabolism, sulfate reduction, methane production, Archaea

Posted Date: January 19th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-149488/v1

Version of Record: A version of this preprint was published at Environmental Research on January 1st, 2022. See the published version at https://doi.org/10.1016/j.envres.2021.111862.

Abstract

Background: El Chichón volcano is one of the most active volcanoes in Mexico. Previous studies have described the poly-extreme conditions of the lake crater but its bacterial composition and the functional features of the complete microbiome have not been characterized yet.

Methods: This study integrated two approaches to explain the microbiology diversity and abundance, one focused on the environmental genomic potential by metagenomics approach, and other culturomics of enrichment of bacteria and archaea. The microbial diversity of the anaerobic consortia cultivated in was carried out by metabarcoding analysis, the metabolic capacity by metabolomics fluxes of carbon and enzymologic techniques for the analysis of sulfate reduction in laboratory-grown prokaryotic cells.

Results: This work provides new information on the taxonomic and functional diversity of the Archea representative phyla Crenarchaeota and Euryarchaeota as well as the phyla Thermotogales and Aquificae for Bacteria. Through the analysis of microbial consortia cultivation and the genetic information collected from the natural environment sampling, metabolic interactions were identified between the microorganisms that support the life of the microbiome under multi-extreme conditions. A close relationship is proposed between the cycles of carbon and sulfur in an active volcano.

Conclusions: This research contributes to the understanding of microbial metabolism under extreme conditions and potential knowledge of "microbial dark matter" that can be applied in biotechnological processes and evolutionary studies.

Background

Man-hostile environments have been described as extreme. Some of them have been considered too extreme to support microbial life, such as cold Arctic water, brines, deserts, and hydrothermal vents [1]. Active volcanic sites have also been of concern on regard of combining more than one extreme characteristics. Some of these volcanoes have a lake-crater that is the superficial expression of volcanic activity in the upper part of complex magmatic-hydrothermal systems [2]. This is the case of the active volcano El Chichón (17.36 ° N, 93.23 ° W; 1100 m above mean sea level, MAMSL) which is a geothermal system located in the northwest of the State of Chiapas, Mexico with a crater-lake formed after the last eruptive process in 1982. Hydrothermalism is possibly generated by the constant volcanic activity of the Modern Chiapaneco Volcanic Arc (MCVA). In the area near this volcano there are constant seismic episodes, hydrothermal zones and recently, new sites with geothermal potential in the periphery have been described [3].

El Chichón is one of the most recent and important volcanic systems in Mexico [3], it is considered a highly dynamic system due to the constant variations in the geochemical composition, shape and water-level of the lake, as well as a high dynamism in temperature and pH [4]. Because of geothermal activity, different minerals [5], metals [6] and sulfur are present, where the most predominant forms of the latter

are sulfide [7], which confers acidic conditions and a highly toxicity and hostility for the survival of microbial life.

Extreme natural environments are a source of microorganisms of geological and biotechnological interest; however, in “El Chichón” only the taxonomic diversity of thermoacidophilic bacteria has been examined so far, as fifteen identified phyla were found in the sediment at 50 ° C such as *Actinobacteria* (33.1%), *Proteobacteria* (29.1%) and *Acidobacteria* (20.1%); nine phyla were found in the sediment at 92 ° C where *Firmicutes* (52.7%, mostly *Alicyclobacillus* and *Sulfobacillus*) and Proteobacteria (44.8%, mostly *Bradyrhizobium*, *Methylobacterium*, *Sediminibacterium*) were the most abundant [8]. However, to our knowledge, there are no reports dealing with the diversity, culture and metabolism of poly-extreme archaea in crater-lake active volcano, presumably the predominant microorganisms in these environmental conditions, and which isolation and study remain elusive. Understanding the mechanisms used by extremophiles microorganisms to tolerate and thrive in multi-extreme conditions may provide useful information to assess habitability on earth, as well as its application in biotechnological processes.

In this work, by using complete metagenome analysis, enzyme activities and metabolic fluxes determination, molecular and biochemical evidence related to the energy metabolism in this novel multi-extreme environment is reported for thermoacidophilic bacteria and archaea for the first time. This study provides new insights regarding the geobiochemical role of volcanic microorganisms.

Methods

The physicochemical environmental parameters have been previously described [6]. The samples were collected on November 8th 2018 from El Chichón crater-lake, Chiapas, México.

Whole metagenome analysis

Samples of sediment and water column were taken for metagenomic analysis, samples were placed in three separate sterile tubes, immediately preserved by freezing in liquid nitrogen, and stored at −80 °C until DNA was extracted. In turn, metagenomic DNA was extracted by triplicate using the QIAGEN DNA isolation kit (Hilden, Germany) and the metagenomic DNA was sequenced using Illumina NextSeq platform with 2 X 150 pair-ends reads (Integrated Microbiome Resource, Halifax, Canada). The quality control of the reads was performed with Trimmomatic [9], functional and taxonomical analysis of metagenome was carried out using the co-assembly mode from SqueezeMeta v1.0.0 automatic pipeline [10], Assembly was done using Megahit [11]. Contig statistics were done using prinseq [12]. RNAs were predicted using Barrnap [13], 16S rRNA sequences were taxonomically classified using the RDP classifier [14], tRNA/tmRNA sequences were predicted using Aragorn [15]. ORFs were predicted using Prodigal [16]. Similarity searches for GenBank [17], eggNOG [18], KEGG[19], were done using Diamond [20]. HMM homology searches were done by HMMER3 [21] for the Pfam database[22]. Read mapping against

contigs was performed using Bowtie2 [23]. Binning was done using MaxBin2 [24], Binning was done using Metabat2 [25], Combination of binning results was done using DAS Tool [26], Bin statistics were computed using CheckM [27].Pathway prediction for KEGG [19] and MetaCyc [28], databases was done using MinPath [29]. Gene abundance was estimated mapping individual reads to the reference co-assembly contigs annotation. Finally, Squeezemeta manages the results and generates a table of genes (taxonomy, function, origin of contig and bin, abundance in samples and amino acid sequence), a table of contig, which gathers the data of the contigs (taxonomy, affiliation of bin, abundance in samples and disparity), and a bin table with information related to the bins (taxonomy, integrity, contamination, abundance in samples and disparity). These three tables and the metadata were used to create a MySQL database which was analyzed using R (version 3.4.4, Inc., Boston, MA, U.S.A). The metagenomic data obtained were used to build metabolic schemes which were carried out using specific genes involved in the metabolism of carbon and sulfur (see Additional file Table 1). Using an independent analysis system and using KEGG as a reference base[19] The identification of metabolic processes was carried out by comparing the genes of the metagenome with the KEEG database. Illustrations were made using CorelDrawn software (version 2016) [30].

Methanogenic and sulfate-reducing bacteria enrichment

The culture system is called ´Lab-Lake´ which was carried out under strict anoxic conditions in the culture medium reported by Sowers et al. (1993)[31] modified by the addition of a mixture of carbon sources commonly used in the cultivation of Extremophiles under laboratory conditions: 10 mM glucose, 10 mM maltose, 10 mM glycerol, 20 mM glyceryl triacetate, 20 mM sodium pyruvate, 100 mM sodium acetate and 100 mM methanol [32] *plus* 100 µM $FeSO_4$. The culture media were prepared in an anaerobic chamber (COY, Michigan, USA) that contained an atmosphere of 80 % N_2, 15 % CO_2, 5 % H_2 (v / v). The oxygen dissolved in the culture media was removed by gas exchange (constant bubbling with the gas mixture) for four hours and the addition of cysteine. Each experimental unit consisted of a 100 mL vial with 50 mL of operation, hermetically sealed and sterilized by humid heat for 40 min.

The first stage of cultivation was carried out *in situ* the crater of the volcano and was started with the addition of approximately 50 mL of water column of the volcanic lake collected in various sections of the crater lake at different temperatures (30, 35, 40, 45 and 82 ° C) injected using sterile syringes in sealed 100 mL bottles containing 10 mL of Lab-lake medium. These samples were kept at 60-70 ° C during their transport to the National Institute of Cardiology in Mexico City, which took 72 hours. After 8 days of incubation, 10 ml were transferred to 50 ml of mixture of fresh medium and sterile volcanic water and cultured at 37 ° C or 70 ° C without shaking. After repeating this procedure three times, 190 culture bottles resulted, from which methane production and CO_2 fluxes were determined at different times during 24 days. From the continuous transfer to fresh media for 2 months and based on the growth curves obtained (production of CH_4, CO_2 and protein content), enriched samples were obtained that were finally grouped into two representative sets: mesophilic and hyperthermophilic consortia and subsequently, the microbial growth parameters were each determined for 500 hours.

Cell harvesting

After 15 days of growth, cell cultures were harvested under anaerobic conditions at 5000 *x g* for 15 min at 4°C. Cell pellet (1 mL) was resuspended and washed in 10 volumes of a fresh solution containing 50 mM Tris, 20 mM $MgCl_2$, 200 mM NaCl and 1 mM EGTA at pH 7.2 (TME-Na buffer) and further centrifuged at 5000 *x g* for 15 minutes at 4°C. After this, 5mM DTT, 1 mM PMS and 10% glycerol (v/v) were added to the cell pellet. Cells were frozen and kept at -80°C until use.

Metabarcoding analysis

DNA metagenomic was extracted of thawed washed cells of mesophilic and hyperthermopilic consortia after 15 days of growth. For bacterial identification the variable V4-V5 regions of the 16s rRNA were amplified with primers 515Fw (5'-GTGYCAGCMGCCGCGGTAA-3') and 926Rw (5'-CCGYCAATTYMTTTRAGTTT-3') (Walters et al., 2016), it was also analyzed the regions V6-V8, the same gene for archaea identification with primers 956Fw (5'- TYAATYGGANTCAACRCC-3') and 1401Rw (5'-CRGTGWGTRCAAGGRGCA-3') [33]. Barcoded DNA libraries were sequenced through Illumina MiSeq 2x300 platform. 16S rRNA reads were processed using MiSeq SOP Mothur [34], for reducing sequencing and PCR errors, sequencereads with < 50 nucleotides, > 1% and 7% of homopolymers and those with mitochondrial or chloroplast origins were discarded. Chimeric sequences were removed using default parameters in UCHIME [35]. Sequences were aligned with SILVA database version 132 [36] at 80% confidence threshold [37]. The aligned data sets were clustered into Operational Taxonomic Units (OTUs) at 98% sequence similarity using a pairwise distance matrix. The relative abundance of taxas was as a percentage of the number of sequences affiliated with the specific taxon against the total number of sequences obtained for that sample.

Biochemical characterization of microbial cultures

Carbon metabolism was determined by measuring CO_2 and CH_4 fluxes in a gas chromatography equipment (Shimadzu GC2010, Japan) as described previously by Santiago-Martínez et al (2015, 2016) [38,39], while carbon sources consumption was determined by spectrophotometry, gas chromatography (see Additional file Text 1).

Sulfate-reduction pathway was determined throughout the growth curve by measuring the synthesis of sulfide by the cell consortia. All steps were carried out under strict anaerobiosis: cell-free supernatant and washed cells were used to determine the extra and intracellular sulfide and mixed with a solution containing 23.7 mM zinc acetate, 60 mM NaOH, 0.18 mM N,N-dimethyl-p-phenylenediamine (DMPD) dissolved in 5 N HCl and 0.1 mL of sample, or different amounts of sulfide and 2.8 mM $FeCl_3$. Sulfide was quantified by the methylene blue formation [40] Limit of sensitivity was 3 nmol and linear up to 350 nmol [41].

Enzymatic determination of the dissimilatory sulfate reduction pathway

Enriched cytosolic and membranal fractions of cells among its logarithmic growth phase were collected afterwards its lysate by sonication with five 20 seconds intervals with a minute rest in which anaerobiosis was preserved by a constant flux of N_2 (10 mL/min). Enzymatic activity was carried out in anaerobic conditions in a reaction mixture containing 0.1 mg of protein of each cellular extract, 5mM ATP and 1mM GSH and 100 µM NADPH as electron donors. Reaction was started with the addition of 50 µM $FeSO_4$ in a final volume of 1 mL of HKE-Na buffer. The assay was attained at 37°C for 30 min (reaction was lineal until 60 min). Sulfide produced was determined by the described spectrophotometric method for methylene blue.

Statistical Analysis

Statistical analyzes and graphs of metabolism were performed in Origin 5.0 (www.OriginLab.com). For metabarcoding diversity based on Operational Taxonomic Units (OTUs) were done in STAMP [42], the bar plot visualization was constructed with a relative abundance of the genus or phyla level > 0.05% of abundance count table and rest summarized in others. Venn diagram and metagenomic analysis were performed with Rstudio software[43].

Results And Discussion

Environmental general features of El Chichón volcano

The physicochemical characteristics of water column and sediment along the crater-lake were variable depending on the location. A priori perimetric exploration allowed sampling of sediment and water column at different temperatures. The sites analyzed and parameters determined in this study are shown in Table 1 and Figure 1.

Table 1
In situ parameters of samples for metagenomic analysis and microbial culture.

Sample	Geographic Location	Altitude (masl)	T (°C)	pH
1	17°21´35´´N 93°13´41´´ W	888	35	3.3
2	17°21´33´´N 93°13´41´´ W	888	40	3.7
3	17°21´39´´N 93°13´41´´ W	886	45	3.5
4	17°21´40´´N 93°13´41´´ W	885	82	3.1
5	17°21´37´´N 93°13´44´´ W	883	ND	ND

ND=not determined physicochemical parameters. Samples 1 to 5 used for Lab-Lake culture. Samples 3 and 4 used simultaneously for metagenomics analysis. masl=meters above sea level.

Metagenome analysis

Water column and sediment of crater El Chichón volcano were analyzed using high throughput next-generation sequencing to identify the microbial biodiversity and functional potential, the statistical and parameters are presented in Table 2.

Table 2
Data details of metagenome analysis of water column and sediment of El Chichón active volcano.

	Water column	Sediment
Reads	10 767 992	32 227 870
Contigs	4 775	24 312
N50	26 634	9 633
Total assembly length (Mb)	6.13	47.3

Each metagenomic sample was sequenced in triplicate and the sequences were pooled for bioinformatics analysis.

Taxonomic classification environmental and lab culture

The environmental metagenomic analysis enable to identify members of the three domains of life, this information is a precedent for the rapid evolution that this extreme environment has had since its last eruption in 1982, after this geological event and studies in which the absence of life was reported. [44]. Unlike these reports, in this study, a set of methodologies with a multi-omic approach was used. Starting with a metagenomic study to achieve characterization in microbial cultures.

It is of great interest to deepen the study of microbial diversity in this environment, in order to have a better understanding of the microbiology from El Chichón volcano and its highly dynamic environment, sediment and water column samples from the crater lake were analyzed by metagenomics. It is exhibited in the natural environment that the hyperthermophilic water column was not very diverse (Shannon's index = 0.58), 80% corresponds to Archaea represented by the *Candidatus* Aramenus genus and 20% bacterium domain of the *Hydrogenobaculum* genus, both microorganisms are characteristic of hot springs and rich in sulfur. *Candidatus* Aramenus is a thermophilic and acidophilic candidate genus that has been identified only in the geothermal site Los Azufres, Michoacán [45], and which has a role in sulfur metabolism and *Hydrogenobaculum* a chemolytoautotrophic bacterium, which has been identified in solphatara pools and volcanic environments, and is characterized by obtaining ATP using H_2 as an electron donor in the presence of a reduced sulfur source [46]. Regarding the mesophilic sediment, greater microbial diversity was considered (Shannon index = 1.41) finding the presence of the bacterial phylum *Proteobacteria* represented mainly by *Acidithiobacillus* and *Desulfurella*, the former, a microorganism normally found in acidic environments such as Rio Tinto [47] [48]) Copahue volcano [49] and Rio Agrío [50]; where they play a crucial role in the fixation of CO_2 and production of H_2SO_4, it also obtains the energy from the oxidation of Fe^{2+} by means of an “upward potential gradient” [51,52]. On the other hand, *Desulfurella* has been found in acid mine residues [53], Rio Tinto sediments [54], among other thermophilic and acidic environments. In these ecological sites they are of great importance due to its role in the disproportionation of sulfur, given that it can use sulfide or thiosulfate as an electron acceptor, and as a donor acetate, pyruvate, propionate, among others. CO_2 / H_2 is presented as a product of the oxidation of these substrates. Another *phylum* identified in these samples is Firmicutes, highlighting the presence of the genera *Thermoanaerobacterium*, *Ruminococcus*, and *Ignavibacterium*. The dominant bacterial taxon in this sample is *Thermotogae*, which is represented by *Athalassotoga*, an anaerobic, heterotrophic, acidophilic and moderately thermophilic, which has been identified in similar environments as a hot-spring in Japan [55]; and in greater abundance *Mesoaciditoga*, this microorganism has also been reported in the Copahue-Caviahue volcanic system [56]. Regarding to the diversity of archaea, the presence of the phylum *Euryarchaeota* stands out, represented by Candidatus Methanofastidiosa, and the *Thermoplasmatales*: *Ferroplasma*, *Thermoplasma*, *Cuniculiplasma* and *Acidiplasma*. The *Thermoplasmatales* order has been identified at other volcanic sites, such as the Tenorio volcano, Costa Rica, where its main metabolic contribution is the oxidation of reduced sulfur compounds (for example, H_2S) to sulfate [57]. It was also found the presence of the *phylum Chlorophyta* composed of green algae, this phylum is dominated by *Trebouxiophyceae*. Some microalgae belonging to this group have been identified in extreme conditions, for example, *Endolithella mcmurdoensis* in the McMurdo valleys, desert in Antarctica [58].

Another aspect of great relevance is the study of Extremophilic viruses. In this study, it was possible to identify the Mimiviridae and Pithoviridae families using the Lowest Common Ancestor (LCA) algorithm with a >80% bit-score identity [59] compared contigs to GenBank; *Mimiviridae* corresponds to a family of double-stranded DNA viruses, which is associated with protozoa. These viruses stand out for their participation in theories of the origin of life [60]. On the other hand, *Phithoviridae* is a single-stranded RNA

virus, which also plays an important role in understanding evolutionary theory [61]. The Phytoviridae viruses rely on very diverse eukaryotic hosts, which include protists, algae, vertebrate animals, and insects[62].

In addition to the taxonomic classification, the information generated from the metagenomic analysis allowed to identify the metabolic and functional potential of the microbiome, and with this information microbial culture strategies and metabolic and kinetic analyzes were developed to evaluate the participation of microorganisms in key biogeochemical processes such as carbon and sulfur metabolism. To understand these processes with greater precision, a culturomic stage was developed, in which it was possible the enrichment of *Firmicutes* (genera *Ruminococcus*, *Thermoanaerobacter*, *Tepidanaerobacter*) and archaea of the *Euryarchaeota* type (genera *Methanobacterium*, *Methanothermobacter*) (Fig 2b). Based on the sequences obtained from the analysis of the 16S rRNA gene, it was identified by prediction of genomic potential that the microorganisms present in laboratory cultures developed roles of vital importance in the preservation of the biogeochemical balance of sulfur and carbon (see Additional File Table 1 S1). Therefore, it is possible to use microbial cultures to understand the participation of microorganisms in the biogeochemical cycles of carbon and sulfur in El Chichón volcanic system.

Carbon metabolism

Based on the genomic potential of the natural environment, a metabolic map of the carbon cycle is proposed (Figure 3), considering the acquisition and intermediate metabolism of carbon in the extreme natural environment El Chichón.

The microbiome showed genomic characteristics that allow to carry out most of the metabolic pathways described for obtaining of a carbon source. The presence of the semi-SWEET system was identified that allow the internalization of sugars, as well as glycerol transporters and glycerol-3P molecules that can be used as carbon sources by the extremophilic microorganisms. The abundance of metabolic pathways for CO_2 fixation was also observed, these results suggested that this is one of the most important mechanisms for obtaining carbon in this extreme environment; besides low concentration of sugars (or not) has been determined in the lake (data not show). On this regard, CO_2 production was 5 ± 0.4^a and 4 ± 0.1^a mmol CO_2 for mesophilic and thermophilic cultures, respectively. This type of metabolism has been reported in environments homologous to El Chichón volcano, such as Yellowstone National Park, where the presence of archaea from the *Euryarcheota* and *Crenarchaota* phyla has been reported, which at high temperatures carry out processes of methanogenesis and the metabolic cooperation of these microorganisms with bacteria has even been reported to facilitate the obtaining of CO and CO_2, which they use as an electron donor and carbon source [63].

The dynamics of assimilation of carbon sources of the lab-culture showed that the mesophilic microorganisms consumed 54 ± 8^a % of the substrates, while the hyperthermophilic culture consumed

only 41± 3^b % carbohydrates present in the growth media (Glc and maltose) were mainly consumed over the rest of substrates (glycerol, glyceryl triacetate, sodium pyruvate, sodium acetate and methanol) 90 and 35-60 % respectively of these substrates. Additionally methanogenesis was evaluated as it was the last step in the total degradation of organic matter, in addition to a mechanism for obtaining energy under anaerobic conditions [64]. Mesophilic cultures were observed to have higher methanogenic activity than hyperthermophiles (1.8 ± 0.3^a and 0.5 ± 0.07^b mmoles of CH_4 after 15 days of culture). Results based on metabolic evidence show differences between mesophilic and hyperthermophilic cultures, and therefore allow an inference of the metabolic dynamics that takes place in this extreme natural environment, this suggests that glycolysis and CO_2 fixation for methanogenesis are energy mechanisms most used by mesophilic consortia.

Sulfur metabolism

The functional analysis of the microbiome expressed in terms of functional orthologs using the KO database revealed that a high percent of the KOs was assigned to the KEGG metabolism pathway to sulfur metabolism, with this information the presence of potential to perform sulfate reduction and sulfur oxidation (SOX system) (Figure 4).

Volcanic systems are dominated by sulfur oxidizing microorganisms, while the presence of sulfate-reducing bacteria has not been described in detail [65–67]. It is important to consider that the active volcano El Chichón also presents acidic characteristics (pH 2-6), microorganisms have been described that have the capacity to carry out sulfate reduction processes under these conditions such as *Desulfurella*, *Thermodesulfobium*, *Desulfurococcus*, *Desulfosarcinacetonica* [68–70]. Regarding El Chichón volcano, there is the presence of *Desulfurella* sulfate reducing bacteria and *Candidatus* Aramenus, a sulfur oxidizing archeon (Fig 2a).

The sulfate reduction is a process of biogeochemical relevance associated with volcanic environments, this metabolism can follow two routes: i) the assimilatory pathway that is carried out mainly for the synthesis of biomolecules [71] and ii) the non-assimilatory pathway that is the anaerobic process in which sulfate is used as a terminal electron acceptor, allowing the oxidation of organic and inorganic compounds, in this process a large amount of sulfide is produced, which is re-oxidized to sulfate by microbial activity [72,73]. These processes are abundantly distributed in El Chichón volcano microbiome.

Several sulfur reducing extremophilic microorganisms contain in their genome a set of genes involved in sulfate reduction: sat, aprBA, dsrABCMK [72]. These genes were identified in the metagenome of the mesophilic and hyperthermophilic environmental samples from El Chichón volcano. In addition to the biogeochemical relevance of sulfate reduction, reducing sulfate bacteria use this process to obtain energy [74]. The bioenergetic mechanism of this process has not been fully elucidated. Recently, it has been proposed that the QrcABCD complex of *Desulfovibrio vulgaris* is electrogenic, and the mechanism is due to the balance of electrons and protons on opposite sides of the membrane, it is suggested that it is

not an H^+ pump but a proton channel instead [74]. It has been evidenced as a new respiratory system in prokaryotes mediated by an electrogenic complex that involves a redox loop where the menaquinone (a low redox potential quinone) and the substrate sites are on the same side of the membrane, defining a new type of prokaryotic respiratory system [74]. The lab-culture microorganisms from El Chichón volcano showed evidence of carrying out processes of reduction of $FeSO_4$ and formation of sulfide, which can be excreted from the cell interior (see Additional Table 2). Hyperthermophilic microbial consortia showed 1.6 times more ability to reduce sulfate to sulfide in microbial cultures (Supplemental Table S3). This is reproducible when evaluating the flux of the non-assimilatory sulfate reduction metabolic pathway in mesophilic and hyperthermophilic microbial consortia in cytosolic and membrane fractions (Table 3).

Table 3
Enzymatic activity of the non-assimilatory sulfate reduction pathway in El Chichón volcano culture.

Activity in	Mesophilic microorganisms (nmoles of H_2S / min mg protein)	Hyperthermophilic microorganisms (nmoles of H_2S / min mg protein)
Enriched cytosolic fraction (ATP + GSH + NADPH)	20.5 ± 2.5 ^{bB}	399.6 ± 36.4 ^{bA}
Enriched membrane fraction (ATP + GSH + NADPH)	168 ± 30.2 ^{aB}	886.7 ± 31.6 ^{aA}
Enriched cytosolic fraction (-ATP -GSH -NADPH)	10 ± 2 ^{bA}	< 1 ± 0.3 ^{cB}
Enriched membrane fraction (-ATP -GSH -NADPH)	128 ± 42.3 ^{aA}	< 1 ± 0.4 ^{cB}

Mean values ± standard deviation (n = 3). Significant differences ($p \leq 0.01$) using ANOVA/Fisher's least significant difference (LSD) test, are indicated in column by different lowercase letters (a, b, c) between cell fractions, and capital letters (A, B) indicated significant differences ($p \leq 0.01$) in row between treatments.

The results suggested that the highest activity of the non-assimilatory reduction sulfate pathway in the microbial consortia of El Chichón volcano is found in the membrane fraction; in the case of mesophilic consortia there is 8.2 times more than in the cytosolic fraction. Similar to that determined in hyperthermophilic cultures where there is 2.2 times more activity in the membranes than in the cytosol, which suggest that hyperthermophilic consortia use the non-assimilatory pathway for sulfate reduction as an alternative mechanism for obtaining energy.

It is necessary to consider that, energetically, sulfate is a poor electron acceptor for microorganisms since the sulfate-sulfite redox pair is E 0 '-516 mV, which is too negative to allow reduction by NADH or Fed_{red},

which are the main intracellular electronic mediators. To overcome this problem, the sulfate is first converted to APS by the enzyme ATP sulfurylase (Sat), at the cost of a single ATP molecule. The APS-sulfite redox pair has an E 0 'of -60 mV, which allows APS to be reduced by NADH or reduced ferrodoxin using the enzyme adenylyl sulfate reductase (Apr), which requires the input of 2 electrons. In the final step, the sulfite is reduced by dissimilatory sulfate reductase (Dsr) to form sulfide, requiring the input of 6 electrons. However, it has recently been shown that the conservation of energy through sulfate reduction processes are due to the transfer of protons from cytochrome c3 to the menaquinone group, through the Qrc complex. Additionally, the presence of this respiratory system in the prokaryotic membrane has been reported [74].

Conclusion

Our results demonstrated the presence of a complex microbiome, composed of archaea, viruses, bacteria, and microalgae. The phyla *Crenarchaeota*, *Aquificae* and *Termotogae* dominated the microbiome of the crater lake of the El Chichón volcano, while the lab-cultures are dominated by the phyla *Firmicutes* and *Euryarchaeota*. Reducing and methanogenic sulfate populations were enriched in the lab-culture system, poorly represented in the natural environment, with which we detected important differences in the functions of mesophilic and hyperthermophilic microbial cultures, these differences are related to carbon assimilation and energy metabolism of the extremophiles from El Chichón volcano. Future experiments should, therefore, aim to determine regulatory mechanisms in energy metabolic pathways to make proper correlations with the volcanic system.

Abbreviations

Fed_{red}: Reduced ferredoxin

NADH: β-Nicotinamide adenine dinucleotide, reduced

GSH: Reduced glutathione

APS: Adenylyl sulfate

TAG: Glyceryl triacetate

Declarations

Ethics approval and consent to participate

This article does not contain any studies with human participants or animals performed by any of the authors.

Consent for publication

Not applicable

Availability of data and material

All data presented in this article is available under request.

Competing interests

The authors declare that they have no conflict of interest.

Acknowledgements

The authors thank Fernando Puente-Sánchez from the Microbiome Analysis Laboratory, Systems Biology Department, Centro Nacional de Biotecnología, CSIC, who participated in the bioinformatic analysis of the metadata and Prof. Rafael Moreno-Sanchez, Head of the Biochemistry Department, INCICH who participated in the discussion of some experimental results. We also acknowledge the support of Elizabeth Lira-Silva in some experimental activities.

FUNDING

This work was supported by grants # 156969 (RJ-C), 239930 (RM-S) and CB-253281(VM-RV) from CONACyT, Mexico, Project No. 8216.20-P ‘Tecnológico Nacional de Mexico’ (TecNM, México), PID2019-104205GB-C22 CAMELIA-MICRO and RTI2018-094867-B-I00 MINELIFE (EG-T). We would like to thank CONACyT for the doctoral fellowship assigned to Betsy A. Peña-Ocaña (No. 465358), C.I. Ovando-Ovando (No. 476981) and W. Gutiérrez-Sarmiento (No. 636047)

Author information

Affilations

Tecnologico Nacional de México / IT Tuxtla Gutierrez, Tuxtla Gutiérrez, Chiapas, Mexico

Betsy Anaid Peña-Ocaña, César Iván Ovando-Ovando, Wilbert Gutiérrez-Sarmiento & Víctor Manuel Ruíz-Valdiviezo.

Microbiome Analysis Laboratory, Systems Biology Department, Centro Nacional de Biotecnología, CSIC, Madrid, Spain

Javier Tamames

Laboratorio de Microbiomica, Escuela Nacional de Estudios Superiores Unidad Morelia, UNAM, Morelia, Michoacan, Mexico

Luis Eduardo Servín-Garcidueñas

Centro de Astrobiología, CAB (INTA-CSIC), Torrejón de Ardoz, Spain

Elena González-Toril

Departamento de Bioquímica, Instituto Nacional de Cardiología, Mexico City, Mexico

Ricardo Jasso-Chávez

CONTRIBUTIONS

BAPO: Developed experiments; BAPO, VMRV and RJC: designed the study. BAPO, ClOO, WGS and VMRV: Participated the collection of the biological; BAPO, ClOO, RJC, LESG, JT and EGT: analyzed all the data. All authors discussed, read, and approved the final manuscript.

References

1. Stetter KO. Extremophiles and their adaptation to hot environments. FEBS Lett. 2003; 452:22–5.
2. Candela-Becerra LJ, Toyos G, Suárez-Herrera CA, Castro-Godoy S, Agusto M. Thermal evolution of the Crater Lake of Copahue Volcano with ASTER during the last quiescence period between 2000 and 2012 eruptions. J Volcanol Geotherm Res. 2020;392:106752.
3. López-Loera H, Macías JL, Espíndola JM, Arce JL, Layer PW, Torres-Gaytan DE. The Santa Fe Intrusion and Other Magmatic Bodies Under the Chichón Volcano Area (Mexico): Inferences from Aeromagnetic and New Petrologic-Geochronologic Data. Geophys.2020; 859–95.
4. Armienta MA, De la Cruz-Reyna S, Ramos S, Ceniceros N, Cruz O, Aguayo A, Hydrogeochemical surveillance at El Chichón volcano crater lake, Chiapas, Mexico. J Volcanol Geotherm Res. 2014; 285:118–28.
5. Cuoco E, De Francesco S, Tedesco D. Hydrogeochemical dynamics affecting steam-heated pools at El Chichón Crater (Chiapas - Mexico). Geofluids. 2013;13:331–43.
6. Peña-Ocaña BA, Velázquez-Ríos IO, Alcántara-Hernández RJ, Ovando-Ovando CI, Rincón-Rosales R, Gutiérrez-Miceli FA, Changes in the concentration of trace elements and heavy metals in el chichón crater lake active volcano. Polish J Environ Stud. 2020;30:295–304.
7. Casas AS, Armienta MA, Ramos S. Sulfur speciation with high performance liquid chromatography as a tool for El Chichón volcano, crater lake monitoring. J South Am Earth Sci. 2016;72:241–9.

8. Rincón-Molina CI, Hernández-García JA, Rincón-Rosales R, Gutiérrez-Miceli FA, Ramírez-Villanueva DA, González-Terreros E, et al. Structure and Diversity of the Bacterial Communities in the Acid and Thermophilic Crater-Lake of the Volcano “El Chichón”, Mexico. Geomicrobiol J. 2019;36:97–109.
9. Bolger AM, Lohse M, Usadel B. Trimmomatic: A flexible trimmer for Illumina sequence data. Bioinformatics, Oxford University Press. 2014;30:2114–20.
10. Tamames J, Puente-Sánchez F. SqueezeMeta, a highly portable, fully automatic metagenomic analysis pipeline. Front Microbiol. 2019;10.
11. Li D, Liu C-M, Luo R, Sadakane K, Lam T-W. MEGAHIT: an ultra-fast single-node solution for large and complex metagenomics assembly via succinct de Bruijn graph. Oxford University Press. 2015;31:1674–6.
12. Schmieder R, Edwards R. Quality control and preprocessing of metagenomic datasets. 2011;27:863–4.
13. Seemann T. Prokka: Rapid prokaryotic genome annotation. 2014;30:2068–9.
14. Wang Q, Garrity GM, Tiedje JM, Cole JR. Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl Environ Microbiol. 2007;73:5261–7.
15. Laslett D, Canback B. ARAGORN, a program to detect tRNA genes and tmRNA genes in nucleotide sequences. Nucleic Acids Res. 2004;32:11–6.
16. Hyatt D, Chen GL, LoCascio PF, Land ML, Larimer FW, Hauser LJ. Prodigal: Prokaryotic gene recognition and translation initiation site identification. BMC Bioinformatics. 2010;11:119.
17. Clark K, Karsch-Mizrachi I, Lipman DJ, Ostell J, Sayers EW. GenBank. Nucleic Acids Res. 2016;44:D67–72.
18. Huerta-Cepas J, Szklarczyk D, Forslund K, Cook H, Heller D, Walter MC, et al. EGGNOG 4.5: A hierarchical orthology framework with improved functional annotations for eukaryotic, prokaryotic and viral sequences. Nucleic Acids Res. 2016;44:D286–93.
19. Kanehisa M, Goto S. KEGG: Kyoto Encyclopedia of Genes and Genomes. Nucleic Acids Res. 2000. p.27–30.
20. Buchfink B, Xie C, Huson DH. Fast and sensitive protein alignment using DIAMOND. Methods. 2014. p. 59–60.
21. Eddy SR. Accelerated profile HMM searches. PLoS Comput Biol. 2011;7.
22. Finn RD, Coggill P, Eberhardt RY, Eddy SR, Mistry J, Mitchell AL, et al. The Pfam protein families database: Towards a more sustainable future. Nucleic Acids Res. 2016;44:D279–85.
23. Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. Nat Methods. 2012;9:357–9.
24. Wu YW, Simmons BA, Singer SW. MaxBin 2.0: An automated binning algorithm to recover genomes from multiple metagenomic datasets. 2016;32:605–7.
25. Kang DD, Li F, Kirton E, Thomas A, Egan R, An H, et al. MetaBAT 2: An adaptive binning algorithm for robust and efficient genome reconstruction from metagenome assemblies. 2019;2019:e7359.

26. Sieber CMK, Probst AJ, Sharrar A, Thomas BC, Hess M, Tringe SG, et al. Recovery of genomes from metagenomes via a dereplication, aggregation and scoring strategy. Nat Microbiol. 2018;3:836–43.
27. Parks DH, Imelfort M, Skennerton CT, Hugenholtz P, Tyson GW. CheckM: Assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. Genome Res. 2015;25:1043–55.
28. Caspi R, Billington R, Fulcher CA, Keseler IM, Kothari A, Krummenacker M, et al. The MetaCyc database of metabolic pathways and enzymes. Nucleic Acids Res. 2018;46:D633–9.
29. Ye Y, Doak TG. A parsimony approach to biological pathway reconstruction/inference for genomes and metagenomes. PLoS Comput Biol. 2009;5.
30. Neuweger H, Persicke M, Albaum SP, Bekel T, Dondrup M, Hüser AT, et al. Visualizing post genomics data-sets on customized pathway maps by ProMeTra - Aeration-dependent gene expression and metabolism of Corynebacterium glutamicum as an example. BMC Syst Biol. 2009;3:82.
31. Sowers KR, Noll KM. Techniques for anaerobic growth. In: Fleischmann EM (eds.), editor. Archaea, A Lab Man. 1995. p. 15–55.
32. Wang X, Xia K, Yang X, Tang C. Growth strategy of microbes on mixed carbon sources. Nat Commun. 2019;10.
33. Comeau AM, Douglas GM, Langille MGI. Microbiome Helper: a Custom and Streamlined Workflow for Microbiome Research. mSystems.2017;2.
34. Kozich JJ, Westcott SL, Baxter NT, Highlander SK, Schloss PD. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the miseq illumina sequencing platform. Appl Environ Microbiol.2013;79:5112–20.
35. Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics.2011;27:2194–200.
36. [https://www.arb-silva.de/no_cache/download/archive/release_132/]. Accessed on 22-10-20
37. Lukhele T, Selvarajan R, Nyoni H, Mamba BB, Msagati TAM. Diversity and functional profile of bacterial communities at Lancaster acid mine drainage dam, South Africa as revealed by 16S rRNA gene high-throughput sequencing analysis. 2019;23:719–34.
38. Santiago-Martínez MG, Encalada R, Lira-Silva E, Pineda E, Gallardo-Pérez JC, Reyes-García MA. The nutritional status of Methanosarcina acetivorans regulates glycogen metabolism and gluconeogenesis and glycolysis fluxes. FEBS J. 2016;283:1979–99.
39. Jasso-Chávez R, Santiago-Martínez MG, Lira-Silva E, Pineda E, Zepeda-Rodríguez A, Belmont-Díaz J. Air-Adapted *Methanosarcina acetivorans* Shows High Methane Production and Develops Resistance against Oxygen Stress. PLoS One. 2015;10:e0117331.
40. King TE, Morris RO. [98] Determination of acid-labile sulfide and sulfhydryl groups. Methods Enzymol. 1967;10:634–41.
41. Lira-Silva E, Santiago-Martínez MG, García-Contreras R, Zepeda-Rodríguez A, Marín-Hernández A, Moreno-Sánchez R, et al. Cd2+ resistance mechanisms in Methanosarcina acetivorans involve the

increase in the coenzyme M content and induction of biofilm synthesis. Environ Microbiol Rep. 2013;5:799–808.

42. Parks DH, Tyson GW, Hugenholtz P, Beiko RG. STAMP: Statistical analysis of taxonomic and functional profiles. Bioinformatics. 2014;30:3123–4.
43. Yang L, Xiu H, Cheng-xu Z, Yu-juan Z. Application of R language graphics in biological research. J East China Norm Univ Sci. 2019;2019:124.
44. Casadevall TJ, de la Cruz-Reyna S, Rose WI, Bagley S, Finnegan DL, Zoller WH. Crater lake and post-eruption hydrothermal activity, El Chichón Volcano, Mexico. J Volcanol Geotherm. 1984;23:169–91.
45. Servín-Garcidueñas LE, Martínez-Romero E. Draft genome sequence of the Sulfolobales archaeon AZ1, obtained through metagenomic analysis of a Mexican hot spring. Genome Announc. 2014;2.
46. Hedrich S, Johnson DB. Acidithiobacillus ferridurans sp. nov., an acidophilic iron-, sulfur- and hydrogen-metabolizing chemolithotrophic gammaproteobacterium. Int J Syst Evol Microbiol. 2013;63:4018–25.
47. González-Toril E, Santofimia E, López-Pamo E, García-Moyano A, Aguilera Á, Amils R. Comparative microbial ecology of the water column of an extreme acidic pit lake, Nuestra Señora del Carmen, and the Río Tinto basin (Iberian Pyrite Belt). Int Microbiol. 2015;17:225–33.
48. Abramov SM, Tejada J, Grimm L, Schädler F, Bulaev A, Tomaszewski EJ, et al. Role of biogenic Fe(III) minerals as a sink and carrier of heavy metals in the Rio Tinto, Spain. Sci Total Environ.2020;718.
49. Lopez Bedogni G, Massello FL, Giaveno A, Donati ER, Urbieta MS. A Deeper Look into the Biodiversity of the Extremely Acidic Copahue volcano-Río Agrio System in Neuquén, Argentina. Microorganisms. 2019;8:58.
50. Massello FL, Chan CS, Chan KG, Goh KM, Donati E, Urbieta MS. Meta-analysis of microbial communities in hot springs: Recurrent taxa and complex shaping factors beyond ph and temperature. Microorganisms. 2020;8:1–18.
51. Johnson CM, Beard BL, Roden EE. The Iron Isotope Fingerprints of Redox and Biogeochemical Cycling in Modern and Ancient Earth. Annu Rev Earth Planet Sci. 2008;36:457–93.
52. Zhang C, He M, Ouyang W, Lin C, Liu X. Influence of Fe(II) on Sb(III) oxidation and adsorption by MnO2 under acidic conditions. Sci Total Environ.2020;724:138209.
53. Sun R, Li Y, Lin N, Ou C, Wang X, Zhang L, et al. Removal of heavy metals using a novel sulfidogenic AMD treatment system with sulfur reduction: Configuration, performance, critical parameters and economic analysis. Environ Int. 2020;136:105457.
54. Florentino AP, Brienza C, Stams AJM, Sánchez-Andrea I. Desulfurella amilsii sp. nov., a novel acidotolerant sulfur-respiring bacterium isolated from acidic river sediments. Int J Syst Evol Microbiol [Internet]. Microbiology Society. 2016;66:1249–53.
55. Itoh T, Onishi M, Kato S, Iino T, Sakamoto M, Kudo T, et al. *Athalassotoga saccharophila* Nov., sp. Nov., isolated from an acidic terrestrial hot spring, and proposal of mesoaciditogales ord. nov. and mesoaciditogaceae fam. Nov. in the phylum thermotogae. Int J Syst Evol Microbiol. 2016;66:1045–51.

56. Willis G, Nancucheo I, Hedrich S, Giaveno A, Donati E, Johnson DB. Enrichment and isolation of acid-tolerant sulfate-reducing microorganisms in the anoxic, acidic hot spring sediments from Copahue volcano, Argentina. FEMS Microbiol Ecol. 2019;95.
57. Arce-Rodríguez A, Puente-Sánchez F, Avendaño R, Martínez-Cruz M, de Moor JM, Pieper DH, et al. Thermoplasmatales and sulfur-oxidizing bacteria dominate the microbial community at the surface water of a CO 2 -rich hydrothermal spring located in Tenorio Volcano National Park, Costa Rica. Extremophiles. 2019;23:177–87.
58. Martins TP, Ramos V, Hentschke GS, Castelo-Branco R, Rego A, Monteiro M, et al. The Extremophile *Endolithella mcmurdoensis* et sp. nov. (Trebouxiophyceae, Chlorellaceae), A New Chlorella -like Endolithic Alga From Antarctica. Verbruggen H, editor. J Phycol. 2020;56:208–16.
59. Luo H, Lin Y, Gao F, Zhang CT, Zhang R. DEG 10, an update of the database of essential genes that includes both protein-coding genes and noncoding genomic elements. Nucleic Acids Res. 2014;42.
60. Forterre P. The origin of viruses and their possible roles in major evolutionary transitions. Virus Res.2006. p. 5–16.
61. Carroll SA, Towner JS, Sealy TK, McMullan LK, Khristova ML, Burt FJ, et al. Molecular Evolution of Viruses of the Family Filoviridae Based on 97 Whole-Genome Sequences. J Virol.2013;87:2608–16.
62. Sharma V, Colson P, Chabrol O, Pontarotti P, Raoult D. Pithovirus sibericum, a new bona fide member of the “Fourth TRUC” club. Front Microbiol.2015;6.
63. Zeikus JG, Ben-Bassat A, Hegge PW. Microbiology of methanogenesis in thermal, volcanic environments. J Bacteriol. 1980;143:432–40.
64. Lyu Z, Shao N, Akinyemi T, Whitman WB. Methanogenesis. Curr. Biol. 2018. p. R727–32.
65. Löhr AJ, Bogaard TA, Heikens A, Hendriks MR, Sumarti S, Van Bergen MJ, et al. Natural pollution caused by the extremely acidic crater lake Kawah Ijen, East Java, Indonesia. Environ Sci Pollut Res Int. 2005;12:89–95.
66. Medrano-Santillana M, Souza-Brito EM, Duran R, Gutierrez-Corona F, Reyna-López GE. Bacterial diversity in fumarole environments of the Paricutín volcano, Michoacán (Mexico). Extremophiles. 2017;21:499–511.
67. Ward L, Taylor MW, Power JF, Scott BJ, McDonald IR, Stott MB. Microbial community dynamics in Inferno Crater Lake, a thermally fluctuating geothermal spring. ISME J. 2017;11:1158–67.
68. Brito EMS, Villegas-Negrete N, Sotelo-González IA, Caretta CA, Goñi-Urriza M, Gassie C, et al. Microbial diversity in Los Azufres geothermal field (Michoacán, Mexico) and isolation of representative sulfate and sulfur reducers. Extremophiles. 2014;18:385–98.
69. Inskeep WP, Rusch DB, Jay ZJ, Herrgard MJ, Kozubal MA, Richardson TH, et al. Metagenomes from high-temperature chemotrophic systems reveal geochemical controls on microbial community structure and function. PLoS One. 2010;5:e9773.
70. Sánchez-Andrea I, Rodríguez N, Amils R, Sanz JL. Microbial diversity in anaerobic sediments at Río Tinto, a naturally acidic environment with a high heavy metal content. Appl Environ Microbiol. 2011;77:6085–93.

71. Wang CL, Maratukulam PD, Lum AM, Clark DS, Keasling JD. Metabolic engineering of an aerobic sulfate reduction pathway and its application to precipitation of cadmium on the cell surface. Appl Environ Microbiol. 2000;66:4497–502.
72. Rabus R, Venceslau SS, Wöhlbrand L, Voordouw G, Wall JD, Pereira IAC. A Post-Genomic View of the Ecophysiology, Catabolism and Biotechnological Relevance of Sulphate-Reducing Prokaryotes. Adv Microb Physiol. 2015. p. 55–321.
73. Rückert C. Sulfate reduction in microorganisms — recent advances and biotechnological applications. 2016. p. 140–6.
74. Duarte AG, Catarino T, White GF, Lousa D, Neukirchen S, Soares CM, et al. An electrogenic redox loop in sulfate reduction reveals a likely widespread mechanism of energy conservation. Nat Commun. 2018;9.

Figures

Figure 1

Location in the Crater-Lake of the active volcano El Chichón. ● Sample 1 (35 °C), ▲Sample 2 (40 °C), ■ Sample 3 (45 °C), ♦ Sample 4 (82 °C) and ▼ Sample 5 not determined.

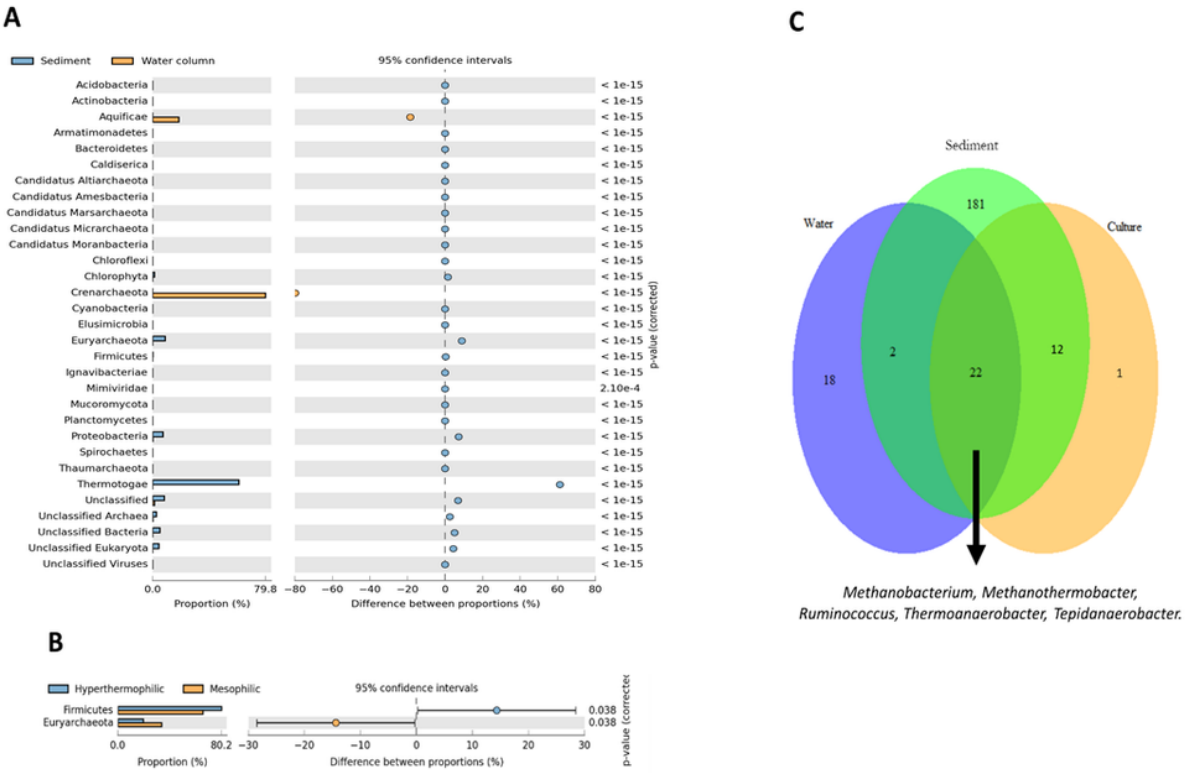

Figure 2

Volcano community composition. A Relative abundance of taxonomic phyla identified from environmental site (Whole metagenomics analysis) and B microbial cultures in the laboratory (Metabarcoding analysis). C Illustrative Venn diagram of common generates in both environmental and laboratory culture samples.

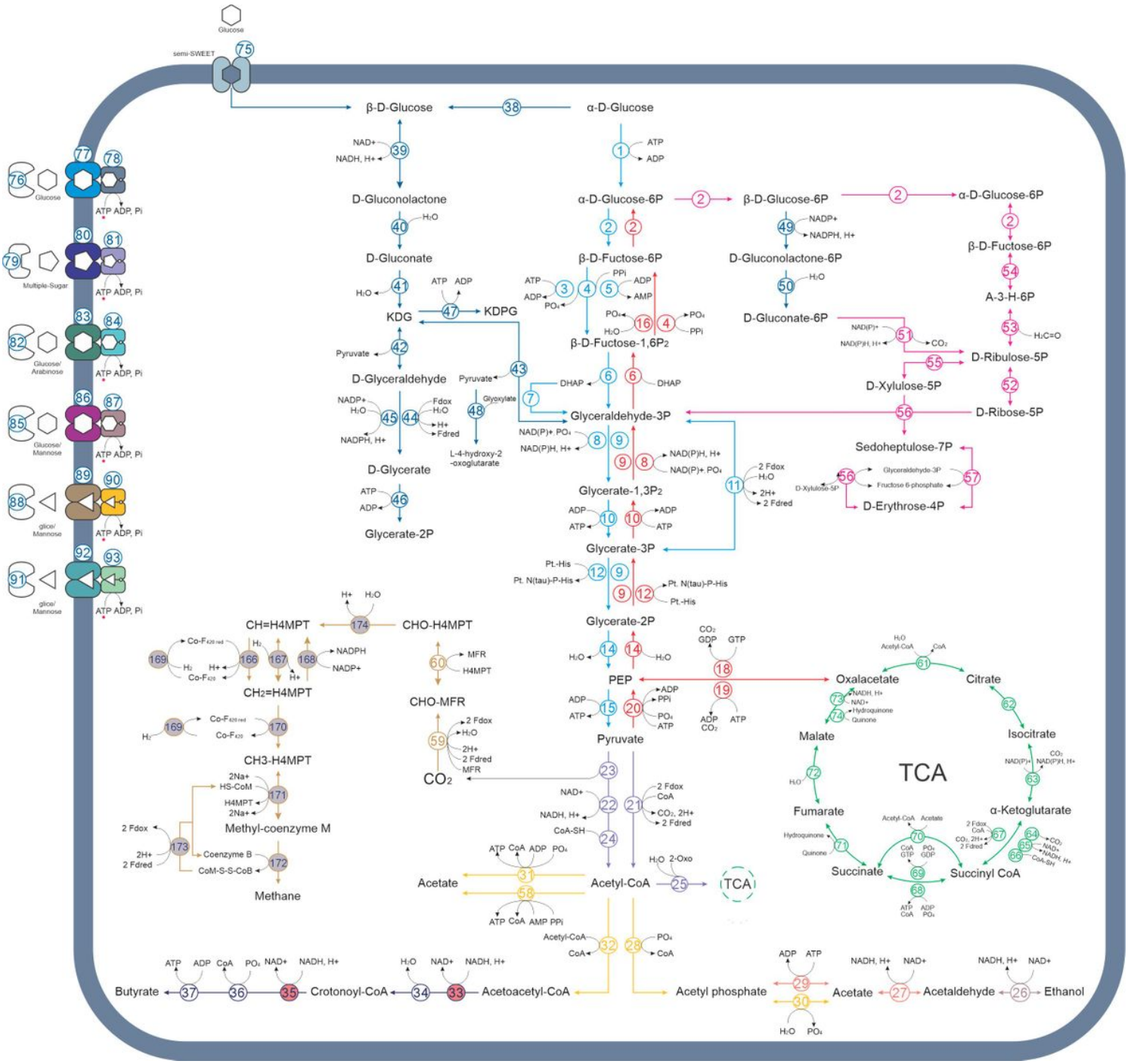

Figure 3

Metabolic map constructed from genomic evidence recovered from mesophilic and hyperthermophilic samples from the natural environment. In light blue, via glycolysis. In pink, the Via Entner-Doudoroff. In red, gluconeogenesis. In green, the tricarboxylic acid cycle (Krebs). In yellow, acetogenesis. In purple and pink the alcoholic and butyric fermentation. In orange, methanogenesis. Each enzyme is represented by a number, which can be found in Additional Table 1. To initiate carbon metabolism, it is necessary to internalize the carbon source into the cell, a process that can be carried out by active transport (ATP dependent), to phosphorylate the substrate (represented in 77-93). The end process of the degradation of organic matter occurs in methanogenesis (59, 60, 89-96), which is an energy-generating process in

methanotrophic archaea, in addition, the methane produced is re-oxidized to CO2 by methanotrophics, and in this way, there is reincorporation of CO2 into the environment and the carbon cycle is maintained favoring CO2-fixing microorganisms.

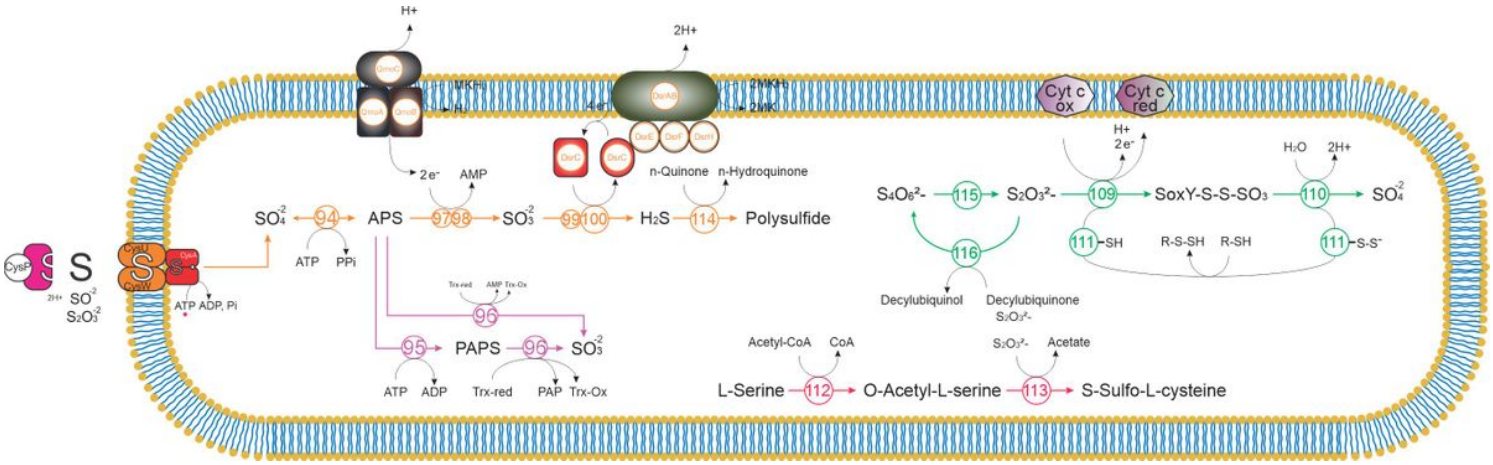

Figure 4

Metabolic map, sulfate reduction and sulfide oxidation, constructed from genomic evidence recovered from mesophilic and hyperthermophilic samples from the natural environment. In orange, the non-assimilatory (dissimilatory) reduction sulfate pathway. In purple, the assimilatory reduction sulfate pathway. In pink, the synthesis of sulfur amino acids. In green, sulfide oxidation. Each enzyme is represented by a number, which can be found in Additional Table 1.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- AdditionalTable1.xlsx
- AdditionalFileTable2.docx
- AdditionalFileText1.docx