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Bacterial Composition of a Geothermal Site in Central Mexico

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ABSTRACT

Bacteria is the most diverse group of cellular organisms on earth. Next-generation gene sequencing technologies allow us to cost-effectively and accurately estimate the diversity of bacteria in multiple samples. Bacterial composition is affected by environmental conditions. Once these conditions are changed, complex bacterial populations will differ from the original composition for a long period of time, thus giving us a larger timeframe to identify environmental changes than water quality analysis alone. Here, we used 16s rRNA sequencing to identify the bacterial composition for two geothermal springs in the zone of Acoculco, Mexico during the rain and dry seasons of 2018. Overall, 91.8% of sequences were assigned at the Phylum level, 67.4% to genus and 30.8% to species. During both seasons, proteobacteria was the dominant phylum (Dry: 44%, Rain: 57%), followed by Firmicutes (Dry: 14%, Rain: 9%) and Actinobacteria (Dry: 11%, Rain: 9%). Cyanobacteria represented 11% of identified species during the dry season, but only 1% during the wet season. Four Phyla (Verrucomicrobia, Nitrospirae, Chlorobi and Thermosulfobacteria) were only found during the wet season. Physicochemical analysis indicates acid superficial waters with temperatures of 20.0 ºC to 31.8 ºC for Los Azufres springs, meanwhile, basic waters where found for La Alcaparroza with range of temperatures 18.0 ºC to 14.7 ºC. Dissolve oxygen were slightly different between ponds and seasons from both geothermal springs, meanwhile, total dissolve salt and conductivity different between seasons and springs. As result of a high bacterial activities in spring waters, high content of nitrite and low concentration of phosphorus compounds were found. A total of 20 chemical elements were determinate in springs waters, high sulfur and iron content were found in Acoculco springs with ranges of 36.7 to 358 mg/L and 102.0 to 12110 mg/L respectively, representing an acid-sulfuric waters with iron clusters expressions. This result agrees with Tello-Hinojosa (1994), Rocha-Lopez (2006) and Viggiano-Guerra (2011) studies, were presence of pyrite rocks were dominant and acid-sulfuric waters where found in the Acoculco area.

1. INTRODUCTION

Microorganisms are the most abundant, ubiquitous and diverse living things in the planet. It is estimated that only 1% of the microorganisms are cultivable (Amann, 2000). Also, microbes have the ability to adapt to the most extreme environments. "Geothermal springs are harsh and rare habitats colonized by prokaryotic organism adapted to live in these communities (Zakaria and Abdulrahman, 2007). These microbial population has been study with interest as a source of bioactive molecules (Grether-Beck et al., 2008), as potential producers of biodiesel (Onay et al., 2014), as biological indicators for monitoring aquatic environments (Maznah and Wan, 2010) and for other biotechnological purposes (Davis et al., 2003).

1.1 Microorganism in geothermal environments

Bacteria in geothermal sites have structural and metabolic modifications that allow them to survive under environment pressures, such as low pH, high salinity, eutrophication, and low and high temperatures (Siliakus et al., 2017). Geothermal sites can present different states of temperature and physicochemical parameters, depending the origin and age of the site, likewise the bacteria diversity may fluctuate according those parameters. Species of *Synechococcus, Chloroflexus, Phormidium and Mastigocladus* have been reported in sites with temperatures that fluctuates from 73ºC to 30ºC (Carbonell et al., 2019). Microbes like the archeas *Sulfolobus sp., Thermoplasma sp.* and the bacteria *Mycobacterium parascrofulaceum,* can live in extreme pH conditions, and are found in environments with pH 2 to pH 3. Likewise, *Synechococcus spp., Methanobacterium thermoautotrophicus* and *Thermocrinis rubber* had been reported in high alkalinity sites (Inskeep et al., 2013).

Since most bacteria cannot be cultivated, in order to study the bacterial diversity and function we can rely in genomics, an interdisciplinary field that studies the structure, function and evolution of the genomes. Metagenomics is an important tool for the study of microbial ecology. With the advent of Next-Generation Sequencing technologies we can obtain the sequence of genes and genomes in an efficient way form environmental samples (Kim et al., 2013). This can allow us to evaluate microbial diversity in extreme environments, better understand the role microorganisms they play in those communities, their metabolic capacity, and to recognize the relation between microbial diversity and environmental characteristics.

1.2 Acoculco, Puebla an Enhanced Geothermal System

Enhanced Geothermal Systems(EGS) are systems that exploit the geothermal heat from the surface, it is done by drill wells at different distances and water is injected under pressure to enhanced the cracks and fracture the rocks, the water is heated as it descends to the next well. In Mexico, the federal electricity commission (CFE) considers the zone of Acoculco like a potential site to develop an EGS applying hydraulic fracturing or chemical techniques due the low permeability of this area (CRE, 2009).

The Acoculco geothermal field comprises and area of 1290 km^2 and has two hydrothermal altered areas with acid-sulphate cold springs and some mofettes, Los Azufres-El Potrero Colorado, and La Alcaparroza (Tello-Hinojosa, 1994 and Rocha-Lopez, 2006). Acoculco field is associated to Tulancingo-Acoculco caldera complex, located in the eastern portion of the Trans-Mexican Volcanic Belt, and is geological characterized for a 2000 m thick of Tertiary-Quaternary volcanic rocks, Cretaceous metamorphized limestone and even Cretaceous granite (Gutiérrez-Negrín and Quijano-León, 2007). Tello-Hinojosa (1994) characterized 39 geothermal expressions from this area, study found superficial waters with temperatures of 13ºC to 49ºC, and acid-sulphate cold springs originate by geothermal H_2S and superficial water mix. According to geological studies, the presence of low temperatures and few geological expressions is due to the senile stage of the area (Viggiano-Guerra et al., 2011).

Acoculco, is a locality in the municipality of Chignahuapan, Puebla. This area is located 180 km NE away to the Mexico City, and has an average elevation of 2800 and 2900 m a.s.l, Relief zone includes south-north mountains complex with peaks of 3400 m a.s.l., hills and west-east down valleys with 2200 m a.s.l., this topography allows the growth of mighty rivers during the rainy season, which are small stream during the dry season (INAFED, 2019). Acoculco present a semi-humid cold weather, with average annual rainfall of 40 mm and 5 ºC temperature, with rain intense in July to October (up to 80 mm), and dry seasons with minimums of 14.3 mm and 30.1 ºC (February to March) (CONAGUA, 2019). The main economic activities of the region are agriculture and the sale of wood. The excess of these activities has caused the deforestation, loss of soil by agrochemicals and contamination of the waters of aquifers.

Water quality monitoring has been one of the most important aim in the last twenty-five years (Cairns, 1995), the World of Health Organization is an international agency who has been implemented national water standard for chemical content and regulations for costumers (WHO, 2016). Water body studies consist in physicochemical analysis and trace metal content, and for the last fifth-teen years these studies have been complemented with biological composition (Beyene et al. 2009).

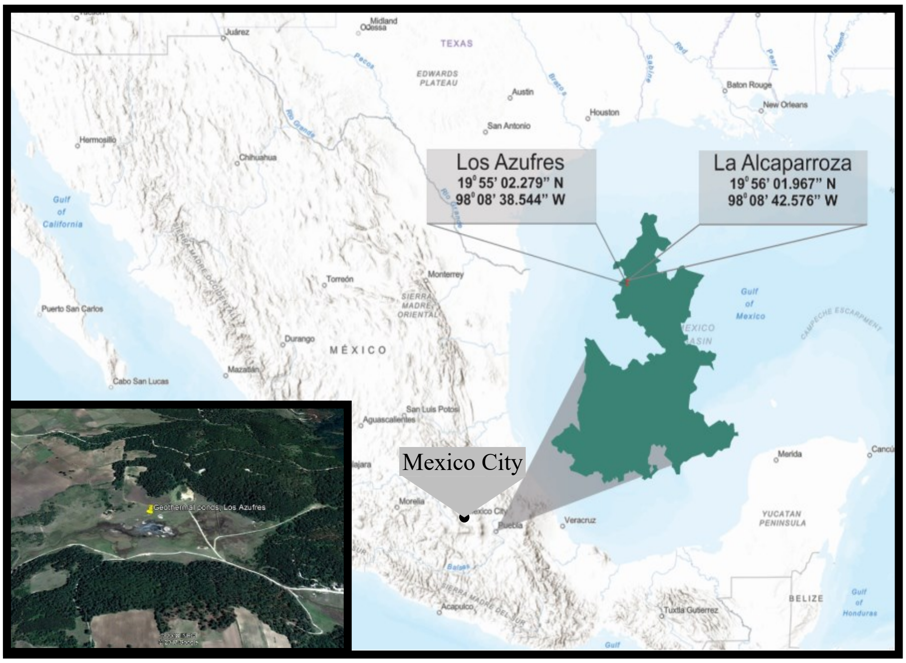

Figure 1: Location of Los Azufres and La Alcaparroza geothermal springs. Green area represents the municipality of Acoculco, Puebla. The Acoculco zone is located in the eastern portion of Trans-Mexican Volcanic Belt, 180km south-east of Mexico City. Box shows an aerial view of Los Azufres.

2. WATER QUALITY OF GEOTHEMAL SPRINGS

The Acoculco geothermal system includes two geothermal springs: Los Azufres-El Potrero Colorado, commonly known as "Los Azufres", and "La Alcaparroza". These sites were sampled during April and November of 2018, representing dry and rain seasons, respectively. Five samples were directly collected from each pond present in Los Azufres spring, pond water presented colorful formation of microbial mats and different expressions of geothermal influence, as blue color, bubbling water, black iron clusters and acid-sulfurous smell. La Alcaparroza spring, has one geothermal expression, both springs presented an "open system" with constant water emission, covering a bigger ponds area during rainy season.

2.1 Collected data

For chemical analysis, water sampling was performed in triplicate. 500 mL per sample were collected in sterile, food-grade plastic containers and stored in the dark at room temperature. Water sample were transported to the laboratory and filtered through 0.22 µm cellulose filters. The filtrate was transferred to 60 mL plastic bottles and 1.2 mL of nitric acid (trace metal grade) was added for elemental analysis. Filters were stored at -80ºC until DNA extraction.

2.2 Physicochemical analysis

Physicochemical parameters (pH, electrical conductivity (EC), total dissolve solids (TDS) and dissolved oxygen (DO)) were measured *in situ* to avoid the effects of high biological demand. Nitrites, nitrates, ammonia, phosphates, chlorides and sulfates were determined *ex situ* using oxidation colorimeter test kits.

The physicochemical parameters of both springs are summarized in Table 1. Los Azufres and La Alcaparroza springs showed different range of superficial temperatures. Los Azufres presented higher temperatures, ranging from 20.0ºC to 31.8 ºC, compared to La Alcaparroza with a range from 14.7ºC 18.0ºC to throughout both seasons. Los Azufres ponds presented an acid pH in superficial waters (outlet), with a range from 3.40 to 6.38. Areas with microbial mats exhibited increased pH values (3.75 to 6.0). On the other hand, La Alcaparroza presented mostly neutral pH values year-round, with a range of 7.13 to 6.8 for superficial waters and 7.50 to 6.50 for areas with microbial biofilms. DO values only varied slightly between ponds and seasons. Meanwhile, TDS and EC variations were higher between seasons and springs. Nitrite salts exhibit relatively high concentrations in springs waters, which could be the result of high bacteria nitrification activities which consume ammonium and produce nitrite salts (Nielsen et al., 2014). Contrary to this, phosphates had the lowest. This could also be explained by microbial activity, which consume phosphorus compounds for the synthesis of nucleic acids and membrane lipids.

Table 1. Summary of physicochemical parameters and nutrient content at Los Azufres (AZ 1.1 – AZ 1.5) and La Alcaparroza (AL1) springs during dry and rainy seasons. Mean values of three independent measurements are shown.

	GPS location	Elevation (m a.s.l.)	Season	Temp. (ºC)	pH		Cond. (µS/cm)	TDS (mg/L)	DO (mg/L)	NO^{2-} (mg/L)	NO^{3-} (mg/L)	NH_4^{3-} (mg/L)	PO_4^{3-} (mg/L)
					Oulet	Biomat							
Spring													
Los Azufres													
AZ 1.1	19º 55'22.16"N	2841	Dry	24.3	4.20	4.29	1556.0	396.0	6.15	0.50	0.53	0.10	0.33
	98º 8'39.16"W		Rain	22.7	4.80	5.00	14.3	226.0	5.80	17.33	0.20	0.27	0.01
AZ 1.2	19º 55'21.08"N	2834	Dry	20.0	3.40	3.75	4.0	592.0	6.24	6.60	1.36	0.00	0.00
	98º 8'38.13"W		Rain	28.0	3.40	5.40	28.0	574.0	6.22	12.60	0.50	0.00	0.00
AZ 1.3	19º 55'20.56"N	2846	Dry	25.0	6.49	4.68	5.4	860.0	6.22	16.60	0.16	0.16	0.02
	98º 8'38.45"W		Rain	24.9	3.90	4.90	24.9	504.0	6.09	0.00	0.86	0.57	0.00
AZ 1.4	19º 55'20.60"N	2838	Dry	28.0	3.42	6.11	5.5	800.0	6.23	0.00	0.43	0.12	0.02
	98º 8'38.96"W		Rain	26.2	5.10	4.00	26.2	462.0	5.86	34.33	0.10	0.43	0.00
AZ 1.5	19º 55'21.25"N	2836	Dry	26.2	6.38	5.03	7.0	970.0	6.21	3.30	0.30	0.28	0.05
	98º 8'39.47"W		Rain	31.8	5.10	6.00	31.8	634.0	5.40	37.66	1.00	0.45	0.00
La Alcaparroza													
AL1	19º 56'2.09"N	2864	Dry	18.0	7.13	7.50	646.0	128.0	6.30	11.00	1.20	0.00	1.50
	98º 8'42.97"W		Rain	14.7	6.80	6.50	606.8	39.3	7.80	110.00	39.80	0.34	0.35

2.3 Metal content analysis

A total of 20 chemical elements were determinate for Los Azufres and La Alcaparroza spring waters pre-filtrated and acidified contented in 60 mL plastic bottles. Chemical analysis was performed by ICP-MS at SWAMP Laboratory from Alberta University, Canada. All values from chemical analysis were presented in tables as mean values with standard deviation.

2.3.1 Mayor ions

Concentrations of six mayor ions in geothermal springs water samples are given in Table 2. The results show increased salinity for both springs during the rainy season. Los Azufres pond AZ 1.3 show a higher ions concentration between present ponds, but La Alcaparroza waters exhibit a higher sulfur content. Salts concentrations in geothermal spring were present in order S>Ca>Na>K>Mg. Representing an acid-sulfuric waters, with high content of calcium and sodium, these results agree with those obtained by Tello-Hinojosa (1994) and Rocha-Lopez (2006).

Table 2. Mayor ion content at Los Azufres (AZ 1.1 – AZ 1.5) and La Alcaparroza (AL1) springs during dry and rainy seasons. Values shown are in ppm ± standard deviation. Mean values of three independent measurements are shown.

		Ca	K	Mg	Na	S
	LOD	mg/L 0.003	mg/L 0.009	mg/L 0.003	mg/L 0.002	mg/L 0.025
Spring	**Season**					
Los Azufres						
AZ 1.1	Dry	12.623 ± 2.042	10.889 ± 1.266	3.132 ± 0.409	19.322 ± 2.471	36.728 ± 4.294
	Rain	18.601 ± 1.241	12.069 ± 0.317	4.572 ± 0.255	23.953 ± 1.916	52.432 ± 4.132
AZ 1.2	Dry	21.857 ± 0.522	8.718 ± 0.118	5.474 ± 0.077	10.203 ± 0.231	71.264 ± 0.755
	Rain	32.858 ± 1.931	9.005 ± 0.372	8.502 ± 0.577	12.806 ± 0.467	94.547 ± 3.433
AZ 1.3	Dry	43.676 ± 2.726	29.181 ± 0.449	11.886 ± 0.680	68.371 ± 4.871	60.958 ± 4.951
	Rain	42.553 ± 3.216	11.722 ± 0.169	9.817 ± 0.124	26.143 ± 6.389	104.769 ± 2.584
AZ 1.4	Dry	36.426 ± 2.265	10.109 ± 0.682	8.449 ± 0.532	14.986 ± 1.059	96.876 ± 6.686
	Rain	41.034 ± 1.072	12.632 ± 0.177	10.095 ± 0.104	23.744 ± 0.047	104.622 ± 2.429
AZ 1.5	Dry	66.231 ± 1.458	25.495 ± 0.825	18.317 ± 0.408	78.288 ± 1.463	100.228 ± 2.290
	Rain	49.209 ± 2.590	15.437 ± 0.983	12.674 ± 0.852	37.753 ± 3.675	114.411 ± 8.907
La Alcaparroza						
AL1	Dry	11.402 ± 6.771	2.979 ± 0.890	5.311 ± 2.247	10.870 ± 8.531	11.618 ± 15.093
	Rain	16.048 ± 2.680	8.790 ± 1.243	4.034 ± 1.284	14.161 ± 4.543	358.635 ± 93.570

2.3.2 Trace elements

Concentrations of 14 trace elements in water samples were detected and are show in Tables 3 and 4. The highest trace element content in all samples was iron. The presence of this chemical element was in order AZ 1.2> AZ 1.4> AZ 1.1>AZ 1.5> AZ 1.3 for Los Azufres ponds in the dry season, but, in La Alcaparroza, iron expression where the higher of all trace elements during ran season. The order of trace elements for concentration for both springs were in order Fe>As>Mn>Zn>Se>Ni>Co>V>Cr>Cu>Sb>Pb>U>Cd. Iron expression were detected during sampling with the presence of iron cluster in waters of ponds AZ 1.4 and AZ 1.2., the highest iron concentrations were found in La Alcaparroza with values of 12110 µg / L, these results agree with the Acoculco geology studies of Viggiano-Guerra (2011) where the presence of pyrite rocks were dominant in this zone.

Table 3. Trace elements (As to Mn) content at Los Azufres (AZ 1.1 – AZ 1.5) and La Alcaparroza (AL1) springs during dry and rainy seasons. Values shown are in ppb ± standard deviation. Mean values of three independent measurements are shown.

		As	Cd	Co	Cr	Cu	Fe	Mn
		µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L
	LOD	0.003	0.001	0.000	0.000	0.001	0.002	0.009
Spring	**Season**							
Los Azufres								
AZ 1.1	Dry	98.389 ± 18.366	0.009 ± 0.004	0.236 ± 0.013	0.249 ± 0.028	0.345 ± 0.254	5397.726 ± 685.420	167.058 ± 24.504
	Rain	85.061 ± 74.843	0.021 ± 0.018	0.334 ± 0.159	0.594 ± 0.151	1.476 ± 1.117	3613.663 ± 1968.502	257.765 ± 15.547
AZ 1.2	Dry	13.630 ± 0.719	0.017 ± 0.000	4.324 ± 0.049	0.227 ± 0.000	0.233 ± 0.055	7944.169 ± 54.977	872.103 ± 8.696
	Rain	6.422 ± 0.350	0.185 ± 0.004	9.102 ± 0.399	0.292 ± 0.029	1.123 ± 0.413	3761.193 ± 140.913	1448.168 ± 65.743
AZ 1.3	Dry	988.083 ± 51.019	4.964 ± 0.274	0.154 ± 0.114	0.114 ± 0.085	0.150 ± 0.007	285.247 ± 199.690	771.285 ± 12.484
	Rain	78.736 ± 20.004	0.006 ± 0.002	5.522 ± 0.242	0.374 ± 0.093	0.316 ± 0.202	3312.461 ± 111.452	1502.976 ± 38.836
AZ 1.4	Dry	133.451 ± 72.389	0.034 ± 0.017	3.142 ± 0.672	0.324 ± 0.052	0.186 ± 0.127	5852.408 ± 446.403	1364.136 ± 93.791
	Rain	146.366 ± 8.472	0.008 ± 0.000	5.202 ± 0.100	0.269 ± 0.023	0.471 ± 0.131	3127.271 ± 53.210	1414.887 ± 18.708
AZ 1.5	Dry	1912.769 ± 29.143	0.001 ± 0.000	0.646 ± 0.154	0.074 ± 0.009	0.486 ± 0.124	2033.099 ± 26.447	1137.651 ± 72.253
	Rain	280.375 ± 1.620	0.002 ± 0.001	5.147 ± 0.438	0.224 ± 0.049	0.231 ± 0.119	3082.705 ± 121.516	1459.496 ± 101.493
La Alcaparroza								
AL1	Dry	2.796 ± 3.638	2.762 ± 3.901	0.263 ± 0.074	0.268 ± 0.270	0.360 ± 0.180	102.411 ± 8.452	65.782 ± 16.391
	Rain	1.578 ± 0.252	0.133 ± 0.056	3.594 ± 2.762	3.421 ± 1.304	0.506 ± 0.372	12110.199 ± 6315.654	453.836 ± 195.370

Table 4. Trace elements (Ni to Zn) content at Los Azufres (AZ 1.1 – AZ 1.5) and La Alcaparroza (AL1) springs during dry and rainy seasons. Values shown are in ppb ± standard deviation. Mean values of three independent measurements are shown.

		Ni	Pb	Sb	Se	U	V	Zn
		μg/L	μg/L	μg/L	μg/L	μg/L	μg/L	μg/L
	LOD	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Spring	**Season**							
Los Azufres								
AZ 1.1	Dry	0.505 ± 0.130	0.094 ± 0.022	0.063 ± 0.007	0.807 ± 0.093	0.014 ± 0.003	1.051 ± 0.217	6.456 ± 2.831
	Rain	0.896 ± 0.368	0.336 ± 0.254	0.099 ± 0.035	0.987 ± 0.234	0.028 ± 0.010	1.094 ± 0.149	16.158 ± 9.080
AZ 1.2	Dry	4.874 ± 0.042	0.155 ± 0.029	0.014 ± 0.001	6.019 ± 0.236	0.010 ± 0.001	0.457 ± 0.025	86.839 ± 1.243
	Rain	7.426 ± 0.344	0.223 ± 0.014	0.022 ± 0.003	10.859 ± 0.372	0.031 ± 0.003	0.442 ± 0.028	161.124 ± 9.439
AZ 1.3	Dry	0.283 ± 0.022	0.052 ± 0.008	0.722 ± 0.191	0.122 ± 0.000	0.009 ± 0.000	0.440 ± 0.020	4.806 ± 0.859
	Rain	4.762 ± 0.269	0.057 ± 0.045	0.006 ± 0.003	16.907 ± 1.201	0.015 ± 0.004	0.460 ± 0.032	118.216 ± 8.110
AZ 1.4	Dry	3.191 ± 0.509	0.340 ± 0.069	0.122 ± 0.008	10.426 ± 1.372	0.019 ± 0.004	1.166 ± 0.499	103.264 ± 35.787
	Rain	4.443 ± 0.093	0.053 ± 0.026	0.021 ± 0.009	15.289 ± 0.574	0.012 ± 0.000	0.693 ± 0.079	111.775 ± 3.639
AZ 1.5	Dry	1.005 ± 0.051	0.023 ± 0.004	1.304 ± 0.052	0.266 ± 0.004	0.012 ± 0.001	0.295 ± 0.018	6.621 ± 0.251
	Rain	4.558 ± 0.332	0.038 ± 0.021	0.009 ± 0.005	14.809 ± 0.942	0.025 ± 0.004	0.617 ± 0.048	105.451 ± 10.097
La Alcaparroza								
AL1	Dry	0.613 ± 0.574	0.045 ± 0.011	0.095 ± 0.119	0.166 ± 0.000	0.059 ± 0.000	1.450 ± 1.243	1.427 ± 0.643
	Rain	4.740 ± 3.495	1.063 ± 0.093	0.110 ± 0.110	21.293 ± 5.612	0.026 ± 0.003	4.700 ± 2.029	63.885 ± 29.270

3. BACTERIAL COMPOSITION IN GEOTHERMAL SPRING WATER

Bacterial composition was estimated by sequence analysis of the V3-V6 region of the 16s rRNA subunit. Amplicons were amplified using primers F 5´-CCTACGGGDGGCWGCA-3´ and R 5´-CTGACGACRRCCRTGCA-3 (Liu et al., 2013). Pair-ended fragments 2X300 were sequenced using a MiSeq sequencer (Illumina, San Diego Ca.) with v3 chemistry. De-multiplexed sequences were paired and trimmed using Trimmomatic (Bolger et al., 2014) with a 5nt sliding window and a PHRED33 value of 20. An average of 440,000 reads were kept for rain season samples while an average of 320,009 reads were retained for the dry season samples. Taxa was assigned by using the Ribosomal Database Project Naïve Bayesian Classifier (Wang et al., 2007). Only bacterial sequences were used for the analyses. Taxa with fewer than 100 corresponding reads were discarded. A total of 79 species were identified for the dry season and 162 for the rain season. Table 5 shows a summary of sequencing results.

Table 5. Summary of sequencing results. Showing the percentage of reads assigned to different taxonomic levels and the total number of species identified.

	RAIN (440k reads)		DRY (320k reads)	
Level	**Classified**	**Unclassified**	**Classified**	**Unclassified**
Phylum	90.97%	9.03%	92.93%	7.07%
Class	86.28%	13.72%	85.77%	14.23%
Order	83.09%	16.91%	80.91%	19.09%
Family	79.19%	20.81%	62.28%	37.72%
Genus	75.26%	24.74%	56.72%	43.28%
Species	35.30%	64.70%	24.66%	75.34%
# Species	162		79	

Overall, 91.8% of sequences were assigned at the Phylum level, 67.4% to genus and 30.8% to species (Table 5). During both seasons, proteobacteria was the dominant phylum (Dry: 44%, Rain: 57%), followed by Firmicutes (Dry: 14%, Rain: 9%) and Actinobacteria (Dry: 11%, Rain: 9%). Cyanobacteria represented 11% of identified species during the dry season, but only 1% during the wet season. Four Phyla (Verrucomicrobia, Nitrospirae, Chlorobi and Thermosulfobacteria) were only found during the wet season. (Figure 2)

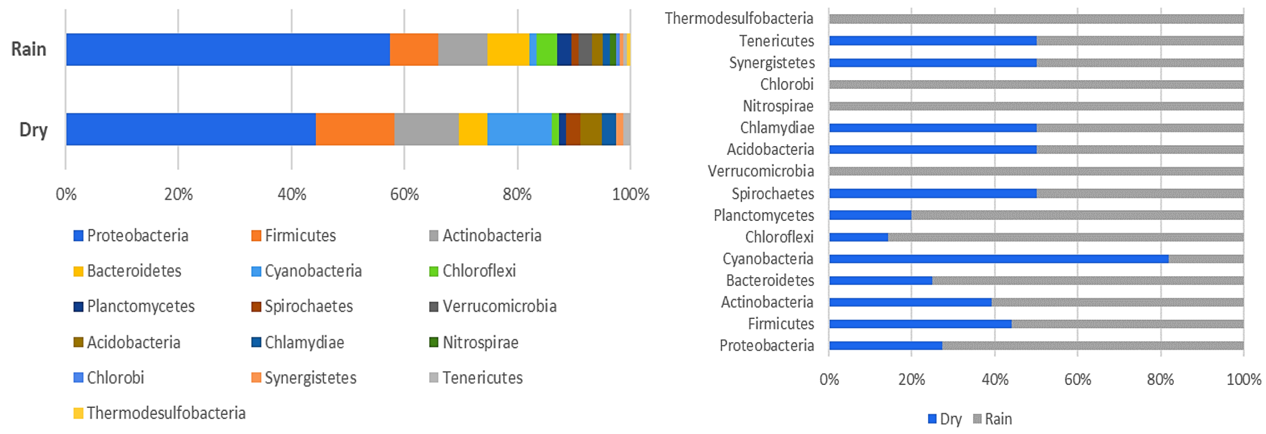

Figure 2. Seasonal representation of Phyla in geothermal springs at Acoculco, Puebla. México.

3.1 Seasonal bacterial composition

Bacterial composition differed significantly during both seasons, with the rainy season showing a higher richness and diversity than the dry season (**Figure 3-A**). During the rainy season 16 phyla were represented opposite to 12 phyla with representation during the dry season. Twelve classes were only represented during the rainy season while two (Oscillatoriophycideae and Nitriliruptoria) were only detected during the dry season. Twenty-four orders that were represented during the rainy season did not show representation during the dry season, while five orders were only identified during the dry season, including two orders of Cyanobacteria, Nostocales and Synechococcales, which can be attributed to the organisms ability to survive in the high concentrations of salts, TDS, and trace metals associated with desiccation.

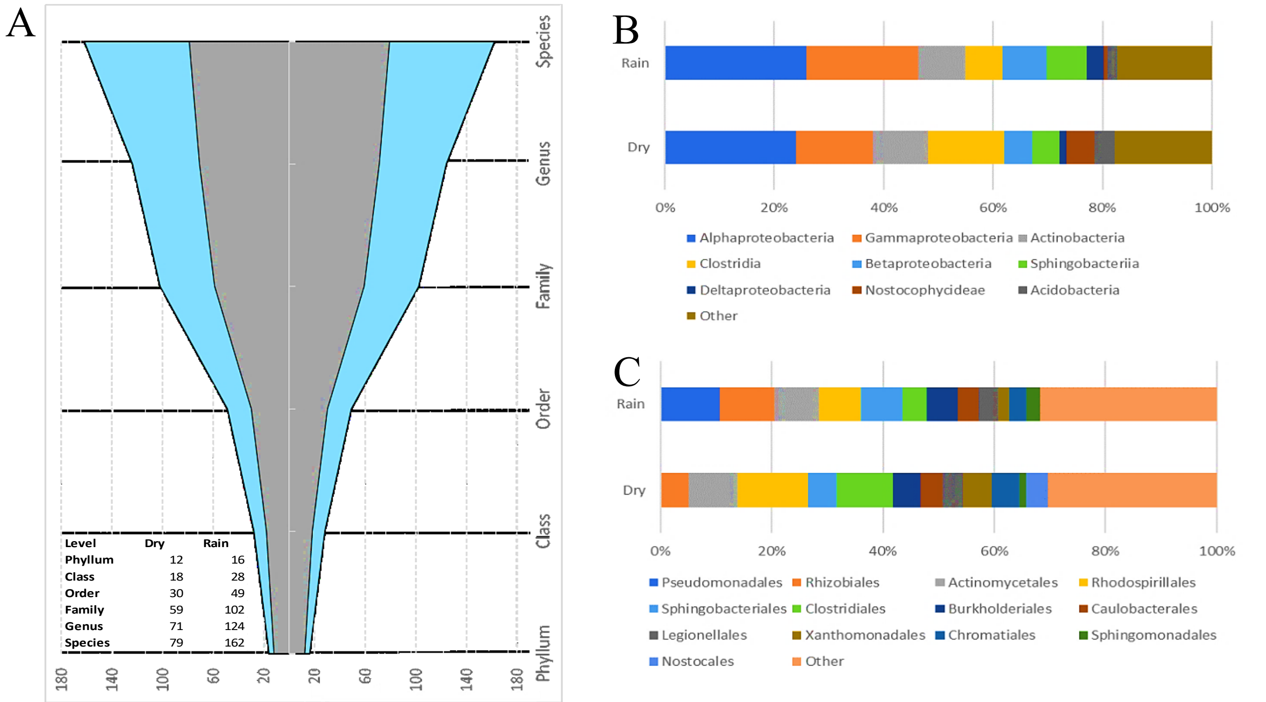

Figure 3: Representation of microbial diversity during the dry (grey) and rain (blue) seasons showing an increased diversity during the rainy season. *A*: Representation across different taxonomical levels. Data left of the vertical axis is mirrored from the data on the right. Table shows the number of represented taxa within each taxonomical level. *B*: Distribution of Classes. *C:* Distribution of Orders.

In total, 135 unique genera were identified. The dry season showed 70 genera while 116 genera were detected during the rainy season. Nineteen genera were only detected during the dry season whereas 65 genera were exclusive to the rainy season. (Figure 4) Interestingly, the genus Pseudomonas was the most represented during the rainy season and was not present during the dry season. Since Pseudomonas are abundant in soils, this indicates soil transport to the springs from adjacent fields. Not surprisingly, many of the bacteria identified are associated with sulfur and iron metabolism. This allows them to survive in geothermal springs and contributes to the geochemistry of the site.

Genus	Dry	Rain
Pseudomonas		13
Legionella	2	5
Acinetobacter		4
Rhizobium		4
Gluconobacter	2	3
Nostoc	3	
Novosphingobium	1	3
Phenylobacterium	3	2
Thiomonas	2	3
Actinoallomurus	1	1
Actinomadura	1	1
Azospirillum	2	2
Clostridium	1	1
Devosia		2
Hyphomicrobium	1	2
Janthinobacterium		2
Luteibacter	1	2
Peptoniphilus	1	2
Rhodanobacter	2	
Roseospira		2
Saccharopolyspora	1	2
Thermogemmatispora		2

Genus	Species
Pseudomonas	
	Pseudomonas benzenivorans
	Pseudomonas brenneri
	Pseudomonas collierea
	Pseudomonas corrugata
	Pseudomonas entomophila
	Pseudomonas jessenii
	Pseudomonas lundensis
	Pseudomonas moraviensis
	Pseudomonas parafulva
	Pseudomonas taiwanensis
	Pseudomonas teessidea
	Pseudomonas umsongensis
	Pseudomonas vancouverensis
Legionella	
	Legionella fallonii
	Legionella shakespearei
	Legionella tucsonensis
	Legionella rowbothamii
	Legionella waltersii

Genus	Species
Acinetobacter	
	Acinetobacter gerneri
	Acinetobacter marinus
	Acinetobacter psychrotolerans
	Acinetobacter tjernbergiae
Rhizobium	
	Rhizobium alamii
	Rhizobium etli
	Rhizobium pisi
	Rhizobium tibeticum
Gluconobacter	
	Gluconobacter morbifer
	Gluconobacter kondonii
	Gluconobacter krungthepensis
Nostoc	
	Nostoc flagelliforme
	Nostoc microscopicum
	Nostoc punctiforme
Novosphingobium	
	Novosphingobium hassiacum
	Novosphingobium yangbajingensis
	Novosphingobium acidiphilum

Figure 4: Heath map showing genera with the highest representation across samples and identified species of the seven most-represented genera.

4. CONCLUSIONS

We observed a clear difference in the bacterial composition of the site across the studied seasons, and this bacterial distribution changed more markedly than physicochemical parameters across seasons. Further work is needed to correlate specific environmental parameters to bacterial composition. The diminishing cost of sequencing and availability of accessible data analysis pipelines make it possible to use bacterial composition analyses to assess environmental impact instead of single biological indicators or water quality analysis. Furthermore, the ability to identify species can inform about environmental processes that will be difficult to identify with chemical analysis. Finally, the possibility to infer metabolic capacity of a bacterial population will allow us to understand the biology, ecology and geochemistry of a site.

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