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Bacterial Diversity in an Amazonian Mangrove Ecosystem

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Abstract

Mangroves form a transition zone among terrestrial, marine, and freshwater environments, and they host a striking diversity of macro- and microorganisms. In recent years, increasing anthropogenic impacts have caused widespread degradation of mangrove environments. To analyze the bacterial diversity of Salina Lake, an artificial body of water located in the mangroves of eastern Amazonia, total DNA was extracted from sediment and water samples that were collected from three sites (P1, P2, P3) located within the lake. The 16S rRNA gene was partially amplified, separated using the Denaturing High Performance Liquid Chromatography (DHPLC) method, and sequenced. The DHPLC profiles were quite distinct for the different aquatic and sedimentary bacterial communities. The most abundant groups (> 70%) at all sampled locations were *Proteobacteria* and unclassified bacteria, but other phyla were also identified, including *Bacteroidetes, Cyanobacteria, Acidobacteria, Firmicutes, Actinobacteria, Chloroflexi, Siprochaetes* and *TM7*. A total of 24, 15, and 23 OTUs (Operational Taxonomic Units) were observed in the water samples from P1, P2, and P3, respectively, whereas the sediment samples returned 29, 34, and 52 OTUs. This is the first analysis performed on the bacterial diversity of Salina Lake, which was created by the construction of a highway within a protected mangrove area.

Keywords: Diversity, Bacteria, Mangrove, Lake, 16S rRNA

1. Introduction

Mangrove ecosystems cover vast areas of tropical and subtropical coastal zones, and their composition is influenced by tidal forces as well as high concentrations of dissolved nutrients and decomposing organic matter (Holguin et al., 2001; Holguin et al., 2006). Approximately one-third of the world's mangrove forests have disappeared over the past 50 years as a direct result of the expansion of human populations and increasing anthropogenic pressures on their natural resources (Duke et al., 2007; Alongi, 2002). Mangroves support the trophic network of coastal systems by tidal nutrients (Nedwell et al., 1994). The species composition of mangrove ecosystems is strongly influenced by physicochemical factors, particularly salinity (Lozupone & Knight, 2007; Vieira et al., 2008; Silveira et al., 2011). Microorganisms play a fundamental role in ecosystem processes, such as the decomposition of organic matter (leaves and trees). These processes involve a complex bacterial community that produces nitrogen, phosphorus, and carbon, which combine to form the basis of the system's food chain (Holguin et al., 2001).

With the exception of a few recent studies, little is known about the diversity and ecological function of the bacterial communities found in mangrove ecosystems (Hewson & Fuhrman, 2004; Zhang et al., 2008; Zhou et al., 2009; Gomes et al., 2010; Santos et al., 2011; Silveira et al., 2011). The present study was conducted to analyze the diversity and composition of the bacterial communities of a mangrove lake of Bragança-Pa in the eastern Amazon region. The analysis was performed using sequences of the bacterial 16S rRNA gene and the DHPLC method to infer the taxonomic relationships between the identified members of the community.

1.1 Research Methods

1.1.1 Study Site and Sampling

Lagoa Salina, or Salina Lake, is an artificial body of water that is located within the RESEX Marinha de Caeté-Taperaçú reserve (Figure 1). The lake is set within an extensive area of mangrove forest on the state's Atlantic coast. It has a circumference of 2.3 km, a depth of 1.5 m and is not connected to any rivers or channels. The lake was formed during the construction of the PA-458 highway, which cut through 20 km of the mangrove forest and resulted in the damming of a number of tidal channels. These channels formed permanent lakes where trees died off and decomposed, thereby modifying the structure of the ecosystem and the composition of the local microbial community (Goch et al., 2005; Martins et al., 2006).

In the present study, 1 L samples of water and sediment were collected from Salina Lake on April 18th, 2009, at the following three sites (Figure 1): P1 (00°53'42"S, 46°40'1"W), P2 (00°53'50"S, 46°40'4"W), and P3 (00°54'00"S, 46°40'10"W). The samples were stored at -20°C until processing in the laboratory.

Water samples of 500 mL were collected from each site for chemical analysis (Na^+, Ca^{2+}, K^+, and Mg^{2+}) using atomic absorption spectrometry, and physicochemical parameters, including Cl-, SO_4^{2-} by the titrimetric method, and pH by potentiometer. Salinity, electrical conductivity

(EC), temperature, and concentration of total dissolved solids (TDS) were measured in situ with a probe during sample collection. Dissolved oxygen (DO) was measured according to the Winkler method (see Strickland & Parsons, 1972). All analyses were performed at the hydrochemistry laboratory of the Geosciences Institute of the Federal University of Pará (UFPA).

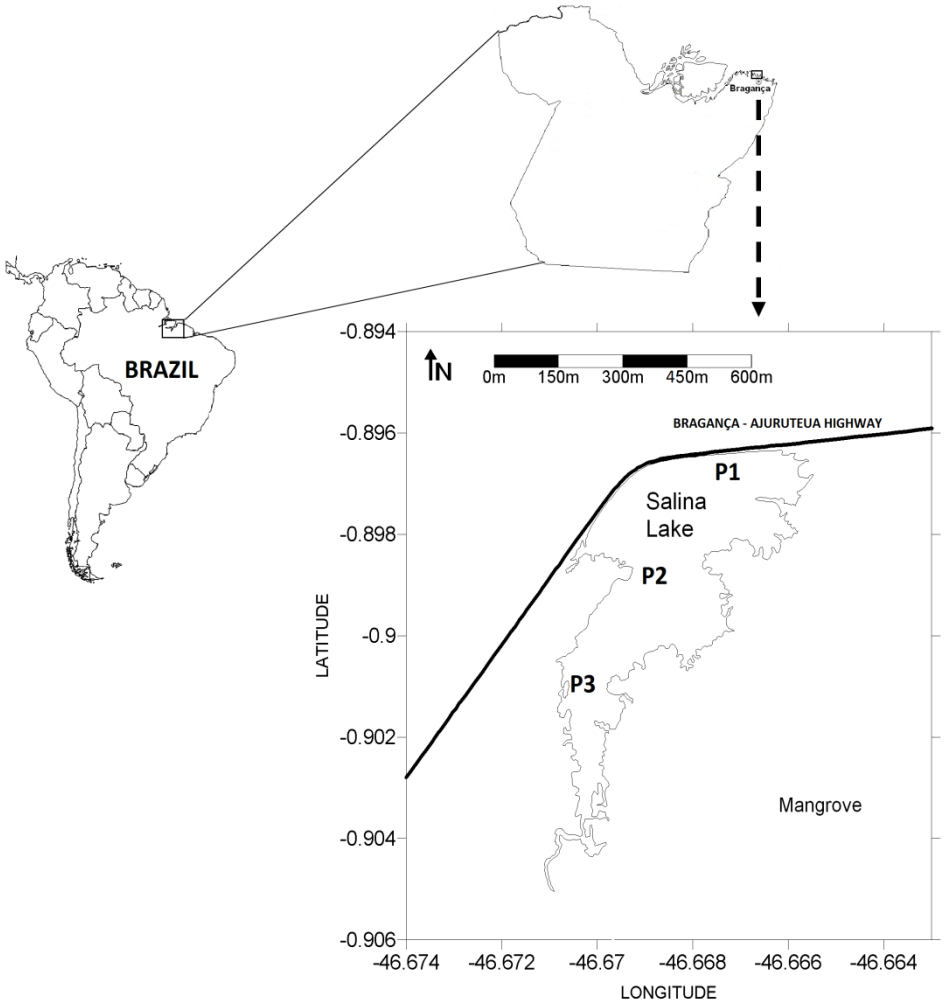

Figure 1. Location of Salina Lake in northeastern Pará

Description: Show the sampling sites P1, P2, and P3 within the area of the lake.

The sediment samples were collected with a metallic dredge and stored in 50 mL Falcon tubes. The water samples were filtered through a 0.8 µm, 0.45 µm and 0.22 µm membranes, respectively (Whatman, Germany). The processed samples were stored in tubes with STE buffer (0.1 M NaCl, 10 mM Tris-Cl, 1 mM EDTA).

1.1.2 Extraction of DNA

The extraction and processing of total DNA were carried out in the tubes containing the 0.22 µm membranes that were used for sample collection. The samples were incubated with STE

buffer under agitation. The cells were precipitated by sedimentation at 2,000 x g for 10 minutes at room temperature. DNA was extracted from the precipitate that formed in the tube and from 0.2 grams of the sediment sample using an UltraCleanTM soil DNA extraction kit (MO BIO Laboratories, USA) according to the manufacturer's protocol. The extracted DNA was quantified by spectrophotometry, and its integrity was confirmed in a 1% agarose gel.

1.1.3 Amplification of Fragments of the 16S rRNA Gene by PCR and DHPLC

Fragments of the 16S rRNA gene were amplified approximately 1,400 bps in length by polymerase chain reaction (PCR) using the following specific primers for the bacterial 16S rRNA gene: 16S-8F (AGA GTT TGA T(CT)(AC) TGG CTC AG), 16S-1407R (GAC GGG GGT G(AT)G T(AG)C AA) and 16S-1492R (CGG TTA CCT TGT TAC GAC TT).

The PCR contained 1X buffer, 2.0 mM MgCl2, 0.5 mM dNTPs, 5 pmol/μL of each primer, and 1 U/μL of Taq DNA polymerase (Invitrogen, USA) in a final volume of 10 μL. Ten reactions at a final volume of 100 μL for each sample were conducted to guarantee the amplification of rare DNA fragments. The cycling conditions for all the reactions were 95°C for 5 minutes, 35 cycles of 1 minute each at 95°C, 1 minute at 60°C, 1 minute at 72°C, and a final extension of 5 minutes at 72°C.

A second PCR using the DNA products generated by the first PCR was necessary for the insertion of the GC clamp into the amplicons. The reaction was performed using the oligonucleotides, DHPLC FP640 (CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG GCC TAC GGG AGG) and DHPLC 534R (ATT ACC GCG GCT GCT GCT GG) (Muyzer et al., 1993), which amplified a fragment of approximately 300 base pairs in length corresponding to the variable V3 region of the 16S rRNA bacterial gene. The cycling conditions for all the reactions were 95°C for 5 minutes followed by 35 cycles of 1 minute each at 95°C, 40 seconds at 62°C, and 32 seconds at 72°C with a final extension of 10 minutes at 72°C.

After the second amplification, the amplicons were processed using a Transgenomic WAVE system (Xiao & Oefner, 2001), at a flow rate of 0.9 mL/minute. Samples were injected into the dHPLC system using a two-eluant buffer system: Buffer 'A' consisted of 100 mM triethylammonium acetate (TEAA), pH 7.0 and 0,025% acetonitrile (Transgenomic, USA) in aqueous solution, and Buffer 'B' consisted of 100 mM triethylammonium acetate, pH 7.0 with 25% (vol/vol) acetonitrile (Transgenomic, USA) and 100 mM EDTA. For the column wash, an aqueous solution with 75% (vol/vol) acetonitrile was used. The detection of the fragments was carried out through fluorescent dyes. The non redundant peaks produced for all of the samples were collected and a new PCR was then performed using the same conditions that were used in the second reaction.

The fragments were first separated, then purified with a GeneJETTM purification kit (Thermo Scientific, USA) and finally cloned using the TOPO TA cloning kit (Invitrogen, USA). A total of 15 libraries were built and twenty-four recombinant clones were selected randomly from each peak within each library for sequencing in an automatic sequencer (3730 DNA Analyzer, Life Technology, USA).

1.1.4 Analysis of Diversity and Phylogenetic Relationships

The sequences were edited with BioEdit v7.0.9 software. The sequences were checked for the presence of chimeric sequences using the Quimera check tool and then compared with the sequences in the Ribosomal Database Project (RDP) (Cole et al., 2009) using the Classifier and SeqMatch tools and GenBank® to determine the closest sequences and their taxonomies. The distance matrix was then generated using the PHYLIP package; the rarefaction curve was constructed, and the Shannon diversity index and Chao species richness estimator were calculated using the Mothur 1.20.3 program (Schloss & Handelsman, 2005). The non-parametric estimator of coverage (C) was calculated using the formula, C = 1-n1/N, where n1 = the number of phylotypes that appear only once in the library and N = the size of the library. The Mothur program was also used to compare the community structure using ∫ -Libshuff.

Finally, a dendrogram was constructed from the unique OTUs with the MEGA4 software (Tamura et al., 2007) using the Neighbor-Joining method. Evolutionary distances were calculated using the Jukes-Cantor model (Saitou & Nei, 1987).

2. Results

2.1 Analysis of the Physicochemical Parameters of the Water

The physicochemical parameters of the water at each sampling site are shown in Table 1. The temperature of the water varied between 26°C and 27°C. The low salinity and reduced concentrations of cations (Na^+, Ca^{2+}, K^+, Mg^{2+}, SO_4^{2-}), total dissolved solids, and Cl- recorded at all the sites can be attributed to the high levels of precipitation in the study area. At all sites, the concentration of dissolved oxygen varied according to the depth of the water (unpublished data), which may be related to the variation in the redox potential at the different sites.

Table 1. Physicochemical parameters of water

Sample	T (°C)	Sal	pH	O_2	Cond (µS·cm^{-1})	SDT	Cl^-	SO_4^{2-}	Na^+	K^+	Ca^{2+}	Mg^{2+}
P1	26.5	0.4	4.6	4.3	844	406	219	42	85	5.8	2.8	8.6
P2	27.1	0.4	5.1	6.5	864	414	219	42	87	5.7	0.2	1.3
P3	26.6	0.3	4.0	3.1	682	326	173	34	68	4.3	1.4	6.2

Description: Physicochemical properties of the water samples collected at sites P1, P2, and P3 in Salina Lake, Bragança, Pará. (O_2, TDS and cations/anions values are in mgL^{-1}).

The water was relatively acidic, with pH values between 4.0 and 5.1 (which are similar to those of rainwater), indicating that the lake is supplied primarily by the year-round precipitation that is typical of the region. Electrical conductivity was relatively uniform (Table 1) with the highest and lowest values recorded at sites P3 and P1, respectively. These sites are located near the outermost extremes of the lake (Figure 1). These values are considerably higher than 100 µS·cm-1 and are influenced by the high levels of dissolved

solids, which are themselves indicative of corrosive waters and an impacted environment.

2.2 Profile of the Communities, Sequencing, Editing, and Taxonomic Analyses

The graphs generated by DHPLC for the aquatic communities obtained from sites P1, P2, and P3 presented 4, 6, and 5 peaks, respectively, with the best resolution observed at a temperature of 64.5ºC (Figure 2a-c). For the sedimentary communities, the plots with the best resolution and separation for sites P1 and P2 each presented 6 peaks at 64.5ºC (Figure 2d-e), and site P3 presented 8 peaks at 65ºC (Figure 2f). The minor peaks were not collected because their lower resolution indicates that they might show the same microbial composition as the adjacent peaks (Figure 2). The distinct profiles obtained from the different samples appear to reflect the considerable diversity of the local bacterial communities to compare with each other.

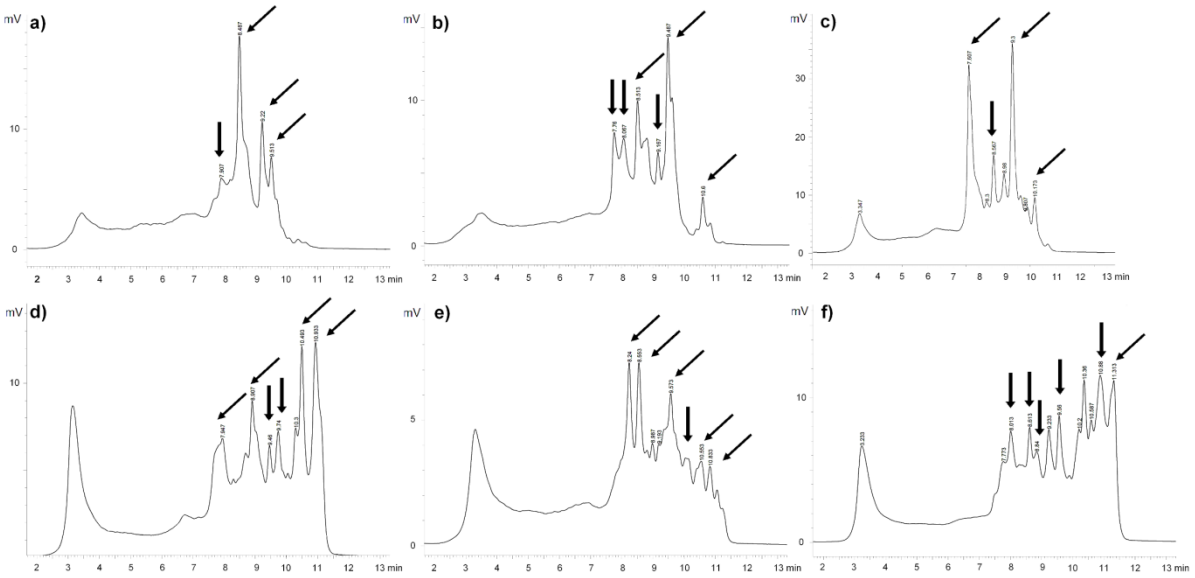

Figure 2. DHPLC analysis of the amplified fragments of the 16S rRNA gene

Description: The profiles of the communities contained in the water (P1 – a, P2 – b, P3 – c) and sediment samples (P1 – d, P2 – e, P3 – f), are characterized by peaks representing specific retention times. The arrows indicate the peaks collected for the investigation of the composition of the microbial population.

The processing of the 284 clones from the collected peaks resulted in 269 sequences that were valid for analysis (accession numbers HE648576-HE648837). Each sequence was approximately 200 bps long. Comparison with the RDP database indicates that theses belong to the *Proteobacteria, Firmicutes, Bacterioidetes, Acidobacteria, Spirochaetes, Actinobacteria, Chloroflexi*, Cyanobacteria, and TM7 phyla; unclassified bacteria were also obtained (Figure 3). The most abundant phylum in the lake water was *Proteobacteria* (53%), whereas in the sediments, the unclassified bacteria predominated (48%). At site P1, the unclassified bacteria were relatively abundant in both the water and sediment samples, whereas at site P2, *Proteobacteria* predominated in both the water and sediment samples.

Proteobacteria also predominated in the sediment sample from site P3. However, in the water sample from P3, unclassified bacteria were the most abundant (Figure 3).

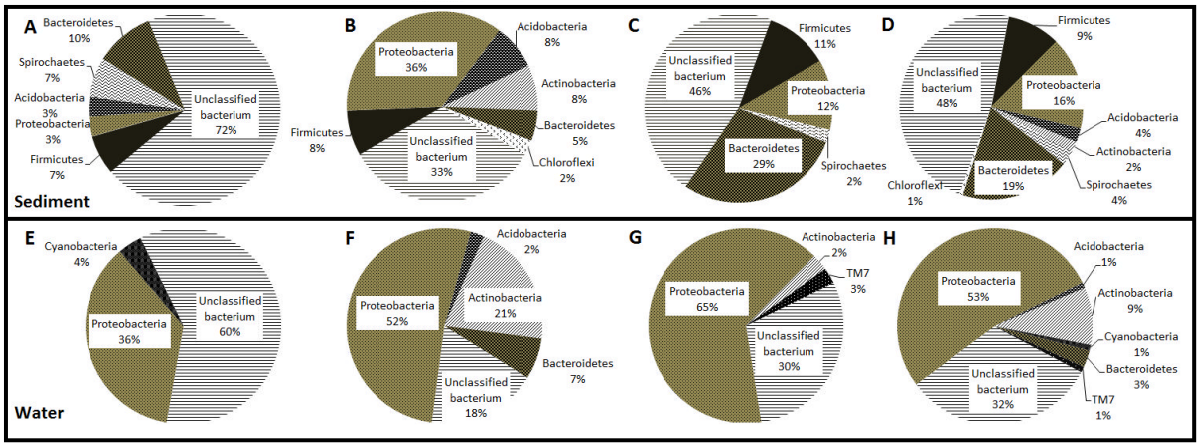

Figure 3. Relative abundance (%) and taxa observed at Salina Lake

Description: This is based on the criterion of 80% similarity with the sequences available in the RDP database, for the three sampling sites, P1 (A, E), P2 (B, F), P3 (C, G), and the total sample (D, H). Sediment samples are shown in the top row, water samples in the bottom row.

2.3 Statistical Analyses

The libraries for the sediment samples from sites P1, P2, and P3 contain 29, 34, and 52 unique operational taxonomic units (OTUs), respectively, whereas those for the water samples contain 24, 15, and 23 OTUs, respectively (with a difference of 0.03) (Figure 4). An analysis of the Shannon's diversity index and the Chao species richness estimator indicated that there was greater diversity in the sediment samples when compared with the samples obtained from the water. The highest values for the sediment were recorded at site P3, and the highest values for the water were recorded at P1. The number of rare phylotypes in both the water and sediment samples may account for this variation, which indicates that the diversity of these communities is so high that the number of OTUs sampled was insufficient to represent the true diversity of species at site P1. This conclusion is supported by the values obtained using the C coverage estimator (Table 2).

Table 2. Diversity indices for bacterial OTUs

Indices	Sediment Sample				Water Sample			
	P1	P2	P3	Total	P1	P2	P3	Total
Shannon[a]	33.7	3.2	36.0	4.5	3.2	2.4	2.8	3.9
Chao	435.0	102.0	1,125.0	367.9	300.0	22.0	108.5	331.2
Coverage	0.0	0.2	0.6	0.4	0.0	0.8	0.5	0.5

a. Non-parametric estimator of Shannon diversity indice.

Description: Shannon (H') and Chao indices with a difference of 3% and coverage for the sediment and water samples from each site in Salina Lake.

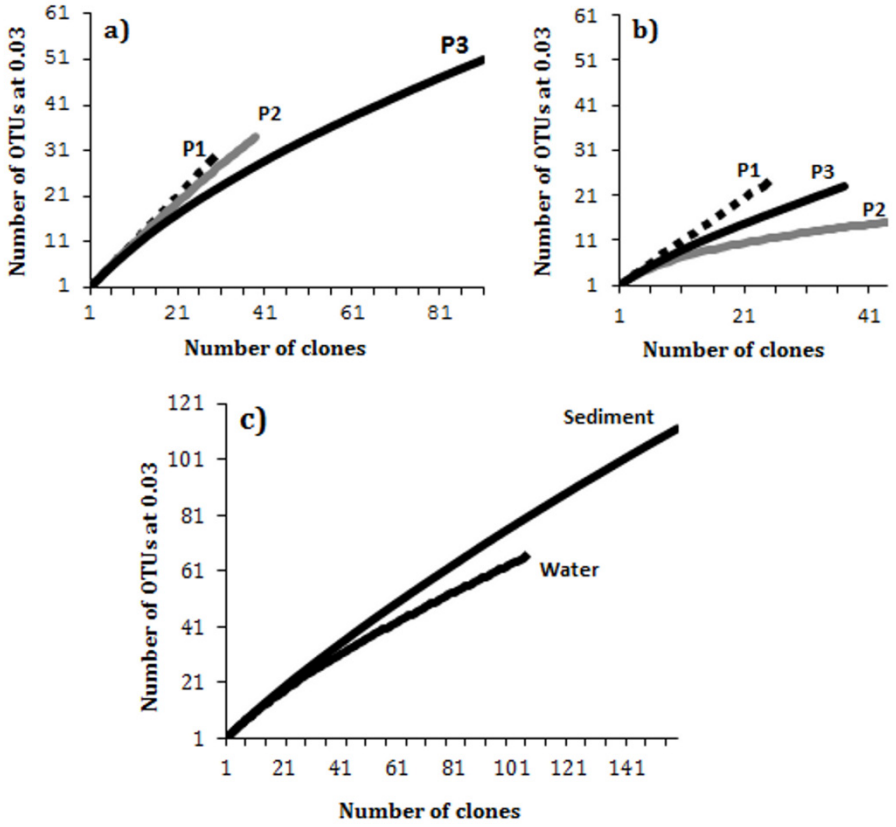

Figure 4. Rarefaction curve for the unique OTUs

Description: The unique OTUs found in the sediment (a) and water (b) of each sampling site, and the aquatic and sedimentary communities as a whole (c).

The C estimator indicates that the sediment and water communities at sites P2 and P3 had a coverage of 20%, 80%, 60%, and 50% (Table 2). Total coverage was 50% for the water samples and 40% for the sediment samples

The dendrograms indicate the degree of relationship among the communities at the different sampling sites (Figures 5 and 6). The *Proteobacteria, Bacteroidetes, Firmicutes, Chloroflexi,* and *Spirochaetes* phyla all formed separate clades with bootstrap values of over 80. The most abundant phylum in the aquatic community was the *Proteobacteria*, which formed a large clade that was well separated from the other phyla with high bootstrap values, except for two sequences that were distinct from both the main group and each other. These two sequences were HE648679, which formed a well-defined clade (bootstrap = 91) with a sequence from the database (Figure 5), and HE648617, which formed a clade with *Bacteriovorax* sp. (bootstrap = 96). Two unclassified cyanobacterial clones (HE648587 and HE648629) were also grouped separately with a high bootstrap value (96). These two clones are from different sampling sites; HE648587 was from P1, which is closer to the highway and suffers greater anthropogenic impact, and HE648629 was from site P2, which is in the middle of the lake.

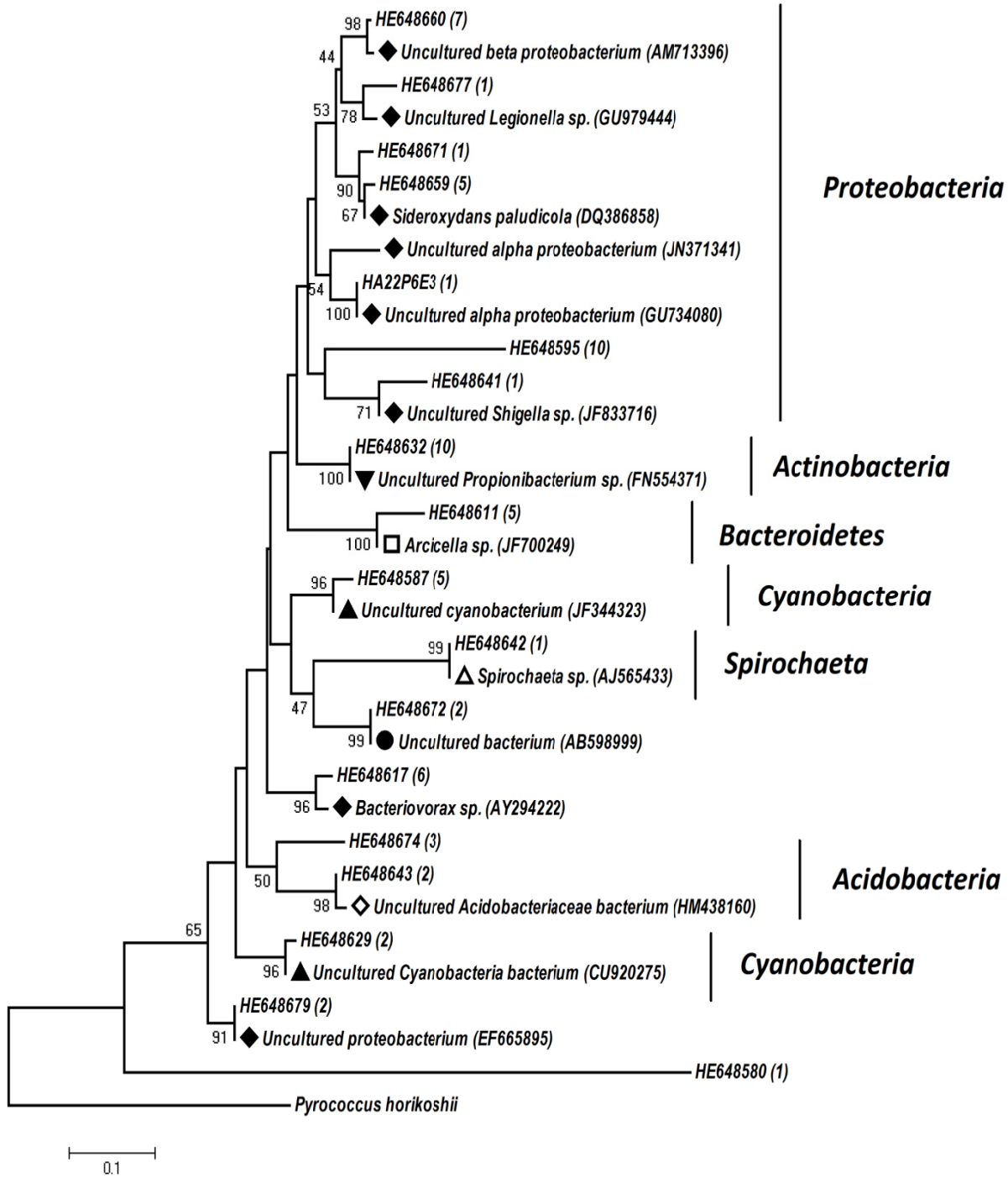

Figure 5. Dendrogram based on the sequences of the 16S rRNA gene for the bacterial phyla found in the water samples collected from Salina Lake

Description: *Proteobacteria* (♦), *Chloroflexi* (○), *Cyanobacteria* (▲), *Actinobacteria* (▼), *Bacteroidetes* (□), *Spirochaetes* (Δ), *Acidobacteria* (◊), *Firmicutes* (■), unclassified bacteria (●). The numbers in brackets beside the name of each sequence indicate the number of clones produced for the respective sequence.

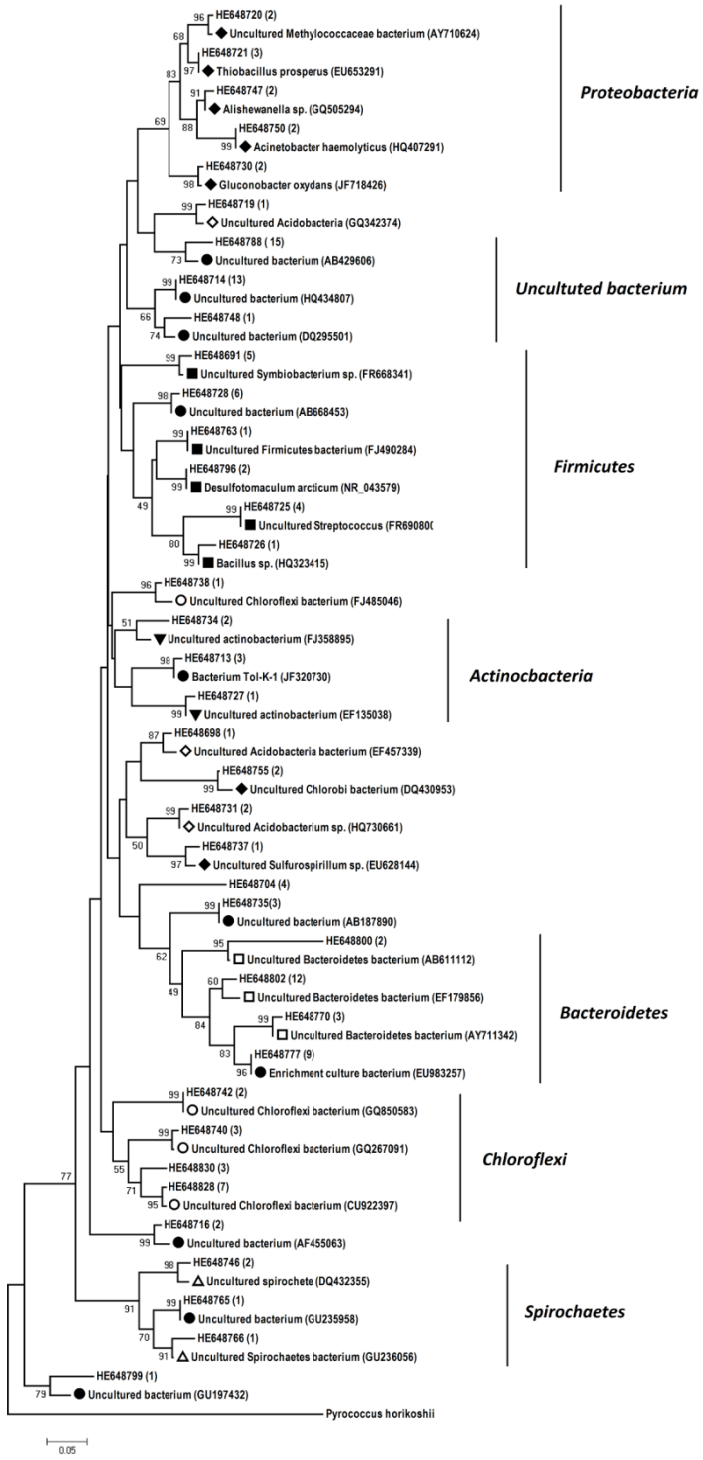

Figure 6. Dendrogram based on the sequences of the 16S rRNA gene for the bacterial phyla found in the sediment samples collected from Salina Lake

Description: *Proteobacteria* (♦), *Chloroflexi* (○), *Cyanobacteria* (▲), *Actinobacteria* (▼), *Bacteroidetes* (□), *Spirochaetes* (Δ), *Acidobacteria* (◊), *Firmicutes* (■), unclassified bacteria (●). The numbers in brackets beside the name of each sequence indicate the number of clones produced for the respective sequence.

The ∫-Libshuff analysis indicated that the structure of the communities from each sampling site (water and sediment) are statistically different from each other ($p < 0.001$), suggesting that each community may be considered to be a distinct ecosystem (Appendix 2).

3. Discussion

The profiles identified in the present study were similar to those reported for complex environmental communities (Wagner et al., 2009; Barlaan et al., 2005), although the chromatogram patterns obtained in the present study showed peaks with better resolution and separation (Figure 2). By comparing the number of peaks in the DHPLC chromatograms with the number of groups observed in the taxonomic graphs there is much similarity (Figures 1 and 2). The represented bacterial groups are *Proteobacteria, Bacterioidetes, Actinobacteria, Acidobacteria, Firmicutes, Chloroflexi, Spirochaetes, Cyanobacteria*, and unclassified bacteria (Figures 5 and 6). These phyla are relatively common in the mangrove systems of southeastern Brazil (Almeida et al., 2009; Gomes et al., 2010; Santos et al., 2011; Silveira et al., 2011) and in other tropical and subtropical countries, such as India, China, Australia, and the United States (Zhang et al., 2008; Zhou et al., 2009), both aquatic and sedimentary communities (Clementino et al., 2008; Vieira et al., 2008).

The distribution of the different bacterial groups within the area of the lake reflect the influence of chemical, physical, and geographical factors, such as pH, temperature, salinity, redox potential, and competition, that favor their development (Lozupone & Knight, 2007; Dimitriu et al., 2008; Caporaso et al., 2011). The local climate is divided into a rainy season between January and July and a dry season between August and December. The principal variations in abiotic conditions are caused by equinoctial tides, which may reach 6 m and cause strong two-way currents and seasonal fluctuations in river discharge. These phenomena exert a considerable influence on the species composition of the local communities (Cohen et al., 1999; Lara & Dittmar 1999). These characteristics represent the occurrence of ecological succession, given that this environment is undergoing restructuring after clearing the lake area and decomposition of tree species.

The represented bacterial groups are involved in a number of different biogeochemical cycles and the decomposition of mangrove vegetation. The organisms function in the liberation of CO2, phosphorus and nitrogen, which are the principal elements in the trophic network (Holguin et al., 2001; Hewson & Fuhrman, 2004; Zhang et al., 2008; Ghosh et al., 2010). For example, *Desulfotomaculum arcticum* (recorded at P3), which survives at high temperatures and is involved in the sulfur cycle. This species is a reducer of sulfates, and like other species of the genus, it liberates hydrogen sulfate into the environment, a feature that is characteristic of mangrove systems (Vandieken et al., 2006).

Several classes from the *Proteobacteria* phylum, including the *Betaproteobacteria, Deltaproteobacteria*, and *Gamaproteobacteria* classes, were the most abundant organisms found in the aquatic community of the lake (at sites P2 and P3). These classes are involved in the cycling of carbon, nitrogen, and sulfur in the ecosystem and include *Desulforhopalus singaporensis*, a sulfur-reducing deltaproteobacterium that was found at site P3, which is close to the undisturbed mangrove. Other important detected organisms include unclassified

cyanobacteria, which are involved in the consumption of nitrogen for the production of oxygen, *Bacteriovorax* sp. and an unclassified betaproteobacterium of the order, *Methylophilales*. Each of the latter organisms is found in areas with salinity concentrations above 0.5% (Baer et al., 2004), which is similar to the concentration recorded in the present study (Appendix 1). Representatives of the *Acidobacteria* were also present, although most of the recognized taxa are still unclassified. The ecology of this phylum is not well understood, although representatives can be found in a range of environments. Organisms from subdivision I, known as true acidophiles, grow at a pH of between 3.5 and 4.5, a range that corresponds to the values recorded at Salina Lake (Table 1) (Davis et al., 2011).

Propionibacterium was found at P2. This genus of pathogenic bacteria causes diseases, such as acne, in humans. The bacterial groups present in the sedimentary community, including the *Alphaproteobacteria, Betaproteobacteria*, and sulfur-reducing groups, such as *Desulfotomaculum arcticum* and *Sulfurospirillum*, are commonly found in estuarine and marine environments (Dimitriu et al., 2008). These organisms play an important role in the biogeochemical cycle of the ecosystem (Jennerjahn & Ittekko 2002). Bacterial groups from the *Spirochaetes* phylum, which cause a number of diseases in humans, were also found in the present study. A representative of the TM7 phylum was found in the water sample from site P3 (Appendix 1).

The composition of the sedimentary community was quite distinct from that of the aquatic group (48%). This indicates that the diversity of species found in the sediment is much higher than that present in the aquatic community. This result was also verified by Shannon and Chao diversity indice and the rarefaction curves. Similar results have been observed in a number of previous studies (Kemp & Aller 2004; Lozupone & Knight, 2007; Caporaso et al., 2011; Graças et al., 2011).

The analysis of community structure (∫-Libshuff) indicated that both the sedimentary and aquatic communities were the most distinct at site P2. These findings may be due to the presence or absence of certain phylotypes in these communities (Schloss & Handelsman 2004), such as the presence of one more phylum in the sedimentary community at P2 than P1 (Appendix 2).

The high diversity at P2 and P3 site, the low coverage observed at site P1 and the evidence of bacterial groups derived from external environments into the lake by tides corroborate the occurrence of ecological succession, with a dynamic recruitment and domain groups regarding the frequency of phyla *Proteobacteria, Firmicutes, Bacterioidetes*, *Actinobactérias, Acidobacteria, Chloroflexi, Espiroquetas* in all sampling points of the Saline Lake (Figure 3). It also indicated a clear shift in the composition of community structure among aquatic environments, sediments and location of each community.

4. Conclusions

The present study provided a preliminary description of the composition of bacterial diversity in the aquatic and sedimentary communities at the Salina Lake of Bragança-Pa, northeast Amazonia. Composition differences of these communities and their probable role in the

ecosystem indicate the occurrence of ecological succession. The difference in the abundance of phyla, species of different ecological groups and unclassified bacteria indicates the differentiation of successional stages. Proteobacteria was the most abundant phylum in aquatic and sediment communities and was recognized as opportunistic and pioneer. However, more seasonal studies will be necessary to understand the true diversity of this biota and its role within the local environment.

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Appendix

Appendix 1. Taxonomic classification

Clone	Sequence Acession no.	Closest relative				
		Ident. (%)	RDP Classifier Phylum/Class/Order	Ident. (%)	BLAST-N Identity	Sequence Acession no.
P1 Site HA23P4A4	HE648587	99	Cyanobacteria	99	Uncultured cyanobacterium	JF344323
		99	Cyanobacteria			
		98	Bacillariophyta			
HA23P6E7	HE648591	98	Proteobacteria	94	*Escherichia coli*	JN180966
		89	Gammaproteobacteria			
		85	Enterobacteriales			
HA23P7D10	HE648578	86	Proteobacteria	89	*Polynucleobacter necessarius*	AB607317
		82	Betaproteobacteria			
		73	Burkholderiales			
HA23P4C4	HE648594	94	Proteobacteria	91	Uncultured betaproteobacterium	EU070245
		88	Betaproteobacteria			
		68	Methylophilales			
SA21P11B1	HE648685	94	Firmicutes	95	Uncultured Firmicutes bacterium	FJ490284
		71	Bacilli			
		71	Bacillales			
SA21P11A1	HE648688	81	Proteobacteria	92	Uncultured Desulfuromonas sp.	JF807003
		78	Deltaproteobacteria			
		61	Desulfuromonadales			
SA21P11F1	HE648698	98	Acidobacteria	96	Uncultured Acidobacteria bacterium	EF457339
		96	Acidobacteria Gp18			
		96	Gp18			
SA21P7D10	HE651023	91	Bacteroidetes	85	Uncultured Bacteroidetes bacterium	EF179856
		49	Flavobacteria			
		49	Flavobacteriales			
SA21P10D10	HE648706	89	Bacteroidetes	85	Uncultured Bacteroidetes bacterium	EF179857
		35	Sphingobacteria			
		35	Sphingobacteriales			
P2 Site HA22P1A1	HE648600	100	Proteobacteria	100	*Polynucleobacter necessarius*	AB607317
		100	Betaproteobacteria			
		100	Burkholderiales			

Clone	Sequence Acession no.	Closest relative				
		Ident. (%)	RDP Classifier Phylum/Class/Order	Ident. (%)	BLAST-N Identity	Sequence Acession no.
HA22P6H3	HE648612	99	Bacteroidetes	95	*Arcicella sp.*	JF700249
		96	Sphingobacteria			
		96	Sphingobacteriales			
HA22P1D1	HE648614	100	Proteobacteria	95	*Bacteriovorax sp.*	AY294222
		100	Deltaproteobacteria			
		100	Bdellovibrionales			
HA22P3G2	HE648619	100	Proteobacteria	100	Uncultured betaproteobacterium	EU070245
		100	Betaproteobacteria			
		100	Methylophilales			
HA22P5H2	HE648625	100	Proteobacteria	99	*Escherichia coli*	JF910107
		100	Gammaproteobacteria			
		100	Enterobacteriales			
HA22P8D4	HE648632	100	Actinobacteria	100	Uncultured Propionibacterium sp.	FN554371
		100	Actinobacteridae			
		100	Actinomycetales			
HA22P6E3	HE648641	100	Proteobacteria	100	Uncultured alpha proteobacterium	GU734080
		100	Alphaproteobacteria			
		91	Rhizobiales			
HA22P9G4	HE648643	99	Acidobacteria	96	Uncultured Acidobacteriaceae bacterium	HM438160
		99	Acidobacteria Gp6			
		99	Gp6			
HA22P7D6	HE648630	67	Proteobacteria	99	Uncultured Cyanobacteria bacterium	CU920275
		65	Deltaproteobacteria			
		52	Desulfuromonadales			
SA23P10B8	HE648715	100	Proteobacteria	98	Uncultured Desulfuromonadales bacterium	JF727697
		100	Deltaproteobacteria			
		94	Desulfobacterales			
SA23P6G1	HE648713	83	Actinobacteria	95	Uncultured bacterium	JF320730
		42	Coriobacteridae			
		42	Coriobacteriales			
SA23P11E8	HE648727	100	Actinobacteria	99	Uncultured actinobacterium	EF135038
		100	Actinobacteridae			
		100	Actinomycetales			
SA23P12B9	HE648719	95	Acidobacteria	92	*Uncultured Acidobacteria*	GQ342374
		95	Holophagae			
		95	Holophagales			
SA23P1F1	HE648721	100	Proteobacteria	99	*Thiobacillus prosperus*	EU653291
		100	Gammaproteobacteria			
		85	Chromatiales			
SA23P6D1	HE648726	99	Firmicutes	100	Uncultured Streptococcus	FR690800
		98	Bacilli			
		92	Bacillales			
SA23P1E1	HE648725	100	Firmicutes	100	Uncultured Streptococcus	FR690800
		100	Bacilli			
		100	Lactobacillales			
SA23P8E7	HE648731	100	Acidobacteria	99	Uncultured Acidobacterium sp.	HQ730661
		100	Acidobacteria Gp1			
		100	Gp1			
SA23P2A4	HE648735	100	Bacteroidetes	100	Uncultured bacterium	AB187890
		100	Sphingobacteria			
		100	Sphingobacteriales			
SA23P1B1	HE648737	88	Proteobacteria	96	Uncultured Sulfurospirillum sp.	EU628144
		82	Epsilonproteobacteria			
		81	Campylobacterales			

Clone	Sequence Acession no.	Closest relative				
		Ident. (%)	RDP Classifier Phylum/Class/Order	Ident. (%)	BLAST-N Identity	Sequence Acession no.
SA23P4E7	HE648740	83	Chloroflexi	97	Uncultured Chloroflexi bacterium	GQ267091
		81	Anaerolineae			
		81	Anaerolineales			
P3 Site HA21P2C1	HE648644	100	Proteobacteria	100	Uncultured beta proteobacterium	EU070245
		100	Betaproteobacteria			
		100	Methylophilales			
HA21P4C1	HE648654	99	Proteobacteria	97	Betaproteobacterium enrichment culture	HQ386537
		99	Betaproteobacteria			
		98	Burkholderiales			
HA21P5H1	HE648668	99	Proteobacteria	98	Uncultured betaproteobacterium	AB539996
		99	Betaproteobacteria			
		78	Rhodocyclales			
HA21P1C1	HE648672	97	TM7	98	Uncultured bacterium	AB598999
		97	TM7			
HA21P5A1	HE648675	98	Proteobacteria	99	Uncultured alphaproteobacterium	JN371341
		97	Alphaproteobacteria			
		96	Rhodobacterales			
HA21P3G1	HE651022	100	Actinobacteria	100	Uncultured Propionibacterium sp.	FN554371
		100	Actinobacteridae			
		100	Actinomycetales			
HA21P7A1	HE648678	99	Proteobacteria	94	*Desulforhopalus singaporensis*	NR_028742
		97	Deltaproteobacteria			
		88	Desulfobacterales			
SA22P4A12	HE648747	100	Proteobacteria	98	*Alishewanella sp.*	GQ505294
		100	Gammaproteobacteria			
		94	Alteromonadales			
SA22P4B11	HE648750	100	Proteobacteria	99	*Acinetobacter haemolyticus*	HQ407291
		100	Gammaproteobacteria			
		100	Pseudomonadales			
SA22P2D4	HE648754	95	Proteobacteria	96	Uncultured bacterium	HQ434807
		95	Deltaproteobacteria			
		88	Desulfuromonadales			
SA22P4D10	HE648762	100	Firmicutes	97	Uncultured Firmicutes bacterium	FJ490284
		87	Bacilli			
		87	Bacillales			
SA22P2H6	HE648766	87	Spirochaetes	92	Uncultured Spirochaetes bacterium	GU236056
		87	Spirochaetes			
		87	Spirochaetales			
SA22P2B6	HE648772	100	Bacteroidetes	98	Enrichment culture bacterium	EU983257
		85	Bacteroidetes_incertae_sedis			
		85	Prolixibacter			
SA22P1A3	HE648792	100	Proteobacteria	97	Bacterium Tol-K-1	AB081545
		100	Deltaproteobacteria			
		92	Syntrophobacterales			
SA22P1F2	HE648796	100	Firmicutes	97	*Desulfotomaculum arcticum*	NR_043579
		100	Clostridia			
		100	Clostridiales			
SA22P3B7	HE648814	90	Bacteroidetes	96	Uncultured Bacteroidetes bacterium	AB611170
		51	Flavobacteria			
		51	Flavobacteriales			
SA22P3E9	HE648793	100	Bacteroidetes	92	*Lactobacillales bacterium*	AY581273
		47	Bacteroidetes_incertae_sedis			
		47	Marinifilum			

Description: Analysis of the partial sequences of the 16S rRNA gene obtained from the peaks generated by the DHPLC for the different sites in Salina Lake, and their tentative taxonomic classification, using two database (RDP and NCBI).

Appendix 2. Libshuff analyses comparison

Comparison	dCXY Score	Significance
Sedimento (P1-P2)	0.00159181	0.7297
Sedimento (P2-P1)	0.04112548	<0.0001
Sedimento (P1-P3)	0.00462641	0.6398
Sedimento (P3-P1)	0.04291301	<0.0001
Sedimento (P2-P3)	0.01496923	0.0067
Sedimento (P3-P2)	0.06626945	<0.0001
Água (P1-P2)	0.00318128	0.3662
Água (P2-P1)	0.15063480	<0.0001
Água (P1-P3)	0.49854240	<0.0001
Água (P3-P1)	0.50566340	<0.0001
Água (P2-P3)	0.95203501	<0.0001
Água (P3-P2)	0.65223309	<0.0001
P1 (Água – Sedimento)	0.02212464	<0.0001
P1 (Sedimento – Água)	0.02664804	<0.0001
P2 (Água – Sedimento)	0.10018914	<0.0001
P2 (Sedimento – Água)	0.03510955	<0.0001
P3 (Água – Sedimento)	0.65223309	<0.0001
P3 (Sedimento – Água)	0.89750832	<0.0001

Description: Results of the Libshuff analyses comparing the libraries for the water and sediment samples from the three sampling sites (P1, P2, P3).

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