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Prokaryotic Diversity of the Tucuruí Hydropower Plant Reservoir in the Brazilian Amazon

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Abstract

To perform the a culture-independent characterization of the microbial diversity observed within a water body in the Amazon, we constructed a library of bacterial and archaeal 16S rRNA genes isolated from environmental samples from the Tucuruí Hydroelectric Plant reservoir. The sampling locations included the upstream compartment (MR), which is located 50 km upstream of the dam, and Itupiranga (ITU), which is located upstream on the Tocantins River at the inlet of the reservoir. The bacterial phyla Cyanobacteria (46%), Proteobacteria (12%), Actinobacteria (9%), Bacteroidetes (4%) and Verrucomicrobia (2%) were identified at the MR. Phyla including the Chloroflexi, Acidobacteria, Planctomycetes and TM6 candidate division were observed at a frequency of <2%. The phyla Cyanobacteria (62%), Proteobacteria (5%) and Actinobacteria (7%) were identified at ITU. The archaeal diversity was limited to the Euryarchaeota and Crenarchaeota phyla; the latter was found in greater abundance and mainly consisted of uncultured phylotypes. In both regions, 25% and 95% of the bacteria and archaea, respectively, that were identified were previously unclassified. The phylum Cyanobacteria was almost exclusively represented by the genus *Synechococcus* sp.; however, a wide variety of microorganisms from the phylum Proteobacteria were identified from the classes alpha, gamma, delta and beta proteobacteria, with the latter being the most abundant. This study demonstrates that high prokaryotic diversity exists in the Amazonian rivers in addition to the previously reported megadiversity of fauna and flora.

Keywords: Amazon, Archaea, Bacteria, Hydropower reservoir, Metagenome, 16S rDNA

1. Introduction

Despite the current knowledge of the megadiversity of animals and plants in the Amazon, little is known about the microbial diversity (Borneman & Triplett, 1997; Fiore *et al.*, 2005; Hungria *et al.*, 2005; Fierer & Jackson, 2006; Lima-Bittencourt, 2007).

The rivers that form the Amazon basin drain an area that is greater than 7 x 106 km2 and discharge approximately 18% of all fresh water in the world into the oceans (Benner, 1995). These freshwater habitats are fundamentally important to humanity and are directly related to the maintenance of the Amazonian megadiversity and the density of rainfall on the continent; these rivers also provide an energy source through the use of hydroelectric power plants (Fearnside, 1989; Junk & Mello, 1990; Fearnside, 1993).

The Brazilian energy matrix is 88% dependent on hydropower, and the Tucuruí Hydroelectric Power Station (HPS-Tucuruí) (3°43' and 5°15' S and 49°12' and 50°00' W) is the primary genuinely national hydropower generator. In addition to the plants that are currently in operation, more than a dozen hydropower plant projects are under construction, and a similar number of new projects are planned for the Brazilian Amazon, which has the potential to provide 52% of the nation's hydropower. The construction of the HPS-Tucuruí resulted in the flooding of approximately 2,430 km^2 of forest and the formation of a reservoir that has a total volume of 50,290 x 106 m^3 and is 180 km in length (Junk & Mello, 1990; Tundisi, 2007).

The construction of hydroelectric dams along the Amazon is responsible for many environmental changes both above and below the dams. Some of the impacts of these buildings are directly related to global climate change through the production of greenhouse gases. For example, the Tucuruí area that is now occupied by the reservoir was previously an ombrophilous forest. When this area was submerged, a large amount of organic matter was converted to CO_2 and CH_4 (Fearnside, 1995; Fearnside, 1997).

The analysis of microbial communities using cloned fragments of collective genomes has been the subject of intensive research in soil (Handelsman, 1998), marine (Venter *et al.*, 2004), river (Gonzalez-Toril, 2003), wetland (Dedysh, 2006) and airborne (Tringe *et al.*, 2008) ecosystems.

Estimates have been performed using DNA reassociation kinetics that predict that 99.9% of the pristine soil microbial diversity is unknown (Gans, 2005). Amann and colleagues (Amann *et al.*, 1995) estimate that our knowledge of microbial diversity in mesotrophic lakes does not exceed 1% of the real diversity. Approaches based on molecular biology techniques have been demonstrated to be the most effective for determining the richness of diversity and the structure of microbial communities (Suzuki, 1998). To study this diversity in the natural environment, pioneering work using 16S rDNA has resulted in a new system for the classification of life based on molecular sequences and structures that was proposed because these data are more revealing than approaches that are dependent on culturing (Woese, 1990). The present work contributes to the knowledge of microbial diversity through the sequencing of 16S rDNA fragments found in ecogenomes from lakes in this region of the world. Thus, we chose to investigate the reservoir that was created by the HPS-Tucuruí using two locations:

a site at the Tocantins river and another site in the middle of the lake.

2. Research Methods

2.1 Location and Sample Collection

The HPS-Tucuruí is located in the Eastern Brazilian Amazon where the river characteristics are suitable for power generation. This reservoir is located in the Araguaia basin on the lower course of the Tocantins River (Fig. 1).

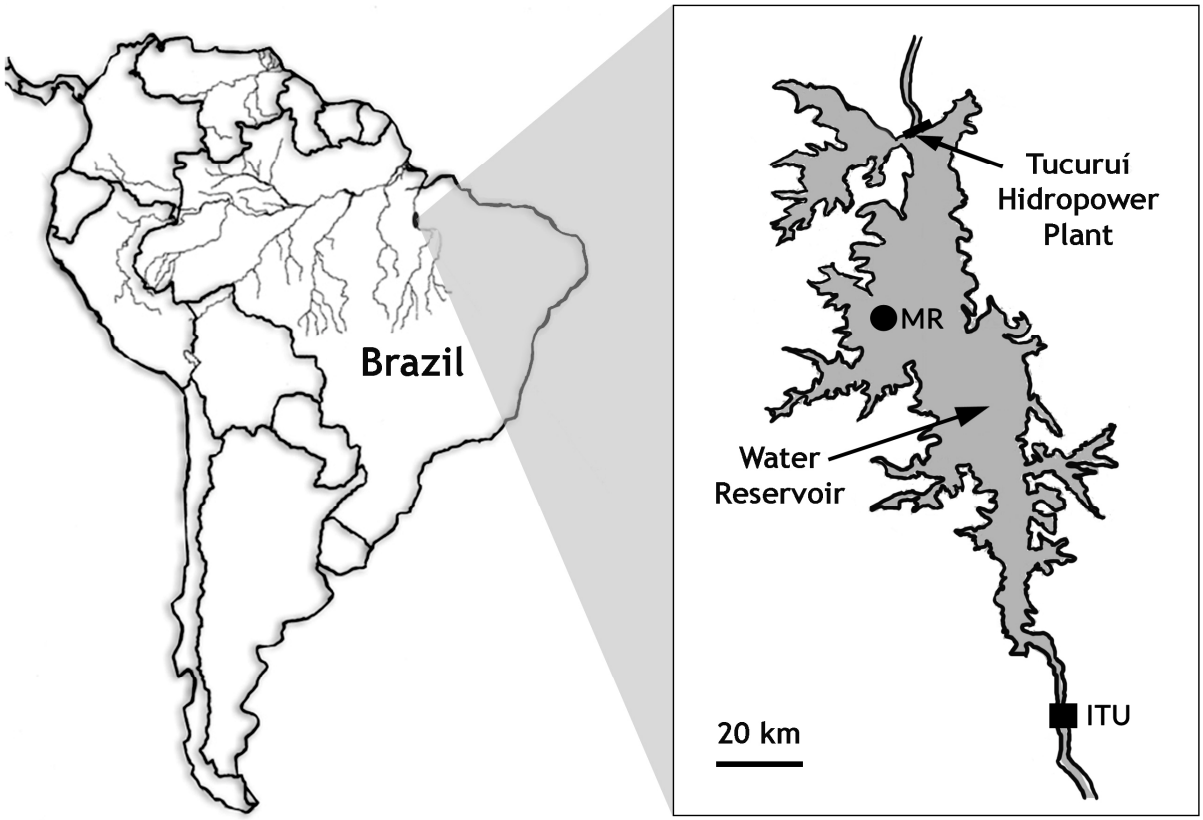

Figure 1. HPS Tucuruí geographical location

Description: Geographical location of the Tucuruí Hydroelectric Power Station on the Tocantins River in northern Brazil with its respective sampling points; the Upstream Compartment (MR ●) and Itupiranga (ITU ■) that were used in the metagenomic analysis.

To analyze the microbial communities and to determine the physicochemical characteristics of the reservoir, specific sampling regions established by the Limnology/Water Quality Program of the HPS-Tucuruí Centre of Environmental Protection (Centro de Proteção Ambiental – CPA) were used. The following sampling points were used in this study: the upstream compartment (MR) (04°13'7''S and 49°42'9.2''W), which is located 50 km from the dam, and Itupiranga (ITU) (05°08'0.2''S and 49°19'14,1''W), which is located upstream of the reservoir (Fig. 1).

A total of 20 L of water was collected from each sampling site. The photic layer was sampled at ITU, and a pooled sample of the photic and aphotic layer was collected at MR (0-20 m); these samples were collected using a Van Dorn bottle and transferred to amber glass flasks.

At the collection site, the water temperature was measured, and sub-samples were taken for the analysis of pH, optical density, ammonium, nitrate, phosphate and turbidity. The collection period in September 2006 corresponded to the Amazonian summer.

2.2 DNA Extraction

The separation of the microorganisms was performed using three parallel filtrations. In the first step we used a Büchner funnel with qualitative paper. The water was subsequently filtered under negative pressure through a sterile nitrocellulose membrane with a pore size of 0.8 µm (Millipore, Brazil) and was filtered again under the same conditions using a 0.22 µm membrane (Whatman, Germany). The membranes were perforated and transferred to a centrifuge tube containing 40 ml of a DNA preservation solution (50 mM Tris HCl, 500 mM NaCl, 125 mM EDTA pH 8.0). The tubes were shaken overnight at room temperature on an orbital shaker. Precipitation of the cells was performed by centrifugation at 4000 rpm for 20 minutes at room temperature.

Environmental DNA was isolated using the UltraClean™ Soil DNA Kit (MoBio, USA) according to the manufacturer's specifications. DNA quantification was performed using UV spectrometry.

2.3 16S rRNA Gene Amplification Using the Polymerase Chain Reaction (PCR)

PCR was performed to amplify 16S rRNA gene fragments. A PTC 200 thermocycler (MJ Research, UK) was used with the primers 8f (5'-AGAGTTTGATYMTGGCTCAG-3'), 1492r (5'-CGGTTACCTTGTTACGACTT-3') and S16.5-F3 (5'-GCCAGCCGCGGTAATAC-3') for bacteria and 341f (5'-CCTAYGGGGYGCASCAGGCG-3') and 1407r (5'-GACGGGGGTGWGTRCAA-3') for archaea. PCR started with a denaturation step at 94°C for 5 min followed by 30 cycles of 94°C for 1 min, 55°C for 1 min and 72°C for 90 s and a final extension of 10 min at 72°C. The amplification solution had a final volume of 50 µL and contained the following reagents: 10 x buffer [22 mM Tris-HCl (pH 8.4), 55 mM KCl], 2.5 mM $MgCl_2$, 0.2 mM dNTPs, 5 µM of each primer and 2 U of Platinum Taq DNA polymerase (Invitrogen, USA).

The amplicons were visualized by electrophoresis on a 2% agarose gel (GE Healthcare, USA). Fragments in the range of approximately 1.5 kb for bacteria and 1 kb for archaea were excised from the gel and purified using the GFX PCR DNA and Gel Band Purification Kit (GE Healthcare, USA).

2.4 Cloning and Sequencing

The amplicons were ligated into the pGEM®-T Easy Vector System (Promega Corp., USA) according to the manufacturer's instructions and were subsequently transformed into DH5α *Escherichia coli* (Stratagene, USA) using an electroporator (GIBCO BRL, USA). The transformed bacteria were plated on semi-solid LB agar (DIFCO, USA) containing ampicillin

(EMS, Brazil) and X-gal (GE Healthcare, USA). Recombinant clones were isolated by blue and white screening and grown in LB ampicillin liquid medium (DIFCO, USA) in deep well plates with shaking in an orbital incubator at 200 rpm for 16 hours at 37°C. The isolation of recombinant nucleic acids was performed using an alkaline lysis miniprep, and the vectors were purified using MAGV N22 membranes (Millipore, USA).

The sequencing reaction was performed using the DYEnamic™ ET Dye Terminator Kit according the manufacturer's instructions (GE Healthcare, USA) and the primers that were used for the PCR step. Sequencing electrophoresis was performed using a MegaBACE 1000 automatic nucleic acid detection system according to the manufacturer's instructions (GE Healthcare, USA).

2.5 Data Analysis

For the bacterial sequences, the Sequencher 4.0.5 (Gene Codes Corp.) and BioEdit (www.mbio.ncsu.edu/BioEdit/bioedit.html) programs were used for the assembly and manual editing of contigs of approximately 1200 bp, and Mallard software (Ashelford, 2006) was used to detect chimeric sequences and possible sequencing and editing anomalies. The sequences were aligned to sequences from the SILVA database using the Sina WebAligner online tool (Pruesse et al., 2007). From this alignment, distance matrices were calculated and corrected using the Jukes-Cantor algorithm in the DNAdist program of the Phylip package (http://evolution.genetics.washington.edu/phylip.html). These files were used as input for the DOTUR program version 1.53 (Schloss & Handelsman, 2005), which was used to calculate the diversity indices of the samples using the Furthest Neighbor method.

The estimated number of operational taxonomic units (OTUs) was calculated using the nonparametric estimators ACE and Chao1. The Good's C (Good, 1953) and Chao et al. C_{ACE} (Chao, 1993) nonparametric coverage estimators were used. Sequence classification was performed using the SINA WebAligner tool, which aligned the experimental sequences with the most closely related sequences in the SILVA 16S rRNA database. Archeal domain sequences were submitted to the MyRDP pipeline (Cole *et al.*, 2007) using PHRED software for quality analysis; only those sequences with a score equivalent to 20 were used. The LUCY software was used to remove sequences derived from the pGEM-T Easy vector.

Dendrograms were constructed using the neighbor-joining method and were generated in the Phylip package using the Jukes-Cantor model to calculate the distance between the sequences. A bootstrap analysis of the arrangements was performed with 1000 replicates, and consensus values greater than 50% were shown at the branches of the cladograms, which were viewed using the Figtree application (http://tree.bio.ed.ac.uk/). The sequences obtained for this study were submitted to GenBank with the accession numbers EU592502 to EU592963

3. Results

The physicochemical parameters for both sampling points are described in Table 1. The low concentration of nitrates at the water surface suggests that ammonium is the primary nitrogen source used by the microorganisms in this layer (Table 1).

Table 1. Physicochemical parameters

	pH	OD (mg/mL)	NH_4^+ (µg/L)	NO_3^- (µg/L)	PO_4^- (µg/L)	Turbidez (NTU)	Temperatura (^oC)
MR 0.00m	7.37	5.96	8.48	0.0*	13.05	11.76	32.60
MR 26.0m	6.69	0.00	1402.21	35.89	72.97	25.30	29.20
ITU 0.00m	7.54	6.99	10.70	0.0*	13.05	22.70	29.80

Description: The measurements were done by the Centre of Environmental Protection as part of the Limnology/Water quality control. *Values below detection level.

The metagenomic library had a total of 345 bacterial clones of approximately 1200 bp; 61 of these clones corresponded to ITU, and 284 corresponded to MR. In addition, a total of 117 partial archaeal sequences of approximately 400 bp were obtained: 37 were collected from ITU, and 80 were collected from MR. The taxonomic relationships demonstrate that the OTUs were affiliated with nine bacterial phyla and two archaeal phyla (Figs. 2 and 3).

In the two sampled areas, approximately 25% of the bacteria and 95% of the archaea were identified as unclassified environmental taxa. The bacterial phylum with the highest relative abundance at both sampling points was the Cyanobacteria. However, a considerable abundance of Actinobacteria and Proteobacteria was also observed. Additionally, Verrucomicrobia and Bacteroidetes were observed only in the MR. The phyla Chloroflexi, Acidobacteria, Planctomycetes and the TM6 candidate division were detected in the metagenome of the MR sampling point. During the characterization of the archaeal phylotypes, representatives from the phyla Euryarchaeota and those corresponding to uncultured archaea were identified from both sampling points (Table 2).

Table 2. Accession number of each unique OTU with their closest relative organism from ARB-SILVA

GenBank Accession	No. of Clones	Maximum Identity (%)	Closest Relative Organism from ARB-Silva	Accession NCBI & CAMERA	Environmental Location of the Closest Relative Organism
EU592838	1	96	uncultured Acidobacteria bacterium	AM292624	Uranium mining waste pile, Germany (unpublished)
EU592601	5	88	Streptomyces flavidofuscus	AY999914	Data not found
EU592628	4	98	uncultured actinobacterium	AJ575501	Rimov reservoir water, Czech Republic (Warnecke, 2004)
EU592635	3	99	uncultured actinobacterium	EU117783	Little Arbor Vitae Lake, Wisconsin, USA (Newton, 2007)
EU592590	3	97	Mycobacteriaceae bacterium	DQ490438	Kilauea volcano deposits, Hawaii, USA (unpublished)
EU592623	3	95	uncultured actinobacterium	EU117723	Hook Lake water, Wisconsin, USA (Newton, 2007)
EU592596	2	97	uncultured actinobacterium	EF520353	Acid-impacted Adirondack Lake, New York, USA (Percent, 2008)
EU592593	2	88	Streptomyces flavidofuscus	AY999914	Data not found
EU592508	1	99	uncultured actinobacterium	EU117871	Mirror Lake water, Wisconsin, USA (Newton, 2007)
EU592625	1	98	uncultured actinobacterium	AJ575506	Lake Schoehsee water, Germany (Wercecke, 2004)
EU592597	1	97	uncultured actinobacterium	AY948059	Parker River, Massachussetts, USA (Crump, 2005)
EU592504	1	97	uncultured actinobacterium	AJ575506	Lake Schoehsee water, Germany (Wercecke, 2004)
EU592634	1	96	uncultured actinobacterium	EU117954	Trout Lake water, Wisconsin, USA (Newton, 2007)
EU592502	1	95	uncultured actinobacterium	EU117730	Ike Walton Lake water, Wisconsin, USA (Newton, 2007)
EU592511	1	95	uncultured actinobacterium	EU117897	Red Cedar Lake water, Wisconsin, USA (Newton, 2007)
EU592624	1	92	uncultured actinobacterium	AJ575506	Lake Schoehsee water, Germany (Wercecke, 2004)
EU592563	5	97	uncultured Bacteroidetes	DQ463716	Lake Tanganyika oxic epilimnion, Tanzania (unpublished)

			bacterium		
EU592570	4	98	uncultured Bacteroidetes bacterium	DQ463729	Lake Tanganyika oxic epilimnion, Tanzania (unpublished)
EU592569	1	98	Flavobacteria bacterium KF030	AB269814	Lake Kasumigaura water, Japan (Watanabe, 2009)
EU592568	1	93	uncultured Bacteroidetes bacterium	AY947919	Ipswich River, Massachussetts, USA (Crump, 2005)
EU592821	1	n/a	uncultured candidate division TM6 bacterium	DQ676372	Suboxic freshwater-pond, France (Briée, 2007)
EU592667	1	91	uncultured Chloroflexi bacterium	DQ501314	Lake Grosse Fuchskuhle water, Germany (unpublished)
EU592712	83	99	Synechococcus sp. MH305	AY224198	Central European Subalpine Lakes (Crosbie, 2003)
EU592688	35	98	Synechococcus sp. 0BB26S03	AJ639899	Bubano Basin, Italy (unpublished)
EU592523	20	99	Synechococcus sp. LBG2	AF330249	Brackish Baltic Sea (Ernst, 2003)
EU592708	10	97	Synechococcus sp. MW28B3	AY151235	Central European Subalpine Lakes (Crosbie, 2003)
EU592527	9	97	Synechococcus sp. 0BB26S03	AJ639899	Bubano Basin, Italy (unpublished)
EU592522	2	97	Synechococcus sp. MW25B5	AY151233	Central European Subalpine Lakes (Crosbie, 2003)
EU592672	2	97	Synechococcus sp. MH305	AY224198	Central European Subalpine Lakes (Crosbie, 2003)
EU592546	1	98	Synechococcus sp. PS721	AF216954	Lake Biwa water, Shiga, Japan (Robertson, 2001)
EU592537	1	98	uncultured bacterium	EU804045	Lake Gatun water, Panama (Shaw, 2008)
EU592532	1	97	Merismopedia sp. CENA106	EF088332	Waste satabilization pond, Brazil (unpublished)
EU592558	1	95	Synechococcus sp. MH305	AY224198	Central European Subalpine Lakes (Crosbie, 2003)
EU592536	1	97	uncultured bacterium	EU804045	Lake Gatun water, Panama (Shaw, 2008)
EU592555	1	95	Synechococcus sp. PS721	AF216954	Lake Biwa water, Shiga, Japan (Robertson, 2001)
EU592543	1	96	uncultured bacterium	EU803469	Lake Gatun water, Panama (Shaw, 2008)
EU592823	1	88	uncultured bacterium	EU803469	Lake Gatun water, Panama (Shaw, 2008)
EU592606	15	99	uncultured bacterium	DQ520197	Hypertrophic Meiliang Bay, China (Wu, 2007)
EU592503	8	99	uncultured bacterium	AB154300	Lake Kasumigaura water, Japan (unpublished)
EU592636	6	99	marine metagenome	AACY0201 73194	Marine metagenome (Venter, 2004)
EU592680	4	96	uncultured bacterium	DQ444431	Lake Tanganyika, Tanzania (Schubert, 2006)
EU592824	4	84	uncultured soil bacterium	DQ298008	Hydrocarbon Contaminated Soil, USA (unpublished)
EU592676	3	98	uncultured bacterium	DQ444431	Lake Tanganyika, Tanzania (Schubert, 2006)
EU592598	3	96	bacterium rJ7	AB021325	Phenol-digesting activated Sludge, Japan (Watanabe, 1999)
EU592673	3	94	uncultured bacterium	AJ538354	Lake Cadagno, Switzerland (unpublished)
EU592685	3	88	uncultured bacterium	DQ906072	Tinto River Rhizosphere, Spain (unpublished)
EU592828	2	100	marine metagenome	AACY0201 79327	Marine metagenome (Venter, 2004)
EU592646	2	98	marine metagenome	AACY0234 37746	Marine metagenome (Venter, 2004)
EU592576	2	97	uncultured bacterium	EU133614	Kessler Farm soil, Oklahoma, USA (Elshahed, 2008)
EU592831	2	93	uncultured Crater Lake bacterium	AF316766	Ultra-oligotrophic Crater Lake, Oregon, USA (Urbach, 2001)
EU592665	2	91	bacterium 005-D	AY661916	Groundwater contamined, USA (Fields, 2005)
EU592643	1	99	uncultured bacterium	EF157202	Asphalts from Rancho La Brea Tar Pits, USA (Kim, 2007)
EU592818	1	99	marine metagenome	AACY0201 79327	Marine metagenome (Venter, 2004)
EU592833	1	99	marine metagenome	AACY0201 74722	Marine metagenome (Venter, 2004)
EU592594	1	98	uncultured bacterium	AJ867924	Alpine Lake Joeri XIII, Switzerland (unpublished)
EU592510	1	98	uncultured bacterium	DQ520196	Hypertrophic Meiliang Bay, China (Wu, 2007)
EU592612	1	97	marine metagenome	AACY0201 81515	Marine metagenome (Venter, 2004)
EU592632	1	97	uncultured bacterium	DQ520196	Hypertrophic Meiliang Bay, China (Wu, 2007)
EU592845	1	97	uncultured bacterium	AB154317	Lake Kasumigaura water, Japan (unpublished)
EU592519	1	97	uncultured bacterium	EF203205	Lake Kastoria Sediment, Greece (unpublished)
EU592587	1	96	unidentified bacterium	AY345540	Lake Wai'ele'ele water, Hawaii, USA (unpublished)
EU592588	1	96	uncultured bacterium	EF590055	Songhua River sediment contamined with nitrobenzene (Li, 2008)
EU592621	1	96	uncultured bacterium	DQ520197	Hypertrophic Meiliang Bay water, China (Wu, 2007)

EU592817	1	96	uncultured bacterium	EF157231	Asphalts from Rancho La Brea Tar Pits, USA (Kim, 2007)
EU592842	1	96	uncultured bacterium	AY963365	Humus and mineral soils forest, China (Chan, 2006)
EU592514	1	96	uncultured bacterium	DQ520165	Hypertrophic Meiliang Bay water, China (Wu, 2007)
EU592651	1	95	uncultured bacterium	AB294330	Deep coal seam groundwater, Japan (Shimizu, 2007)
EU592586	1	94	uncultured bacterium	AY328588	Drinking water distribuition system, Ohio, USA (Williams, 2004)
EU592653	1	94	uncultured bacterium	AY706436	TCE-contaminated groundwater, Ohio, USA (Humphries, 2005)
EU592830	1	92	uncultured Crater Lake bacterium	AF316767	Ultra-oligotrophic Crater Lake, Oregon, USA (Urbach, 2001)
EU592668	1	91	uncultured bacterium	EU134543	Kessler Farm soil, Oklahoma, USA (Elshahed, 2008)
EU592516	1	91	marine metagenome	AACY020174722	Marine metagenome (Venter, 2004)
EU592518	1	91	uncultured bacterium	DQ520177	Hypertrophic Meiliang Bay water, China (Wu, 2007)
EU592820	1	90	uncultured bacterium	EU134543	Kessler Farm soil, Oklahoma, USA (Elshahed, 2008)
EU592517	1	90	marine metagenome	AACY024099985	Marine metagenome (Venter, 2004)
EU592520	1	90	uncultured bacterium	DQ520197	Hypertrophic Meiliang Bay water, China (Wu, 2007)
EU592562	1	89	uncultured bacterium	AY212691	Water 10m downstream of manure (Simpson, 2004)
EU592819	1	88	uncultured bacterium	DQ413113	Data not found
EU592677	1	96	uncultured planctomycete	AJ616263	Elbe River biofilm, Germany (Bruemmer, 2004)
EU592678	1	96	uncultured planctomycete	AJ616263	Elbe River biofilm, Germany (Bruemmer, 2004)
EU592577	6	99	uncultured alpha proteobacterium	DQ501352	Lake Stechlin water, Germany (unpublished)
EU592659	5	99	uncultured beta proteobacterium	AM849452	Oligo-mesotrophic lake Piburger See, Austria (Salcher, 2008)
EU592654	4	98	beta proteobacterium MWH-IPGL7W22	AJ876403	Data not found
EU592645	4	99	uncultured beta proteobacterium	AM849432	Oligo-mesotrophic lake Piburger See, Austria (Salcher, 2008)
EU592644	2	95	Sphaerotilus sp. L19	AB087568	Data not found
EU592584	2	99	uncultured proteobacterium	DQ450165	Shallow atlantic coastal lagoon (Piccini, 2006)
EU592839	2	97	uncultured gamma proteobacterium	AY509440	Freshwater bacterioplankton of eutrophic lakes, Sweden (Eiler, 2004)
EU592574	1	95	uncultured alpha proteobacterium	AJ518772	Uranium mining waste pile, Germany (unpublished)
EU592583	1	99	uncultured alpha proteobacterium	AY948064	Parker River, Massachussetts, USA (Crump, 2005)
EU592589	1	98	uncultured alpha proteobacterium	AY622245	Surface soil of Oak Ridge Reservation, USA (Reardon, 2004)
EU592560	1	97	uncultured alpha proteobacterium	AY948064	Parker River, Massachussetts, USA (Crump, 2005)
EU592561	1	99	uncultured alpha proteobacterium	DQ501352	Lake Stechlin water, Germany (unpublished)
EU592521	1	99	beta proteobacterium MWH-IPGL7W22	AJ876403	Data not found
EU592652	1	93	uncultured beta proteobacterium	AF529336	Data not found
EU592658	1	88	Aquitalea magnusonii	DQ018117	Humic-lake water sample, Wisconsin, USA (Lau, 2006)
EU592669	1	98	uncultured delta proteobacterium	EF520534	Acid-impacted Adirondack Lake, New York, USA (Percent, 2008)
EU592841	1	86	uncultured delta proteobacterium	EU050845	Sediment from the Kings Bay, Svalbard, Arctic (unpublished)
EU592822	1	n/a	uncultured proteobacterium	AF420354	Guaymas Basin hydrothermally active sediments, Mexico (Teske, 2002)
EU592670	1	99	Acinetobacter sp. DQ124	DQ300355	Data not found
EU592671	1	96	Acinetobacter sp. IGCAR-9/07	EF517956	Biofilm formed on modified titanium surface, India (unpublished)
EU592835	4	95	uncultured Verrucomicrobiae bacterium	EF520638	Acid-impacted Adirondack Lake, New York, USA (Percent, 2008)
EU592837	1	95	unidentified eubacterium LD29	AF009975	Lake Loosdrecht water, Netherlands (Zwart, 1998)
EU592846	1	94	uncultured Verrucomicrobia bacterium	AY509518	Freshwater bacterioplankton of eutrophic lakes, Sweden (Eiler, 2004)
EU592947	133	97	marine metagenome	JCVI_NT_1050101565 67	Lake Gatun water, Panama (unpublished)
EU592916	7	90	uncultured euryarchaeote	AB243796	Rice paddy soil, Japan (Sakai, 2007)

Description: Classification of unique OTUs obtained in the Tucuruí ecogenome that grouped with 3% dissimilarity compared with sequences deposited in the SILVA and CAMERA databases.

The statistical calculation of the diversity index was only performed for the bacterial domain because the archaeal diversity consisted of only two phylotypes. In the rarefaction curves, 26 species, 22 genera and eight phyla were identified in the sample collected from ITU (Fig. 4, A), and 77 species, 63 genera and 29 phyla were identified in the sample collected from MR (Fig. 4, B).

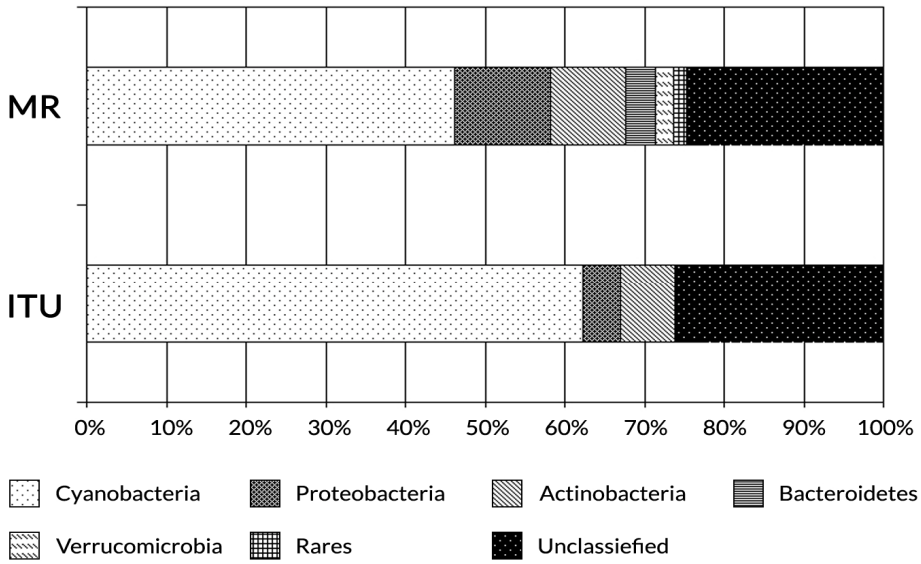

Figure 2. The bacterial diversity at phylum level found at MR and ITU points

Description: Diversity of bacterial phyla described from the different sampling points of the reservoir. *Among the rare phyla (<2%) are Chloroflexi, Acidobacteria, Planctomycetes and a candidate for the TM6 division.

In both libraries, at a distance of 3% between the sequences, the two curves were weakly curvilinear, which suggests that more exhaustive sampling would detect even more phylotypes at the two locations. The curves with 20% dissimilarity reached saturation, indicating that few additional phyla would be found with a greater sampling effort.

The coverages calculated using the Good's C estimator were 64% and 85% for ITU and MR, respectively. Lower values were obtained using the Chao C_{ACE} estimator at 45% for ITU and 72% for MR. For dissimilarities of 3% and 20% between the sequences, the ACE richness estimator detected 210 and 14 OTUs, respectively, in ITU, and 145 and 39 OTUs, respectively, in MR. For dissimilarities of 3% and 20%, the Chao1 richness estimator predicted 142 and 10 OTUs in ITU and 152 and 40 OTUs, respectively, in MR (Fig. 04 C and D). When the OTUs were grouped at 3% dissimilarity, as defined using the DOTUR program, a total of 105 unique OTUs were identified; these OTUs were classified and are described in Table 2.

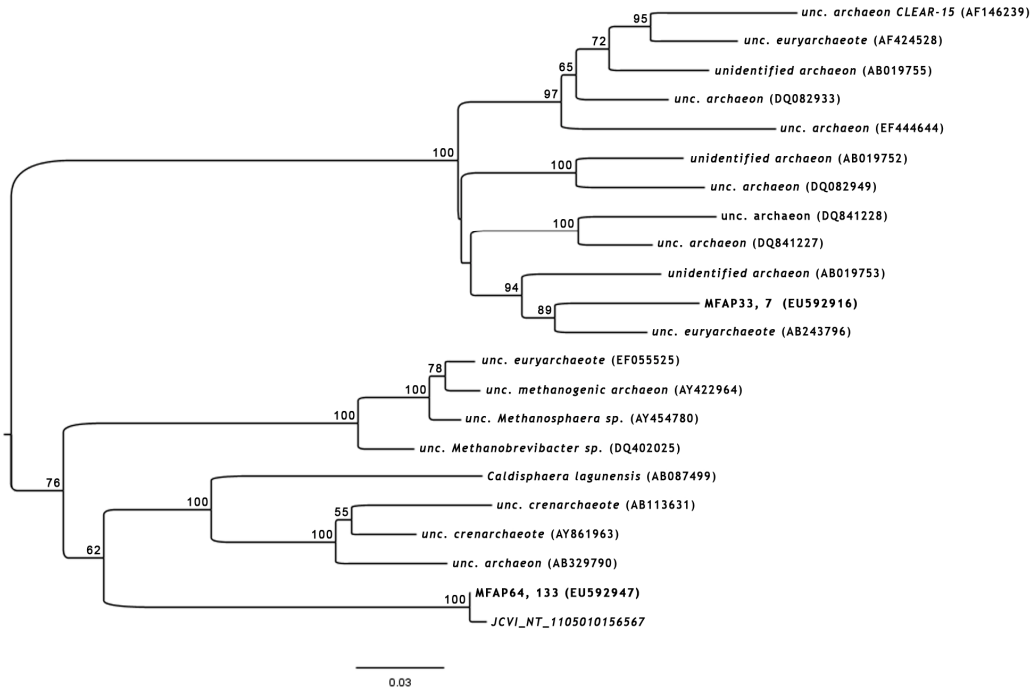

Figure 3. Phylogenetic analysis of Archaea sequences

Description: Archaeal phylogenetic tree showing the diversity found at the sampling points. Sequences that start with MFAP were obtained from the Tucuruí metagenome. After the comma is the number of OTUs with 3% dissimilarity and the GenBank accession number is in brackets.

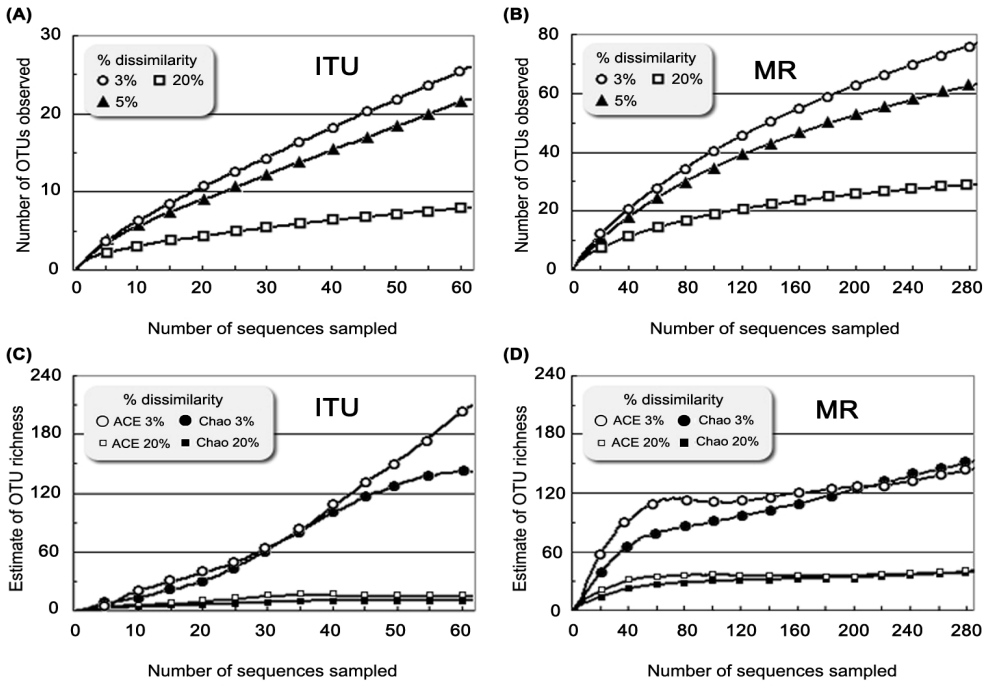

Figure 4. Rarefaction curves and estimators analysis

Description: Rarefaction curves and Ace and Chao1 richness indices for the sampling points ITU and MR. The OTUs with 3% dissimilarity were classified at the species level, those with 5% dissimilarity were classified at the genus level and those with 20% dissimilarity were characterized at the phylum level.

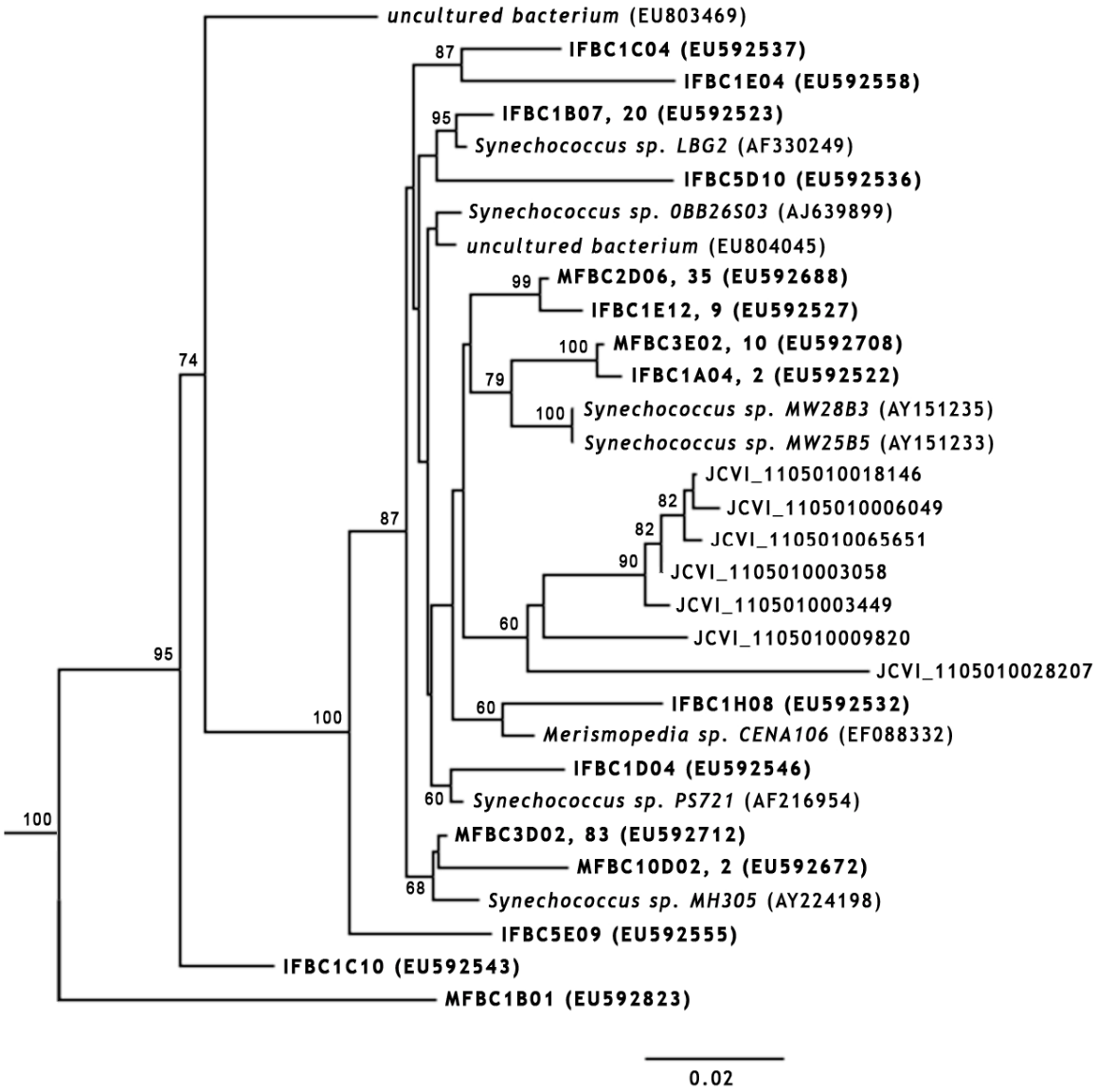

Figure 5. Phylogenetic analysis of Cyanobacteria sequences

Description: Phylogenetic tree of cyanobacteria with the 15 unique OTUs grouped with the closest sequences obtained from the SILVA and CAMERA databases. Sequences that start with MFBC or IFBC are from the Tucuruí metagenome and sequences that start with JCVI were obtained from CAMERA. Next to the name of the clones is the number of sequences that grouped with 3% dissimilarity and in brackets is the GenBank accession number.

4. Discussion

Aquatic environments are less diverse relative to other environments such as soil or sediment and typically have an estimated phylotype richness of less than 200 (Curtis, 2002; Kemp &

Aller, 2004; Nold & Zwart, 1998; Torsivik, 2002). Tucuruí Lake had a diversity of 142 to 210 OTUs at 3% dissimilarity. These values are within the expected range according to Kemp and Aller (2004). These diversity values could increase if the number of chimeras was fewer given that they comprised 7% and 12% of the total contigs obtained from ITU and MR, respectively.

In silico analysis revealed an abundance of bacterial species that corresponded to uncultured microorganisms or that were distant from sequences deposited in the 16S rRNA database. The low diversity of archaea identified in this study is probably due to the low number of sequences deposited in the database, especially with regard to those originating from equatorial areas of the ecosphere. The primary phyla that were characterized in the Tucuruí Lake ecogenome are discussed below.

4.1 Cyanobacteria

Cyanobacteria was the most predominant phylum at both sampling points. Despite its importance in the food chain, little is known about this group in the Brazilian Amazon (Fiore *et al.*, 2005). The cyanobacteria observed in Tucuruí Lake corresponded almost entirely to the genus *Synechococcus*. This group and the genus *Prochlorococcus* are the dominant type of photoautotrophic picoplankton and are major contributors to global primary production (Waterbury, 1986). Nitrogen is one of the most abundant elements in *Synechococcus* cells and accounts for approximately 5-10% of their dry cell weight (Bryant, 1994). The high ammonia concentration and the low frequency of other denitrifying groups may be primarily responsible for the targeted selection of this group. Other determinants for the successful colonization by this group are probably related to the sampling period, which is characterized by high luminosity and temperature (Table 1).

In our analysis, 164 of the 169 sequences that were classified as cyanobacteria belonged to the genus *Synechococcus*. At the MR region, the representatives of this genus are closely related to the *Synechococcus* species described in subalpine lakes of central European (Crosbie, 2003). Although Ernst (2003) suggested that these strains belonged to groups of cyanobacteria inhabiting mainly freshwater and saline lakes, this is contrary to the idea that many species of free-living microorganisms have a global ecological distribution (Finlay, 2002). The marine cyanobacteria described in the analysis by Venter *et al.* (2004) form clades distinct from those found in freshwater lakes including those described in the Tucuruí reservoir, as observed in Figure 05, reinforcing the concept that these species are distinct from the groups found in different environments. Other cyanobacteria that are part of the lake microbial community form distinct clades with high bootstrap values, suggesting a high diversity of phylotypes (Figure 05).

4.2 Actinobacteria

In our study, 15 actinobacteria phylotypes were detected at the two locations. The sequences that are most similar to those within the SILVA database are predominantly from freshwater lakes and belong to uncultivated lineages. The phylum actinobacteria is relatively diverse and contains several subgroups. Furthermore, little is known about their role in the environment.

Several lineages have been defined as acI, acII, acIII and acIV, these clusters are clearly distinct from the lineages found in soil and marine habitats (Warnecke, 2004).

A total of 18 clones that are classified as uncultured bacteria, as represented by the accession numbers EU592606, EU592621, EU592514 and EU592520 (Table 02), are related to the Actinobacteria described in a study that was performed in Lake Taihu in China (Wu, 2007), in which the characterization of the ML-51.2-5 lineage within the Actinobacteria phylum was performed by phylogenetic analysis.

4.3 Proteobacteria

Among the most diverse and well-represented bacterial phylum in the databases is the Proteobacteria. This phylum has more than 460 genera and greater than 1600 species that were known as of 2002, which accounts for 40% of the publications regarding prokaryotic microorganisms (Kersters, 2006) and includes 44% of the bacterial genome sequencing projects in the world (Liolios, 2007). In our analysis, representatives of the alpha- and betaproteobacteria classes were detected in the samples from both ITU and MR. Gamma and delta proteobacteria were also described in MR (Fig. 05). The presence of these classes at MR compared to ITU, which is a region with a less-modified landscape, may be related to the eutrophication of the lake. Eutrophication would influence the microbial community composition by increasing the concentration of compounds that are rich in phosphorus and nitrogen at a depth of 26 m in the MR region (Table 1).

Proteobacteria are involved in many biochemical processes that are vital to the environment. Proteobacteria perform the fixation of CO_2 derived from organic and inorganic substrates via the Calvin-Benson-Bassham cycle; therefore, at lake Tucuruí, Proteobacteria, Cyanobacteria and methanogenic Archaea are responsible for maintaining the biochemical cycle of derived carbon from the organic biomass that was formed when the forest was submerged in the reservoir. Other communities that are important for this process could be best described as being located in the sediment and aphotic layers of the lake.

Alphaproteobacteria are characterized as being a metabolically flexible group and are present in environments with substantial variations in the CO_2 and O_2 concentrations. Betaproteobacteria are generally less flexible with respect to their metabolic strategies (Badger, 2008), and their primary habitat is freshwater that is normally still, photic and enriched with organic nutrients and compounds (Imhoff, 2006), which describes the environment under study and explains the predominance of this class at the reservoir.

In the gammaproteobacteria class, two of the four contigs were most closely related to *Acinetobacter* (96% and 99% similarity). This genus includes gram-negative, non-fermentative bacteria that are found in soil, water, sewage, food and clinical settings as a nosocomial infectious pathogen (Towner, 2006). It should be noted, however, that there is a significant difference between the populations of this genus that are found in the clinical and environmental settings (Vallenet *et al.*, 2008). Finally, members of the deltaproteobacteria class, which is the least abundant class in the reservoir, were classified as uncultivated environmental samples and are closely related to members of the same class found in

metagenomes from acid lakes and Arctic sediments (Table 2).

4.4 Archaea

In the dendrogram, the Tucuruí archaeal reads form two clades that are related to the phyla Euryarchaeota and Crenarchaeota. It is important to detect and identify archaeal sequences in all types of environments to understand the ecological role of these microorganisms, which have long been considered to exist exclusively in extreme environments (Bintrim, 1997). The analysis of 16S rRNA genes have shown that Archaea are distributed in various environments, such as freshwater lakes, soil and marine environments (Schleper, 2005). According to Woese (1990), the phylum Euryarchaeota is represented by the orders Thermococcales, Methanococcales, Methanobacteriales and Methanomicrobiales, as well as the extreme halophiles. In the present study, only seven sequences were identified as belonging to the Euryarchaeota and correspond to the methanogenic archaeal group. The individuals represented by these seven sequences are involved in various biogeochemical cycles, especially the carbon cycle, because they perform methanogenesis, which is the production of methane from carbon dioxide, hydrogen, acetate, formate and other C_1 compounds such as methanol, methylthiols and methylamines (Thauer, 1998). Approximately 133 of the sequences were similar to uncultivated archaea derived from studies involving a shotgun metagenomic approach (Seshadri, 2007), suggesting that despite the tremendous increase in the amount of sequences available in the database, the Archaea domain remains poorly characterized from a genomic perspective when compared to the Bacterial domain. OTU MFAP64 (Figure 03), which is represented by the sequence with accession number EU592947, is phylogenetically similar to an archaeal sequence from an environmental sample from Lake Gatun, Panama. This lake has similar characteristics as the HPS-Tucuruí reservoir, such as the temperature, salinity and depth of the sampling point. Lake Gatun and the HPS-Tucuruí reservoir share another important feature: both bodies of water were artificially created by flooding caused by the building of dams.

5. Conclusions

Amazonia is widely known to have one of the greatest megadiversities of fauna and flora in the world. In addition, the Tucuruí metagenome demonstrates that this biome also has a rich diversity of microbes, many of which are uncultivated. In the present study, we used culture-independent techniques and various bioinformatic tools to demonstrate that it was possible to obtain a profile of the prokaryotic diversity for the two sampling locations of the lake formed by the HEP-Tucuruí. The present study provides knowledge and understanding of the microdiversity that exists in landscapes created by the implementation of mega-hydropower enterprises in the Brazilian Amazon.

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