Alkalo-Thermophilic Microbial Diversity of the Tecozautla Geyser, México

Jesus Alberto Segovia-Cruz
Universidad Politécnica de Pachuca
Valeria Souza
Universidad Nacional Autónoma de México
Yuridia Mercado-Flores
Universidad Politécnica de Pachuca
Miguel Angel Anducho-Reyes
Universidad Politécnica de Pachuca
Genaro Vargas-Hernández
Universidad Politécnica de Pachuca
Alejandro Téllez-Jurado (tejual@hotmail.com)
Universidad Politecnica de Pachuca https://orcid.org/0000-0002-5491-3679

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Abstract

Microbial mats have been studied in many thermal systems; the most iconic is Yellowstone. In Mexico, the information on microbial mats is scarce and therefore novel. In this research, the thermophilic microbial composition of samples from four areas of the Tecozautla geyser, Hidalgo, Mexico, was studied: sediments (GD), salt deposits (GA), and microbial mats (GB and GC). The samples were taken at the outlet of the geyser (94 °C) and in storage pools with temperatures of 61.5-65 °C. Sequencing of the 16S rRNA gene amplicons was carried out, obtaining 1,425,506 readings, and was analyzed through the Quantitative Insights Into Microbial Ecology software package version 2 (qiime2). 32 phyla were identified in the four samples being the most representative for the GA sample: *Armatimonadetes*, *Chloroflexi*, *Cyanobacteria*, and *Thermi*, with abundances of 46.35, 19.18, 3.27, and 1.82 %, respectively. For the GB sample, they were *Proteobacteria*, *Bacteroidetes*, *Cyanobacteria*, *Spirochaetes*, *Thermi*, and *Firmicutes* with abundances of 25.23, 22.04, 20.42, 12.31, 4.56, and 1.32 %, respectively. For the GC sample, abundances of 55.60, 9.85, 7.04, 7.01, and 6.15 % were observed for the phylum *Chloroflexi*, *Armatimonadetes*, *Proteobacteria*, *Cyanobacteria*, and *Acidobacteria*, respectively. Finally, for the GD sample, the most abundant phyla were *Chloroflexi* (36.10 %), *Cyanobacteria* (17.13 %), *Armatimonadetes* (15.59 %), *Proteobacteria* (5.45 %), and *Nitrospirae* with (3.21 %). The metabolic functionality of the microbial communities present in the samples was inferred using the 16S rRNA amplicons. This work represents the first report of the microbial communities present in the Tecozautla geyser.

Introduction

“Extremophilic” microorganisms are defined as those that inhabit environments hostile to humans. Most of the microorganisms capable of growing in extreme conditions of temperature, pH, pressure, radiation, etc., meet the characteristics of being known as extremophilic microorganisms [1]. The evolutionary adaptations that allow them to survive such extreme conditions include modifications in their cellular components, such as the increase in their electrostatic interactions, hydrogen bonds, and hydrophobic interactions in their proteins, to mention a few [2]. Several types of Extremophilic microorganisms can be classified depending on the environment where they grow: high-temperature conditions, high salinity, few nutrients, alkaline and acidic pHs, etc. For example, thermophilic microorganisms are one of them; they grow at temperatures above 45 ° C, as well as hyperthermophiles microorganism that has growth temperature above 80 ° C [1]. Thermophilic organisms find each other distributed in different parts of the planet, mainly in places with volcanic activity, such as hot springs, underwater hydrothermal systems, systems of abyssal thermal openings, geysers, etc.

Most of the microorganisms that grow in extreme environments can associate and, on many occasions, secrete exopolysaccharides whose function is to allow adherence to solid surfaces. These associations generate very complex ecological groups [3]. These mats, in turn, fulfill the function of protecting microorganisms through a physical barrier, contributing to water retention and, due to their anionic nature, allows the exchange with metal cations and nutrients [4].

The word geyser comes from the old Icelandic verb, gjose, which means to erupt. It specifically refers to a hot water tank that intermittently and explosively expels part or all of its contents [5], as is the case with the Tecozautla geyser, located in the municipality the same name in the state of Hidalgo, Mexico. The geyser belongs to the Pathé geothermal zone, located on the border between the states of Hidalgo and Querétaro, about 35 kilometers in a straight line to the northwest with the municipality of San Juan del Río, Querétaro, and 20 km north of the city from Huichapan, Hidalgo state. The area belongs to the physiographic province of the Mexican Volcanic Belt, very close to its limits with the Sierra Madre Oriental [6]. The objective of this work was to study the microbial communities present in the microbial mat formed from the hot springs emanating from the Tecozautla geyser.

Materials And Methods

Description of the sampling and sampling site

The Tecozautla geyser this locates at an altitude of 1665 meters (20 ° 34'40.8 "N 99 ° 41'35.0" W) within the spa known as “El Geiser” in the community of Uxdejhé, belonging to the municipality of Tecozautla, Hidalgo state, Mexico. Samples were taken from four zones within the geyser: salt deposits (GA), microbial mats I (GB), microbial mats II (GC), and sediments + microbial mat (GD), recording temperatures and pH of the sampling zones. The collected samples were placed in sterile 50 mL Falcon tubes and transported in containers with ice. The samples were stored at -20 ° C until analysis.

Water analysis from the Tecozautla geyser and determination of total sulfur

Twenty-three physicochemical parameters of the geyser water were determined: conductivity, pH, TSD, Cl^{-}, NO^{3-}, NO^{2-}, HCO^{3-}, $SO4^{2-}$, ammonia N, Al, As, Ba, Cd, CN-, Cu, Cr, Fe, Mn, Pb, Zn, active substances to methylene blue (SAAM), Na^{+} and Hg, according to NOM-127-SSA1-1994. The samples were analyzed in the Academic Area of Chemistry of the Autonomous University of the State of Hidalgo, Mexico. The determination of total sulfur was carried out by emission spectroscopy with inductive plasma coupling (ICP) in a Perkin Elmer model Optima 8300 XL equipment. All the water samples to be analyzed were taken to 25 mL with deionized water (18 µS/cm). To determine the total sulfur, a standard sulfur stock solution was prepared at a final elemental sulfur concentration of 50 mg/L in 5 % HNO_3. For the calibration curve, dilutions of 2 to 10 mg/L were made, and were read in a spectrophotometer (Thermo) at 182 nm.

Metagenomic DNA extraction

Two DNA extraction methodologies were used since the samples presented different compositions, such as high exopolysaccharides and salts, making them difficult to obtain. The extraction of metagenomic DNA from the GA, GB, and GC samples was carried out with the following methodology: The lysis of the samples was carried out by adding of 0.5 g of glass beads (425–600 µm), 0.5 mL of DNA, 600 µL of a sodium phosphate solution (120 mM, pH 8), 1 % (p/v) of PVP (polyvinylpyrrolidone), 400 µL of Tris-

phenol solution (pH 8), 40 µL of SDS (20 %); followed by 3 cycles of shake-ice on a Tissue Lyses LT (QIAGEN) for 30 seconds at maximum speed (50 Hz). The samples were then centrifuged at 13,000 rpm in 1 minute. The supernatant was transferred to an HTP (hydroxyapatite) column by centrifuging at 1500 rpm for 2 minutes, followed by three continuous washes of the column with 500 µL of 120 mM sodium phosphate pH 7.2, each time centrifuging at 1500 rpm for 6 min. The DNA was eluted by adding 400 µL of a dipotassium phosphate solution (300 mM, pH 7.2), centrifuging at 1500 rpm for 6 minutes. Once eluted, the DNA solution was transferred to a G50 Sephadex column and centrifuged at 13,000 rpm for 1 minute. Subsequently, the DNA was precipitated overnight at -20°C with the addition of 40 µL of a sodium acetate solution (3 M) and 1 mL of absolute cold ethanol. The DNA was recovered by centrifugation at 13,000 rpm for 10 minutes. Subsequently, the pellet was washed with 70 % alcohol and resuspended in a volume of 20–50 µL of DNAase-free water.

For the extraction of metagenomic DNA from the GD sample, the following protocol was carried out. To obtain a homogeneous mixture, approximately 0.5 g of the sample were macerated with liquid nitrogen and extraction buffer (100 mM Tris-HCl, 20 mM NaCl, 100 mM EDTA pH 8, 1 % (p/v) PVP), with 1 % cetyltrimethylammonium bromide (CTAB) at 6 %, following three continuous freeze-thaw cycles with liquid nitrogen and 65°C. Subsequently, the samples were incubated for 30 min at 37°C with lysozyme (30 mg/mL). Then 0.2 mL of 10 % sodium dodecyl sulfate (SDS) and proteinase K (10 mg/mL) were added for 2 h at 65°C. The nucleic acids were extracted twice with a solution of phenol: chloroform: isoamyl alcohol (25:24:1) and once with chloroform: isoamyl alcohol (24:1), recovering the supernatant liquid after each centrifugation (10,000 x g for 10 min). The DNA was precipitated with 0.6 volumes of cold isopropanol, 3M sodium acetate (1/10 v), and centrifuged at 22700 x g for 15 minutes at 4°C. The DNA pellet obtained was washed with ethanol cold to 7 %, centrifuged, and the excess alcohol was allowed to evaporate. The DNA was resuspended in 30 µL of DNAase-free water. Finally, to purify the DNA, the commercial kit Zymo BIOMICS DNA (Zymo) was used. The DNA quality was verified by 0.8 % agarose gel electrophoresis and stained with EpiQuick DNA Stain (10 µL/100 mL). DNA concentrations were determined using a Nanodrop Lite spectrophotometer (Thermo Scientific).

16S rRNA sequencing

The purified DNA samples were used as a template to amplify the 16S rRNA gene using primers from the V3-V5 regions, according to the Illumina MiSeq protocol. The first universal 357-F (5´-CTCCTACGGGAGGCAGCAG – 3´) and CD-R (5´-CTTGTGCGGGCCCCCGTCAATTC-3´) [7] were used.

For the GA, GB, and GC samples a 2x150 run format was used, and for the GD sample 2x300. To carry out the amplification, the reaction mixture was adjusted to a final reaction volume of 25 µL with 5 µL (10 µM) of primer F, 5 µL (10 µM) of primer R, 2.5 µL of DNA (5 ng/µL in 10 mM Tris pH 8.5), 12.5 µL of 2xKAPA HiFi HotStart Ready Mix. The PCR consisted of an initial denaturation at 95°C (3 min), followed by 25 cycles for denaturation (95°C, 30 s) alignment (55°C, 30 s), and extension (72°C, 30 s). A final extension to 72°C, 5 min was used (https://www.illumina.com/content/dam/illumina-support/documents/documentation/chemistry_documentation/16s/ 6s-metagenomic-library-prep-guide-15044223-b.pdf). The Illumina libraries were purified with the DNA Clean & Concentrator purification kit

(Zymo Research, Irvine, CA, USA). The DNA sequencing was done in the National Laboratory of Genomics for Diversity (LANGENBIO, CINVESTAV-Irapuato, México).

Analysis of amplicon readings

The readings obtained were processed by TagCleaner to remove the initiators and ambiguous bases, consecutively Trimmomatic was used to filter the sequences, conserving those that had a length greater than 100 bp ≤ Q20 in the Forward direction of the V3 region, due to their quality and abundance, in addition to the fact that they could not be paired because they were very distant from each other.

QIIME2 v2019.1 software was used, the sequences were imported with Casava 1.8 single-end demultiplexed fastq, using a mapping file (.tsv) with the information of the samples. The analysis started with the DADA2 option, which consists of the purification and trimming of the sequences, carried out at 100 bp to preserve the greatest amount of information, as well as the elimination of errors in the sequencing. The identification and elimination of the chimeric sequences and the “borderline chimeras” were carried out using the uchime-de novo method, grouping them de novo in operative taxonomic units (OTU's) at 97% similarity threshold, using the VSEARCH algorithm for both cases. The taxonomic assignment of the sequences was carried out using a Bayesian classifier with the complement q2-feature-classifier using the Greengenes database (gg-13-8-99-nb-classifier.qza) [8]. Additionally, graphs were made to describe the relative abundance at different taxonomic levels using ggplot2 in the R software (https://www.r-graph-gallery.com).

The diversity analyzes were carried out taking as a parameter the depth of the sampling (rarefaction) starting from the sample with the lowest readings obtained. The alpha diversity, the observed OTU's metrics, Chao 1, Shannon, and Simpson, were determined. In addition, Good coverage was determined (www.qiime2.org). Beta diversity was determined with qiime diversity and plotted by Emperor, through principal coordinate analysis (PCoA), using Bray-Curtis decrease distance matrices. The MetaCoMET web tool was used to determine the central microbiome using an OTU table in BIOM v2.1.0 format that contained the community abundance data, as well as its taxonomy (https://probes.pw.usda.gov/MetaCoMET).

Prediction of metabolic functions with PICRUSt-Galaxy

Prediction of metabolic functions was performed using PICRUSt. The analysis began with the single-end.qza file from where the chimeric sequences and the “borderline chimeras” were eliminated, using the uchime-de novo method, the sequences were grouped to closed-references in operative taxonomic units (OTU's) at 97 % identity. The generated file was exported to the BIOM v2.1.0 format and the taxonomic assignment to 97 % identity with Greengenes data was attached. It was then uploaded to the PICRUSt platform, (http://galaxy.morganlangille.com/), where the functional metabolic prediction was performed (https://forum.qiime2.org/t/how-to-create-a- feature-table-with-qiime2-for-picrust-with-the-taxonomic-assignment/2526/6).

Results

Description of the collection area

The location of the Tecozautla geyser is shown in Fig. 1A. The collection was carried out in June. In Figs. 1B and 1C, the sampling zones in the geyser are shown. The samples were collected in four areas of the Tecozautla geyser. The GA sample was located on the tube of the outlet water of the geyser, it had a composition with a high content of white salts with green areas, registering a temperature of 65°C (Fig. 1B: GA). The GB sample corresponds to a microbial mat found in the soil directly receiving the water from the geyser, it had the characteristic of having white, brown, and green colors in addition to presenting a rigid and fibrous structure (65°C) (Fig. 1B: GB). The GC sample corresponded to a microbial mat of reddish, yellow, green, pink, white, and brown color, with a semisolid structure (muddy) with small fibrous sections, found in a heat flux of 61.5°C on a rock (Fig. 1B: GC). Finally, the GD sample corresponded to the sediments and microbial mats submerged in the left outlet of the geyser, with constant turbulence due to the continuous outlet of water (62°C) (Fig. 1C: GD).

Determination of the physicochemical parameters of the geyser water and total sulfur

The results obtained from the chemical composition of the Tecozautla geyser are presented in Table 1. It was observed that the water presented a conductivity of 1215 µmoh/cm and 82 mg/L of Total Dissolved Solids. The pH was between 9.24 and 10 indicating alkaline waters. The presence of total sulfur (47 ± 2.51 mg/L) sodium (195 mg/L) and chlorides (156.33 mg/L) predominated. Elements such as Al, As, Ba, Cd, Cr, Mn, Pb, Zn, and Hg were not detected. The water temperature at the time of sampling was 94°C at the source. Finally, sulfates (28.6 mg/L), carbonates (16.29 mg/L) and a minimal concentration of nitrates (0.2 mg/L) were detected.

Table 1
Results of the physicochemical characterization of the Tecozautla geyser water.

Parameters	Concentration
Conductivity	1215 µMohs
pH	9.24-10
TSD	82*
Cl^-	156.33*
NO_3^-	0.2*
NO_2^-	0.0*
SO_4^{2-}	28.6*
S total	47.89 ± 2.51*
HCO_3^-	16.29*
N ammoniacal	0.1*
Al	0.0
As	0.0
Ba	0.0
Cd	0.0
CN^-	0.1*
Cu	0.1*
Cr	0.0
Fe	0.1*
Mn	0.0
Pb	0.0
Zn	0.0
SAAM	0.0
Na^+	195*

Parameters	Concentration
Hg	0.0

*mg/L

Taxonomic assignments of the readings and thermophilic diversity

The 4 samples sequenced from the Tecozautla geyser, resulted in a total of 5,660,646 raw paired-end (PE) readings, of which 2,830,823 were found in the forward direction, with these sequences it was carried out with the bioinformatic analysis. After removal of the primers with TagCleaner and quality filtering with Trimmomatic, 1,589,850 readings were obtained. DADA2 was used for removal of chimeras, borderline chimeras, and clustering of sequences de novo at 97 % similarity; obtaining a total of 1,425,506 readings of which 1,178,144 corresponded to GA, 1,668 to GB, 43,769 to GC, and 201,925 to GD. The OTUs obtained for each sample were 434, 75, 130, and 648, for GA, GB, GC, and GD, respectively.

In the 4 samples, a total of 30 bacterial and 2 archeal phyla (in GA and GD), 70 classes, 79 orders, 72 families, and 44 genera were identified and 2 % for sequences not classified in GA and 0.10 % for GD was observed. In addition, bacteria without taxonomic assignment were found with percentages of 26.75 %, 8.40 %, 5.99 %, and 12.83 %, for the GA, GB, GC, and GD samples respectively.

In Fig. 2, the relative abundances are shown at the level of phylum (Figs. 2: A) and class (Figs. 2: B) of the four areas sampled. The bacterial communities within each site were different from each other, where the most representative phyla for the GA sample were *Armatimonadetes*, *Chloroflexi*, *Cyanobacteria*, and *Thermi* with abundances of 46.35, 18.18, 3.27, and 1.82 % respectively. For the GB sample, the abundances were 25.23, 22.04, 20.42, 12.31, 4.56, and 1.32 % for the *Proteobacteria*, *Bacteroidetes*, *Cyanobacteria*, *Spirochaetes*, *Thermi*, and *Firmicutes* communities respectively. In the GC sample, the most abundant phyla were *Chloroflexi* (55.60 %), *Armatimonadetes* (9.85 %), *Proteobacteria* (7.04 %), *Cyanobacteria* (7.01%), and *Acidobacteria* (6.15 %). In the GD sample, *Chloroflexi* (36.10 %), *Cyanobacteria* (17.13 %), *Armatimonadetes* (15.59 %), *Proteobacteria* (5.45 %), and *Nitrospirae* (3.21 %) were the most abundant phyla. The most abundant phylum in the four samples analyzed was *Armatimonadetes*, which constituted 46.35 % of the total number of sequences in the GA sample, GC with 9.85 %, and GD with 15.59 %. The *OS-L* class was present in GA (46.35 %), GC (9.49 %), and GD (14.02 %).

Thermophilic diversity in the Tecozautla geyser

The *SJA-176* class was present in GC (0.05 %) and GD (0.10 %), which is a bacterium not yet cultivated, and finally the *Fimbriimonadia* class was found in the GC (~ 0.02 %) and GD (< 0.01 %), having as the only cultivated species *Fimbriimonas ginsengisoli*. Furthermore, at the class level, a relative abundance of unclassified sequences was obtained in the GC (0.29 %) and GD (1,44 %) samples.

The phylum *Cyanobacteria* is present in all samples of the Tecozautla geyser, with relative abundances of 3.27 %; 20.24 %; 7.01 %, and 17.13 % for GA, GB, GC, and GD respectively.

The class *Gloeobacterophycideae* is present only in the GA sample with 0.10 %, *Nostocophycideae* with 1.78 % in the GC sample, as well as *Oscillatoriophycideae* in the GA, GB, and GC samples with abundances of 5.94x10-3 %, 12.61 %, and 0.03 %, respectively. Finally, the *Synechococcophycideae* class was presented with abundances of 0.01 % for GA; 1.44 % for GB; 4.80 % for GC, and 1.54 % for GD.

The organisms found in the Tecozautla geyser at the genus level within the *Cyanobacteria* phylum were: *Gloeobacter* (GA), *Leptolyngbya* (GA and GB), and *Pseudanabaena* (GA, GC, and GD).

Another abundant group in the Tecozautla geyser samples was the phylum *Chloroflexi* with abundances of 19.28 % for GA, 1.26 % for GB, 55.61 % for GC, and 36.10 % for GD.

Several classes of microorganisms belonging to the phylum *Chloroflexi* were found in the Tecozautla geyser, among them *Anaerolineae* with a single cultured bacterium: *Anaerolinea thermolimosa*. This bacterium presented relative abundances of 18.53 %, 1.02 %, 1.75 %, and 2.84 % for GA, GB, GC, and GD samples, respectively. *Chloroflexia* presented abundances of 0.02, 0.24, 53.66, and 32.18 % for GA, GB, GC, and GD respectively. The class *Thermomicrobia* has an isolated and identified strain; *Thermomicrobium roseum* was found in GD (0.78 %); Two little-known classes were identified *Elin6529* in GD and *TK17* in GC and GD. Unidentified microorganisms were found at the class level with 0.62 %, 0.14 %, and 0.06 % for GA, GC, and GD, respectively.

The phylum *Deinococcus-Thermus* was identified in the Tecozautla geyser with abundances of 1.82 % for GA, 4.56 % for GB, 0.23% for GC, and 1.52 % for GD. Belonging to this phylum, the *Truepera* genus was identified in the GA sample and the *Trueperaceae* family in the GA, GC, and GD samples. The *Proteobacteria* were among the most abundant members with the presence of 0.19 %, 25.10 %, 7.04 %, and 5.45 % for the GA, GB, GC, and GD samples respectively. In the Tecozautla geyser all classes of *Proteobacteria* were identified: Alpha, Beta, Delta, Epsilon, and Gamma, which shows the great diversity in the microenvironments analyzed.

The phylum *Bacteroidetes* was found in all the samples analyzed from the Tecozautla geyser with relative abundances of 0.38 %, 22.04 %, 3.37 %, and 1.94 % for GA, GB, GC, and GD respectively. The phylum comprises the classes *Bacteroidia*, *Cytophagia*, *Flavobacteriia*, and *Sphingobacteriia*. Specifically, the classes of *Bacteroidia*, *Flavobacteriia*, *Rhodothermi* (GA and GB) were found in the geyser under study; *Cytophagia*, *Saprospirae* (GA-GD), and *SM1A07* (GB), in addition to the genera *Rubricoccus* (GA), KSA1 (GB) and *Rhodothermus* (GD).

Other taxonomic groups identified

The *Acidobacteria*, *Actinobacteria*, *Aquificae*, *Chlorobi*, *Dictyoglomi*, *Firmicutes*, *Gemmatimonadetes*, *Nitrospirae*, *Planctomycetes*, *Spirochaetes*, *Synergistetes*, *Tenericutes*, *Thermotogae*, and *Verrucomicrobia* phyla were identified in the Tecozautla geyser, and some groups that do not have cultured members so

their characteristics are unknown, such as *TM7*, *WS6*, *OC31*, *OD1*, *OP1*, *OP11*, *OP9*, *OctSpA1-106*, *NKB19* and *GAL15*, having relative abundances that do not exceed 1 %

Archeas

The archeal phyla found in the Tecozautla geyser were *Crenarchaeota* and *Euryarchaeota* in the GA and GD samples, respectively, with relative abundances < 0.001% in both samples, and with 0.002 % of unclassified sequences in the GD sample.

Taxonomic assignments at the genus level

For the relative abundances at the genus level, lower abundance percentages were observed because some of the sequences could not be classified at a lower taxonomic level. This situation suggests difficulties at DNA extraction due to the high content of exopolysaccharides and salts. Figure 2C shows the relative abundances of the microorganisms with the highest incidence.

In the GA sample, the genus *Caldilinea* showed an abundance of 0.76 %, *Thermus* of 0.24 %, *Gloeobacter* of 0.10 %, and *Truepera* of 0.06 %. The GB sample showed abundances of 6.35 %, 4.55 %, 1.31 %, 0.83 %, and 0.77 % for *Porifericola*, *Thermus*, *Halanaerobium*, *Blvii28*, and *Leptonema* respectively. The GC sample presented abundances for *Roseiflexus*, *Candidatus Chloracidobacterium*, *Thermodesulfovibrio*, and *Spirochaeta* of 51.57 %, 0.79 %, 0.42 %, and 0.36 % respectively. For the GD sample, some of the most abundant genera were *Roseiflexus* (5.81 %), *Chloroflexus* (3.48 %), *Thermodesulfovibrio* (2.03 %) and *Thermus* (1.23 %). The genera found in the 4 samples belong to *Hydrocarboniphaga* (GA), *Rhodovulum* (GC), *Novosphingobium*, *Acinetobacter*, *Geothermobacterium*, and *Desulfomicrobium* (GD).

Analysis of beta diversity and the central microbiome

The beta diversity analysis was carried out using the Bray-Curtis principal coordinate analysis (PcoA), presented in 3D, which determines the fraction of minimum abundance per sample of shared taxa. In Fig. 4A it is observed that the 4 samples are separated from each other, that is, that none of the samples is superimposed. The Venn diagram of the central microbiome (Fig. 4B) shows the set of OTUs shared by the four samples collected from the Tecozautla geyser. It is observed that all the samples share 1 OTU, which is related to unclassified bacterial diversity. The GA, GC, and GD samples share 11 OTUs, the GA, GB, and GD samples share 1 OTU. The GA and GD samples share 4 OTUs, GB and GD share 1 OTU, GC and GD share abundant diversity among themselves with 36 OTUs, possibly due to their physical proximity (sampling location). Each sample presents a large number of OTUs that they do not share; GA does not share 8 OTUs, GB 29 OTUs, GC 14 OTUs, and GD 118 OTUs. This result agrees with what was observed in beta diversity, that is, despite the fact that the physical location of each sample is relatively close, there are large differences in microbial diversity.

Prediction of functional profiles

The prediction of the functional profiles of the microbiome based on the 16S rRNA gene was carried out using the OTU table of assigned taxa and their relative distribution, in order to generate the relative

abundances of the functional categories based on sequenced genomes through analysis with PICRUSt, using the Kyoto Encyclopedia of Genes and Genomes (KEGG). The results obtained from the four samples of the Tecozautla geyser presented similar functions to each other, for level 2 (Fig. 5A). The highest abundances were the pathways related to membrane transport, carbohydrate metabolism, amino acid metabolism, energy metabolism, replication and repair, cofactor, and vitamin metabolism. These results show some of the main routes of microorganisms in the Tecozautla geyser, but at a not so specific level.

The most abundant metabolic pathways obtained for level 3 shown in Fig. 5B, were similar to each other, predominating ABC membrane transport, DNA repair and protein recombination, prediction of general functions, membrane transport, metabolisms of the methane, and purine metabolism. It is observed that oxidative phosphorylation stands out from energy metabolism, followed by routes that intervene in carbon fixation and methanogenic metabolism (Fig. 5C).

Discussion

Chemical composition of thermal water

According to the results obtained, the existence of sulfate ($SO4^{2-}$) may favor the presence of microorganisms that use sulfur in their metabolic pathways. Sulfate is considered one of the most important sources of energy in thermal ecosystems. There is a wide variety of microbial mats that thrive in geothermal hot springs and form unique ecosystems made up of physiologically and phylogenetically diverse microorganisms. In fact, hot springs that contain sulfur, such as the Tecozautla geyser, form microbial mats of various colors (white, gray, yellow, red, pink, green, etc.), which harbor different types of microorganisms such as lithotrophic oxidizing bacteria of sulfur, anoxygenic phototropic bacteria, cyanobacteria, and heterotrophic bacteria. Some of the major members of the microbial mats have been isolated, but most of the existing microbial diversity remains uncultivated and therefore unidentified, thus its physiological and ecological functions remain not fully understood [9].

Arsenic was not detected in the waters of the geyser, unlike that described by Núñez-Benítez [10] who reported 43,772 mg/L. Likewise, this author reported the presence of silice with a concentration of 112.65 mg/L. Although it is water from the same area, the composition of the water can be highly variable since it depends on the path you take from the depth of the geyser and the depletion of the minerals. The chemical composition of the water has a strong impact on the microbial communities since several of the minerals present are a source of energy for them, such as Arsenic, which favors the proliferation of species such as *Thermus aquaticus* and *Thermus thermophilus* who use it as a source of energy [11].

Thermophilic Diversity Analysis

The analysis of the sequences of the Tecozautla geyser allowed the identification of 30 bacterial phyla and only 2 archaea, 70 classes, 79 orders, 72 families, and 44 genera. An important observation is that bacteria without taxonomic assignment with high percentages in abundance were found in the 4 samples

analyzed, some authors have described similar results; the lack of taxonomic assignment can be attributed to the fact that their identification has not been possible due to the difficulty of their isolation preventing identification and characterization. It was observed that the phylum *Armatimonadetes* was the most abundant in the GD sample. The *Chthonomonadetes* class is part of this phylum which is present in the GD sample with ~ 0.02 % of the total sequences, the only cultivated strain is *Chthonomonas calidirosea*, which can grow in an interval of 50–73°C, pH 4.5–5.8 and 2 % NaCl [12]. The *OS-L* class, which corresponds to unidentified microorganisms, was also found in samples GA, GC, and GD, this class was found in Octopus Springs in Yellowstone National Park [13]. The species *Fimbriimonas ginsengisoli*, which belongs to the class *Fimbriimonadia*, can indirectly provide information about the class. It is a strict anaerobic mesophilic microorganism (https://www.genome.jp/Tools-bin/taxsummary).

The phylum *Armatimonadetes* is a moderately abundant and phylogenetically diverse bacterial group, little studied and phylogenetically associated with *Chloroflexi* [12]. Three cultured individuals are known, two previously mentioned and *Armatimonas rosea*. The candidate phylum *OP10* (now *Armatimonadetes*) was first described in the ecology study of Obsidian Pool, a geothermal hot spring in Yellowstone National Park [12].

Armatimonadetes have been identified in different areas of the world as in Tibetan hot springs (32–86°C, pH 3.0-9.5) [14], in Sungai Klah hot springs, Malaysia (50–110°C, pH 7.0–9.0) [15], in sediments and water from Great Boiling Spring, United States (62–82°C, pH 6.63–7.40) [16].

The phylum *Cyanobacteria* is one of the most abundant in most of the known microbial mats of thermal and non-thermal waters, participating in the fixation of carbon and nitrogen [17]. They have been identified in the hot springs of Garga, Russia, with temperatures of 70°C, 55°C, and 45°C; pH 8.0–9.0, with abundances ranging from ~ 10 to 60 %, in the 7 types of microbial mats studied [18]. Likewise, the phylum was found in abundances of 1.2–36.6 %, in all the samples of the microbial mats of the Camargo wetlands (Rhone Delta, southern France) that present temperatures of 13.5–18°C, and in non-thermal waters [19]. In the same way, they have been detected in the thermal areas of the Pakistanis Himalayas in conditions of 60–95°C with a pH of 6.2–9.4 [20], and in El Coquito, located in the Colombian Andes that present temperatures of 29°C in the source and pH of 2.7 [21]. The *Cyanobacteria* class was found in Ghats, India, with abundances of 96.42 % and 87.35 % of the total classes for the AT (58°C, pH 8.56) and TP (48°C, pH 8.76) samples [22].

From the analysis of the phylum *Cyanobacteria* that was detected in the samples from the Tecozautla geyser, the most studied genera are *Gloeobacter*, which contains two species that are *Gloeobacter violaceus* and *Gloeobacter kilaueensis*, the latter isolated in a cave near the lava caldera of the volcano Kilauea in Hawaii. Both are non-thylakoidal and carry out oxygenic photosynthesis [23].

Leptolyngbya is one of the most common and frequent filamentous cyanobacterial genera in thermal environments, occurring in a wide range of terrestrial, aquatic, and extreme environments and equally distributed in Mexico [24]. Finally, the genus *Pseudanabaena* is anoxygenic photoautotrophic thermophilic cyanobacterium, also found in Yellowstone National Park, and is one of the main

responsible for the formation of microbial mats in Chocolate Pool to 52°C, in addition to having been found in geysers in areas that exceed 60°C, as well as in the Amazon River at ~ 30°C, which shows that it can grow at different temperature ranges and habitats [23]. An important point to note about filamentous cyanobacteria is that they are encapsulated by exopolysaccharides, making them difficult to lyse and nucleic acids can be trapped, making them inaccessible for polymerase chain reaction and sequencing [25].

The phylum *Chloroflexi* has been found in the Garga hot spring microbial mats, where the incidence of *Chloroflexi* and *Chlorobi* did not represent > 10 % of the total number of sequences in the Ga2-verh and Ga3-sred samples [26]. The phylum *Chloroflexi* is one of the most found in thermal environments in the world, the presence of *Chloroflexi* predominates in microbial mats with temperatures of 53–65°C and slightly alkaline pH (7.75–7.91), similar to the samples of the Tecozautla geyser [27]. They have also been identified in microbial mats (66°C, pH ~ 6.5), with relative abundances of ~ 18 to 50 % [28], which are similar to the percentages found in the geyser understudy for *Cyanobacteria* and *Chloroflexi*. *Chloroflexi* was identified in microbial mats from hot springs in Costa Rica, constituting 93 % of all readings, the conditions of the sample area were 37–60°C and pH 6.1–7.5 [29]. Likewise, it is known that the phylum *Chloroflexi* is very widespread in microbial mats of hot springs in Japan [30], Yellowstone (USA) [31], Kamchatka, Thailand, Tibet [23], and in the Andes [32].

The phylum *Chloroflexi* currently comprises eight subgroups at the class level: *Chloroflexia*, *Anaerolineae*, *Ardenticatenia*, *Caldilineae*, *Dehalococcoidia*, *Ktedenobacteria*, *Thermoflexia*, and *Thermomicrobia*, which mainly contain chlorophotrophic and non-phototrophic filamentous bacteria [33]. Furthermore, unidentified classes of bacteria are found in this phylum [34].

The class *Chloroflexia* has two orders, *Herpetosiphonales* and *Chloroflexales*, of the latter they are divided into two families: *Roseiflexineae* and *Chloroflexineae* (https://www.genome.jp/tools-bin/taxsummary), although previously they were known as green bacteria without sulfur. Chloroflexal chlorophotrophic organisms are now known as filamentous anoxygenic phototrophs (FAP). They are characterized by their multicellular, filamentous morphology, with lengths of several hundred μm, and an anoxygenic photoheterotrophic/photomyxotrophic lifestyle [30, 33, 34].

The abundances of the two most common chlorophotrophs of *Chloroflexi* (*Roseiflexus castenholzii* and *Chloroflexus spp*.) vary with temperature. At temperatures lower than 60°C, *Roseiflexus spp*. are more abundant, but when temperatures are higher, *Chloroflexus spp*. they are predominant at ~ 70°C [30]. In the Tecozautla geyser, Hidalgo, the genera with the highest relative abundance were *Roseiflexus* and *Chloroflexus*. The first is a photosynthetic, filamentous, thermophilic bacterium lacking chlorosomes, with a single cultured species *Roseiflexus castenholzii* with optimal growth temperature of 45–55°C and pH 7.5–8 [30]. The second genus contains only two species: *Chloroflexus aurantiacus* and *Chloroflexus aggregans*, both with optimal growth temperatures of 55°C, the first with a growth limit of 70°C and the second of 60°C, they are generally found in neutral alkaline hot springs [35]. *Chloroflexus* are photoheterotrophic and chemoheterotrophic, they inhabit microbial mats of thermal waters together with

cyanobacteria, their growth can occur at temperatures of 30 to 70°C. Furthermore, they are facultative bacteria capable of growing photoheterotrophically under anaerobic and chemoheterotrophic conditions under aerobic conditions [33, 35].

The phylum *Deinococcus-Thermus* is considered a group of microorganisms abundant in hot springs [21, 36]. With a presence in hot springs and microbial mats in Tibet (China), Sichuan (China), Mushroom Spring and Calcite Springs, YNP (USA), Siloam (South Africa), Bulgaria (Eastern Europe), Chile (South America) [14, 28]. The phylum *Deinococcus-Thermus* includes the class *Deinococci* and this is currently divided into the orders *Deinococcales* and *Thermales*. The first is made up of the *Deinococcus*, *Deinobacterium*, and *Truepera* genera. All members of *Deinococcus* are radioresistant, with two known thermophiles *Deinococcus geothermalis* and *Deinococcus murrayi* [36]. In the case of *Truepera*, the only cultivated species is *Truepera radiovictrix* resistant to ionizing radiation with optimal growth at pH 7.5–9.5 up to pH 11.2, with the ability to grow in multiple extreme conditions in alkaline, moderately saline, and high-temperature habitats [37]. The *Thermales* order encompasses five genera (*Thermus, Meiothermus*, *Marinithermus*, *Oceanithermus*, and *Vulcanithermus*). Cultured representatives of *Thermus* are thermophilic and hyperthermophilic. The members of *Thermales* have recovered from a large set of natural and man-made thermal environments. These bacteria and their cellular components are of biotechnological interest with possible applications in bioremediation or molecular biology, for example, thermostable enzymes [38]. Their ecological importance stands out in that they play an important role in the carbon, nitrogen, and sulfur cycles, such as *Thermus oshimai*, *Thermus thermophilus* [39], or as *Thermus scotoductus* with oxidizing mixotrophic characteristics of sulfur [40]. In the case of the Tecozautla geyser, only the genera *Thermus* and *Meiothermus* were found.

Regarding the phylum *Proteobacteria*, its presence has been reported in habitats with temperatures of 29–35°C and pH 3.5–6.5, with relative abundances of 60 % which decreases significantly at 68°C (pH 6.9) with < 10 % [32]. They have also been found in microbial mats, sediments, and hot springs in Eritrea (Africa) with relative abundances of 6.2 to 82.3 %, with conditions of temperatures of 44 to 110°C and pH of 6.97 to 7.54 [41], which allows elucidating that *Proteobacteria* inhabit a wide spectrum of environmental conditions. The diversity of *Proteobacteria* is based on the classes, that is, Alpha, Beta, Epsilon, and Gamma, a habitat with the presence of all four classes is an indication of the high diversity of this phylum, as was the case of the Tecozautla geyser that shares the same diversity with the thermal devil's eye [40]. The phylum *Proteobacteria* is home to the largest variety of bacteria in all environments in the world, including phototrophic and anoxygenic bacteria that predominate in many geothermal environments. They are an important part of ecosystems because they participate in the sulfur and carbon cycle such as purple sulfur bacteria (PSB), type I methanotrophs, which belong to *Gammaproteobacteria*, purple sulfur-free bacteria (PNSB) that belong to *Betaproteobacteria* and *Alphaproteobacteria*, in addition to type II methanotrophs. Sulfate-reducing bacteria (SRB) belong to *Deltaproteobacteria*, being the main components in environments related to geothermal, alkaline lakes, and saline environments [20, 25].

The PSBs belong to the *Chromatiales* order in the *Gammaroteobacteria* and within the *Chromatiales* the PSBs are separated into the *Chromatiaceae* and *Ectothiorhodospiraceae* families. All PSB species and their families can perform anoxygenic photosynthesis under anoxic conditions and fix CO_2 by the Rubisco enzyme and the Calvin-Benson-Bassham cycle. PSBs are mesophilic organisms that can also photo-assimilate small organic molecules or grow heterotrophically in the dark. Under favorable conditions, they have the ability to reduce N_2 to ammonia. In addition to converting sulfur into less toxic compounds such as sulfate [33]. Nitrogen fixation is widely distributed among PNSB. Many of them can use sulfur as an electron donor, but can generally only tolerate low sulfur concentrations < 0.5 mM. Like PSBs, PNSBs are ubiquitously found in mesophilic, circumferential neutral aquatic, or terrestrial environments. Exceptions occur, some prefer acidic, alkaline, or hypersaline conditions. Isolates of thermophilic microorganisms with growth > 50°C are unknown; only mildly thermophilic PNSB species have been isolated (*Blastochloris* sp. and *Rhodocista* sp.) which grow up to 47°C, these bacteria were identified in slightly alkaline hot springs [33]. The third physiological group of chlorophotrophic proteobacteria is the aerobic anoxygenic purple bacteria (AAPB), with species belonging to the α, β, and γ proteobacteria. Unlike the other two groups, AAPBs require oxygen and organic molecules for their growth. They lack the ability to use CO_2 as their primary carbon source. However, they can obtain up to ~ 15 % of their cellular carbon by anaplerotic CO_2 fixation reactions [33]. AAPBs are found in freshwater and marine aquatic environments, as well as in soil crusts and microbial mats from hot springs. They have recently been found in the microbial mats of Octopus Springs and Mushroom springs as well as in the Tecozautla geyser.

Another phylum found in the Tecozautla geyser was the *Bacteroidetes* phylum. This phylum is a large group of anaerobic and gram-negative chemoganotrophic organisms that do not form endospores and are not mobile by sliding, with wide distribution in the environment [42], with presence in water samples, wet sediments, and microbial mats from hot springs from Eritrea, Ethiopia with abundances of 2.7 to 8.4 % and with growth conditions of 49.5°C to 100°C and pH of 6.97 to 7.54 [41]. They have also been detected in Himalayan hot springs with relative abundances of 74.28 % at temperatures of 60–80°C and pH 8.0-8.5 [43]. Which suggests high adaptability to alkalo-thermophilic conditions.

Analysis of other identified taxonomic groups

Other phyla with less abundance but no less important were identified, all of them with the capacity to grow in environments with high temperatures and in alkaline waters, characteristics of the Tecozautla geyser. The phylum *Acidobacteria*, *Actinobacteria*, *Aquificae*, *Chlorobi*, *Dictyoglomi*, *Firmicutes*, *Gemmatimonadetes*, *Nitrospirae*, *Planctomycetes*, *Spirochaetes*, *Synergistetes*, *Tenericutes*, *Thermotogae*, and *Verrucomicrobia* were identified, which have been reported for their ability to grow in extreme temperature and alkaline environments [44].

Bacteria of the phylum *Arquea* were also detected, including *Euyarchaeota* and *Crenarchaeota*. *Euryarchaeota* members include halophiles, methanogens, thermoacidophils, and hyperthermophiles. Methanogens are a relatively diverse group that produce methane from CO_2 through hydrogenotrophic

methanogenesis and use H_2 as an electron donor [45]. In the case of *Crenarchaeota*, they have been cultivated at an optimum temperature of 80°C and in the case of extreme thermophiles, at a growth temperature of 65 to 70°C. These bacteria can be acidophilic, predominantly aerobic, or facultative anaerobic, as well as sulfur-dependent thermophilic and ammonium-oxidizing bacteria [46]. These bacteria have been found in the area of the Lake Baikal rupture, where archaea, mainly *Crenarchaeota*, constituted 19.8 % of the total number of sequences in areas with a temperature of 74°C, while for the Ga3-sred sample their abundance was 0.04 % at a temperature of 55°C. These results agree with the results obtained in this work, it was observed that the elevated temperature favors the growth of *Crenarchaeota* because in the sampling area the temperature was between 62 and 65°C, very close to the optimal growth temperature reported by other authors [18].

Alpha diversity analysis

Studies based on the sequencing of 16S rRNA genes have facilitated the understanding of microbial diversity, knowing its composition, function, and dynamics. Although there is no general agreement on which diversity index is the best, in general, it is necessary to use a series of them for greater accuracy [47]. In Fig. 3A, the behavior of the accumulation curves of the 4 samples of the Tecozautla geyser is observed, which describe the relationship that exists between the number of OTUs observed and the sampling effort, the graph provides information on the total of the diversity in the sampled community, to know if the number of sequences obtained represent a significant fraction of the diversity of the community, showing that when the curve has an asymptote, the greatest diversity and the maximum sampling effort have been identified [48].

In the 4 samples of the geyser differences are observed between them, the GD sample of light blue color, presented the greatest diversity, because it does not show a defined asymptote. In the remaining samples a similar behavior is observed among themselves and they follow an asymptotic trend, which suggests that the greatest amount of diversity possible was identified and the sampling effort was adequate as described [48]. The Good coverange graph (Fig. 3B) measures how well the sampling was carried out in an environment and indicates the percentage of individuals sampled in a microbial community, where values greater than 0.9%, evidence that the sequencing effort is sufficient to represent the largest number of species [49]. The result observed in Fig. 3B therefore showed that the sequencing effort was sufficient to represent the largest number of species.

The Chao1 index (richness) (Fig. 3C) estimates the abundance of individuals in a sample, in the so-called species accumulation curve, where the x-axis is the number of individuals sampled or the sampling units examined, which in this case is the sequencing depth, and the y-axis is the number of species observed. The Chao1 index is calculated taking into account the total number of species, as well as the number of rare species and those found twice in the sample.

The results for the four samples of the geyser show different behaviors among themselves, this is due to the fact that in the GD sample (microbial mat-sediments) a greater number of individuals is estimated,

followed by the GA sample (salts), and the two Microbial mats from the GB and GC samples, which have similar behavior to each other, with a smaller number of individuals.

The Shannon-Weiner index (diversity) (Fig. 3D) is defined as the estimator of species richness and uniformity. Typical values are generally between 1.5 and 3.5 in most ecological studies, and the index is rarely greater than 4 [47, 50]. The results reflect that the GD (microbial mat-sediments) and GC (microbial mat II) samples have greater diversity because both exceed the value of 3.5, but the GD sample is even more abundant because it is ~ 5, in addition to that values greater than 4 have greater diversity and are not so common. In the case of the GA and GB samples, they have values around 3 which indicates that they have an average diversity.

The Simpson metric also called dominance index (Fig. 3E), which derives from the probability theory, allows quantifying the diversity of habitat, reflects the probability of finding two individuals belonging to the same species in two successive extractions, that is, the closer the value of this index is to 1, there is a greater possibility of the dominance of a species, and the closer it is to the value of zero, the greater the biodiversity of a habitat. This index gives a higher weight to common or similar species and underestimates rare species [51]. Therefore, in the GB sample, there is a greater probability of predominance of a particular species, followed by the GD sample and finally, the GA and GC samples presented similar percentages.

Beta diversity and the central microbiome

The results obtained from the beta diversity indicated a great microbial diversity in the four samples analyzed. Most of the OTU's were not shared which suggests a great microbial diversity. This result can be explained from the point of view of temperature, the GA sample was taken from the geyser source where the temperature was 94 ºC, while for the GB, GC, and GD samples the temperatures were 65, 61.5, and 62 ºC respectively. It is important to point out that the GD sample was the one that showed the main physical and chemical differences, in it the presence of exopolysaccharides was observed and it was the one that presented the greatest difficulty in DNA extraction. It follows, therefore, that the temperature that determines the microbial diversity in systems similar to the one studied. Similar results have been obtained from other hot spring areas, where it has been observed that microbial diversity depends on the sampling area [52]. Central microbiome analysis is used to understand stable and consistent components across complex microbial ensembles. The results were obtained according to the "affiliation" technique that is based on the presence and absence of OTUs between the different microbiomes that are compared, taking into account 1623 bp for the normalization of the data (rarefaction). It was observed that the 4 samples are very diverse due to the temperature of the sampling, which differentiates the populations. This result shows that the samples have a lower relationship with each other. By detecting different profiles it is deduced that each sample presents a typical and unique diversity.

Functional profiles

The prediction of the functional profiles yielded relevant information on the possible metabolic functions of the studied communities. The most abundant metabolic profile was membrane transport that is

related to ABC transporters, phosphotransferase system, and bacterial secretion system (https://probes.pw.usda.gov/MetaCoMET). Based on the results obtained, the importance of ATP-dependent transporters or ABC transporters that belong to the class of primary transporters is deduced, being very important routes in archaea and bacteria. In the case of the Extremophiles, these transporters are used as a survival mechanism to regulate osmotic pressure, generation of exopolysaccharides and are part of one of the main transport routes at high temperatures. These routes are classified into two groups; carbohydrate and di/oligopeptide absorption transporters. In archaea they can accumulate substrates at much higher concentrations within cells. Hyperthermophilic archaea show important metabolic adaptations for carbohydrate growth under hostile conditions. So far, for carbohydrate absorption, only ABC-type transporters have been described, which are equipped with exceptionally high affinity compared to mesophilic bacterial systems allowing these organisms to efficiently remove all available carbohydrates from the extreme environment [53].

DNA repair, as well as the maintenance of the genome and its expression, is an established mechanism due to growth at high temperatures, where DNA decomposition is accelerated and where some repair proteins are widely conserved, being observed mainly in hyperthermophilic archaea. In addition, under conditions with high levels of radiation including ionizing radiation and UV radiation, repair of massive DNA damage is mediated by energy and protein metabolism, occurring mainly in thermophilic organisms [54]. Regarding energy metabolism, the results agree with the relative abundances found for some bacterial groups where aerobic respiration is the main mechanism for energy generation. In this case, oxidative phosphorylation indicates that electron transfer to a terminal electron receptor, such as oxygen, nitrate, or sulfate, generates ATP. Similar results have been reported in hot springs in Finland [55]. This shows that thermal environments follow a trend in their prediction in metabolic functions, although some of them showed results associated with activities in thermal environments, in others the metabolic capacities of the microbial community were not fully reflected, as is the case of Sulfur metabolism which is very reduced, this can be attributed to a low quantity and quality of the genomes annotated in the databases that are related to the species observed in the thermal water samples [56].

Conclusions

In this research, the microbial communities present in the microbial mats, sediments, and saline accumulations were described for the first time, in the alkalo-thermophilic environment of the geyser of Tecozautla, Hidalgo, Mexico, which presented a temperature of 61.5–65°C in the area of collection.

32 phyla were identified of which *Armatimonadetes*, *Chloroflexi*, *Cyanobacteria*, *Thermi*, *Proteobacteria*, *Bacteroidetes*, *Spirochaetes*, *Firmicutes*, *Acidobacteria*, *Nitrospirae*, *Crenarchaeota*, and *Euryarchaeota* were the most abundant. This demonstrates the great diversity and abundance of microorganisms found in the geyser, which depend on the continuous supply of nutrients from inorganic reduced sulfur compounds, elevated temperatures, and continuous exposure to light, resulting in phototrophic microbial mats.

The functional predictions of the PiCRUSt-KEGG profiles indicated metabolic pathways related to membrane transport, carbohydrate metabolism, amino acid metabolism, energy metabolism, replication and repair, cofactor, and vitamin metabolism. Membrane transport is widely related to the ABC transporters, the phosphotransferase system, and the bacterial secretion system, which contribute substantially to the adaptability of these microorganisms to thermal environments, to name a few. The results of this research provided new prospects for the industrial, scientific and biotechnological potential that can be derived from alkalo-thermophilic microorganisms.

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Author contributions (CRediT roles)

Formal Analysis and Investigation, Writing-original draft: **Segovia-Cruz Jesus Alberto (Thesis Master)**; Conceptualization, Methodology, Resources: **Souza Valeria**; Validation, Visualization: **Mercado-Flores Yuridia;** Software, Data curation: Supervision: **Anducho-Reyes Miguel Angel**; Conceptualization, Visualization: **Vargas-Hernández Genaro**; Conceptualization, Resources, Writing -review & editing: **Téllez-Jurado Alejandro**

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Figures

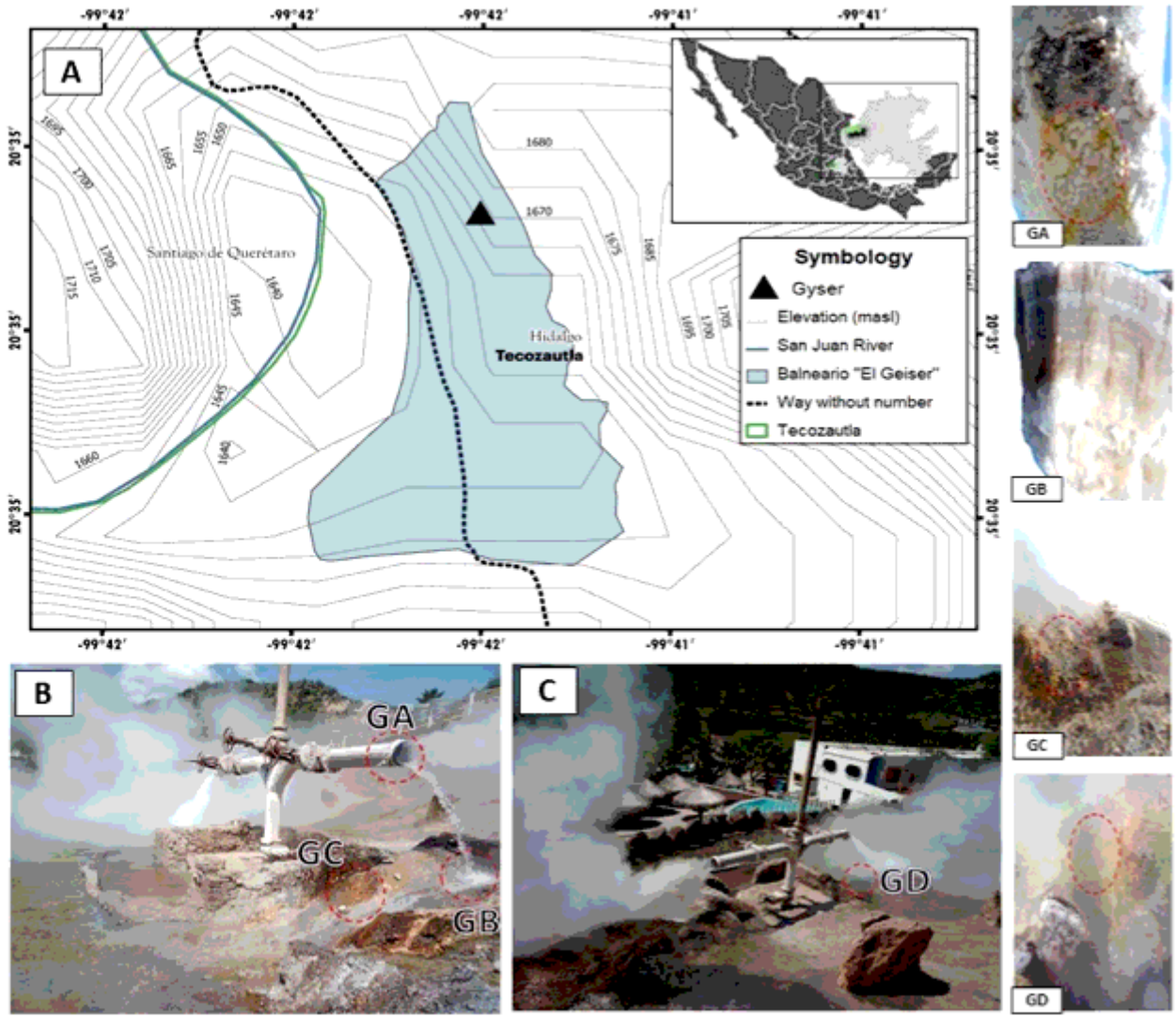

Figure 1

Geographical location of the Tecozautla geyser and location of the areas where the samples were taken. A = Geographical location of the geyser; B = Location of sampling areas for GA, GC, and GB samples; C = Sampling location for GD sample; GA, GB, GC, and GD = Appearance of the sample. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

Figure 2

Relative abundances obtained from the four samples analyzed geyser from Tecozautla. A = At phylum level; B = At class level; C = At genus level.

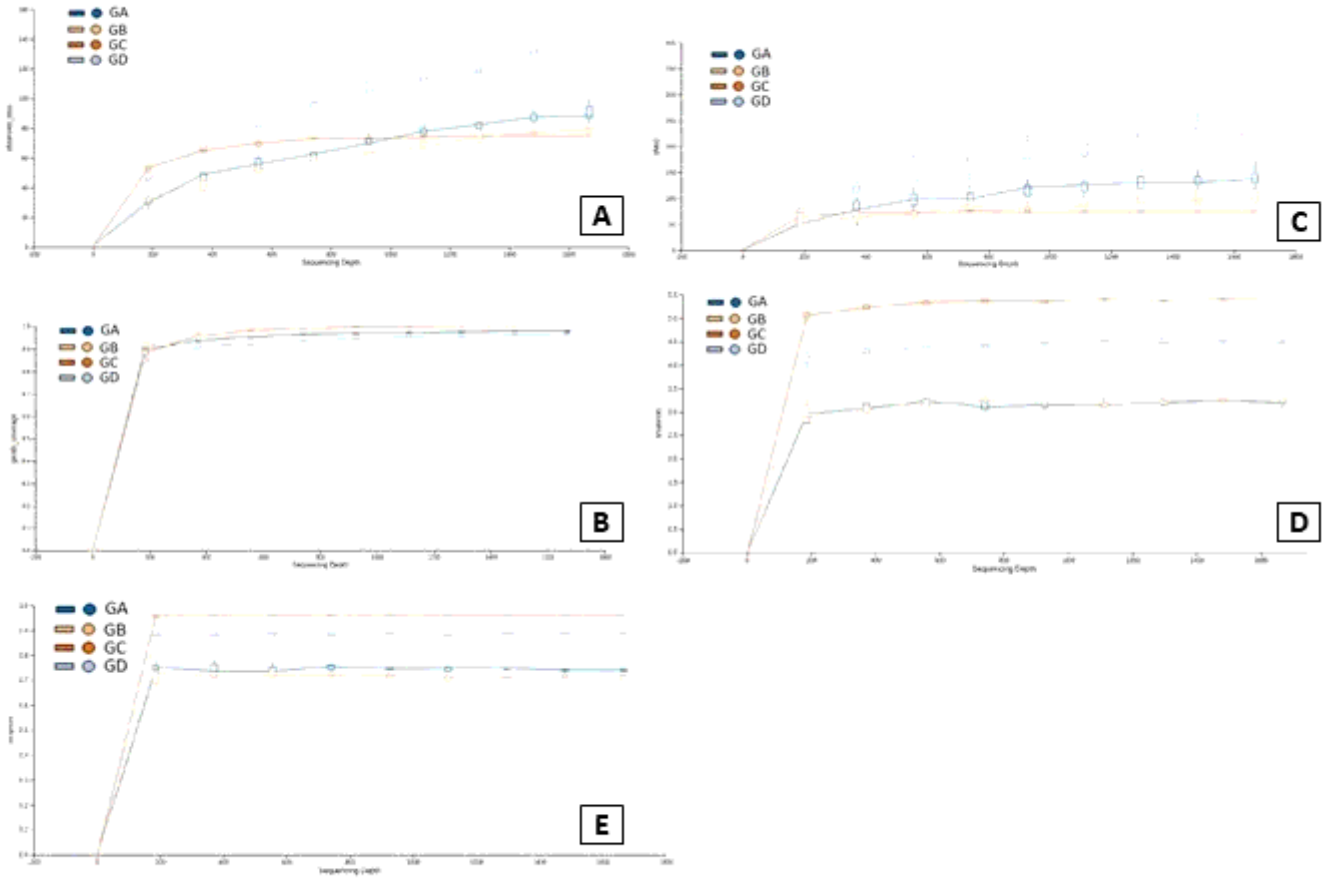

Figure 3

Results obtained from the alpha diversity of the four samples of the Tecozautla gieser. A = OTS´s observed; B = Good coverage; C = Chao index; D = Shannon-Weiner index; E = Simpson index.

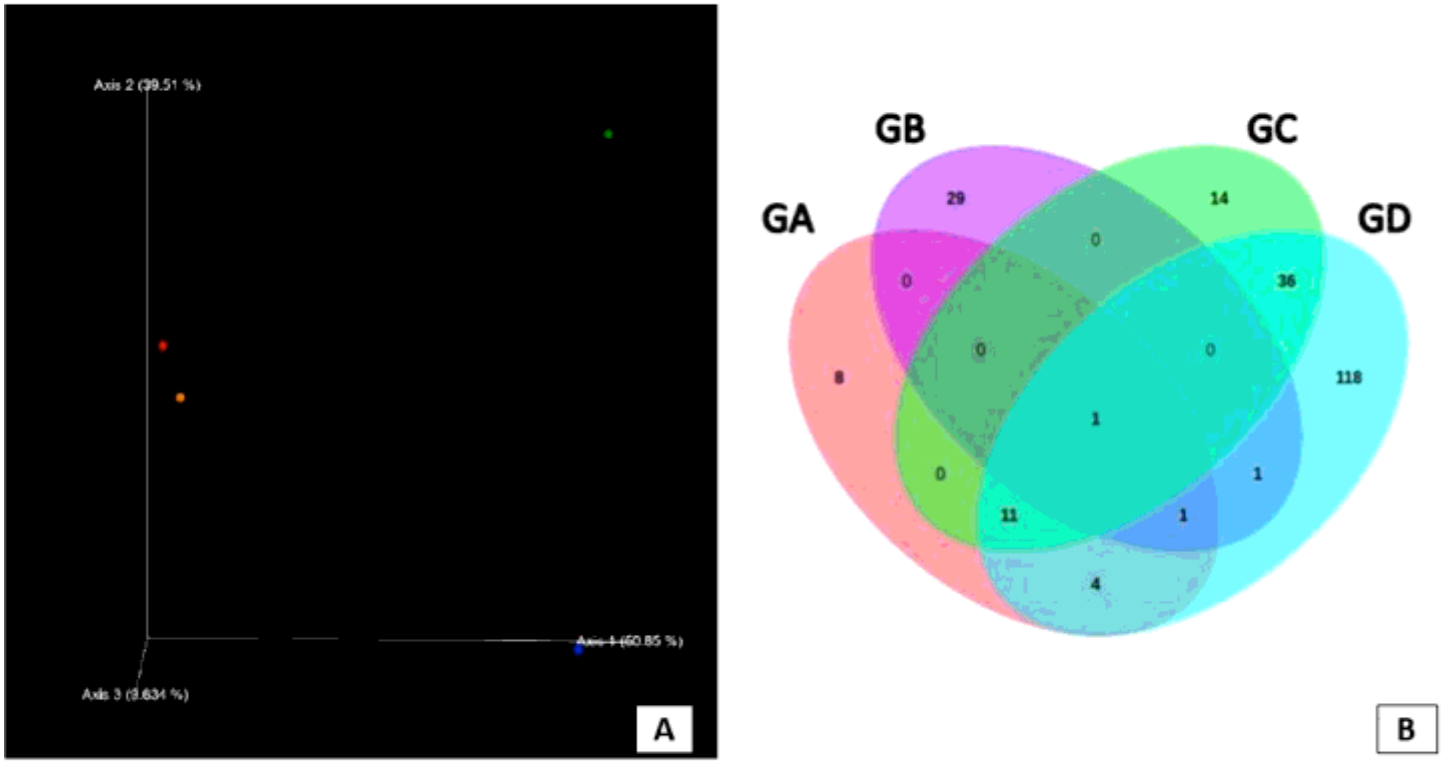

Figure 4

Results obtained from the analysis of beta diversity and the central microbiome of the four samples from the Tecozautla geyser. A = PCoA analysis: blue-GA, green-GB, red-GC, and orange-GD; B = Venn diagram Venn diagram for samples GA, GB, GC, and GD.

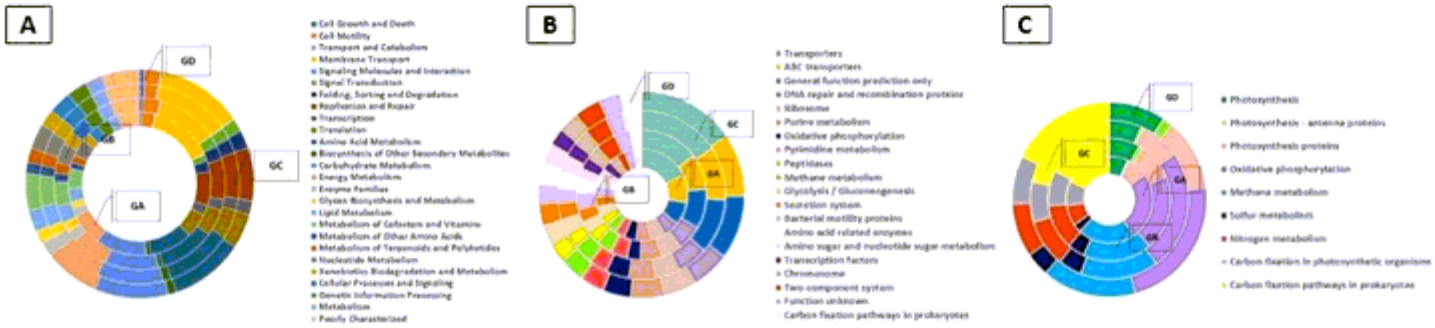

Figure 5

Functional profiles analysis for the studied samples, donut plot of the inference of the KEGG pathways. A = Level 2; B = Level 3; C = Energy metabolism at Level 3.