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ORIGINAL PAPER

Prokaryotic diversity in one of the largest hypersaline coastal lagoons in the world

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Abstract Araruama Lagoon is an environment characterized by high salt concentrations. The low raining and high evaporation rates in this region favored the development of many salty ponds around the lagoon. In order to reveal the microbial composition of this system, we performed a 16S rRNA gene survey. Among archaea, most clones were related to uncultured environmental Euryarchaeota. In lagoon water, we found some clones related to *Methanomicrobia* and *Methanothermococcus* groups, while in the saline pond water members related to the genus *Haloarcula* were detected. Bacterial community was dominated by clones related to *Gamma-proteobacteria*, *Actinobacteria*, and *Synechococcus* in lagoon water, while *Salinibacter ruber* relatives dominated in saline pond. We also detected the presence of *Alpha-proteobacteria*, *Pseudomonas*-like bacteria and *Verrucomicrobia.* Only representatives of the genus *Ralstonia* were cosmopolitan, being observed in both systems. The detection of a substantial number of clones related to uncultured archaea and bacteria suggest that the hypersaline waters of Araruama harbor a pool of novel prokaryotic phylotypes, distinct from those observed in other similar systems. We also observed clones related to halophilic genera of cyanobacteria that are specific for each habitat studied. Additionally, two bacterioplankton molecular markers with ecological relevance were analyzed, one is linked to nitrogen fixation (*nif*H) and the other is linked to carbon fixation by bacterial photosynthesis, the protochlorophyllide genes, revealing a specific genetic distribution in this ecosystem. This is the first study of the biogeography and community structure of microbial assemblages in Brazilian tropical hypersaline environments. This work is directed towards a better understanding of the free-living prokaryotic diversity adapted to life in hypersaline waters.

Keywords Biodiversity · Halophile · Ecology · Biotechnology · Phylogeny · Genetics · Taxonomy · Enzymes · Molecular phylogeny

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Introduction

Hypersaline environments are distributed worldwide, and saturated thalassic brines are among the most physical and chemical demanding habitats on earth. Few groups of

organisms survive in them; rare eukaryotes and many halophilic prokaryotic cells are capable of living in saltern crystallizers. These habitats contain about 37% NaCl, at the limits of biological tolerance for this environmental factor (Ovreas et al. 2003). Despite the unusual biogeochemistry of hypersaline habitats, the composition of their microbiota has not been fully surveyed by classical methods. The use of 16S rRNA clone libraries has provided knowledge of the diversity and composition of microbial communities that live in extreme salt environments (Benlloch et al. 2002). Comparison of 16S rRNA clone libraries obtained from several hypersaline environments of different geographical origin has revealed unexpected uniformity among the microbial communities (Oren 2002).

Araruama Lagoon is a 220 Km^2 ecosystem located between 22°50′ and 22°57′ S; 42°00′ and 42°44′W, in Rio de Janeiro, Brazil. It is one of the largest permanently hypersaline coastal lagoons in the world (Moreira-Turck 2000). This lagoon is followed in size by the lagoons of Coorong, Australia; Dawhat the Sayh, in the Arab Gulf; Bardawil, in the Mediterranean east coast; Madre of Texas and Torrey Pines, USA; Madre de Tamaulipas and Ojo de Liebre, Mexico, and Enriquillo, in the Dominican Republic. However, the microbial assemblages in these halophilic environments have not been well studied.

The Araruama Lagoon is linked to the Atlantic Ocean by the Itajuru channel. A remarkable characteristic of this lagoon system is the high salt content (an average of 5.2% total salts), nearly twice the concentration of seawater. The climate in this region is typically hot and dry, with an annual mean precipitation between 750 and 900 mm and an evaporation of 890 and 1370 mm (Kjerfve et al. 1996). The low rainfall and high evaporation rates in this region favored the development of several salty ponds around the lagoon.

This system is vulnerable to pollution impacts due to its low depth, mostly 2–3 m deep, and limited water renewal (50% every 84 days). The dry climate concentrates the natural salts and also nutrients from the sewage waters. During the last two decades, a rapid change occurred in nutrient availability at the Araruama Lagoon. Five middle-sized cities surround the lagoon with 260,000 residents strongly contributing to the eutrophication process. Despite solid waste removal from domestic and industrial sewage treatment stations in this region, the discharged water still contains high levels of nutrients, mainly nitrogen and phosphorus. This enhances the eutrophication process in the lagoon leading to visible proliferation of macroalgae and cyanobacteria. The chlorophyll levels in 1980 were 1–2 μg l^{-1} and, in 1994, increased to 2–15 μg l^{-1}. In 2002, its concentration was between 30–120 μg l^{-1} (Braga et al. 2003). Since 1999, large algae biomasses of the genus *Enteromorpha* have proliferated, generating many residues from algal decomposition that accumulate in the deep. Frequently, winds suspend the sediments, which retain the elevated concentrations of nitrogen and phosphorus. This process brings these nutrients to the water column, independently adding to the nutrient-rich water discharged into the lagoon system by sewage (Moreira-Turcq 2000).

The purpose of the research described here was to investigate the prokaryotic diversity of water samples from the hypersaline areas of Araruama Lagoon. In addition, we used two *nif*-specific primers to identify microbes in this environment capable of nitrogen- and/or carbon-fixation.

Materials and methods

Sample collection

Water samples were collected at two different sites on 27 August 2004, and immediately kept on ice for transport to the laboratory and processed for analysis. A superficial water sample was collected from the Araruama Lagoon in Praia Seca (P1). Another sample was taken from a saline pond (P2) in the lagoon's margin (Fig. 1).

Determination of abiotic and microbiological parameters

Water temperature and salinity were measured in situ with a field thermometer and a hand-held refractometer (Leica).

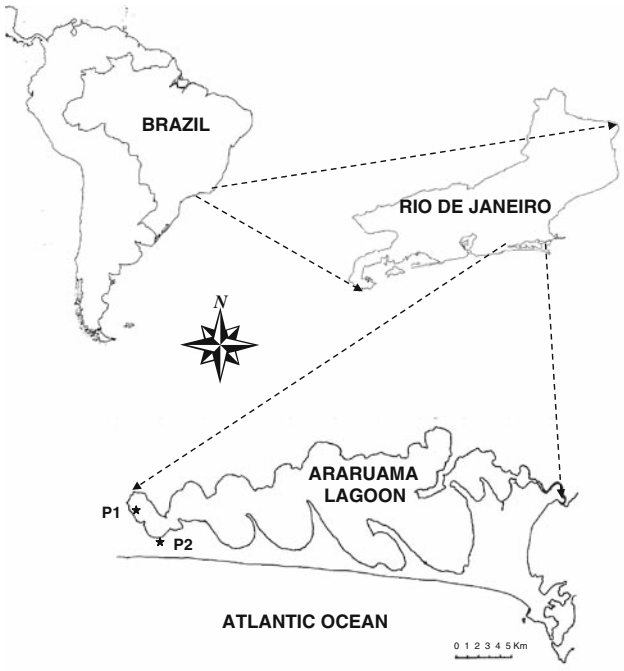

Fig. 1 Location of sampling sites. South America, Brazil, Rio de Janeiro state and *P1 and *P2 sites in Araruama Lagoon

The pH was analyzed by a potentiometric method (Grasshoff et al. 1999). Microbial abundance was determined by flow cytometry after nucleic acid staining with syto13 fluorochrome at 2.5 µM in samples fixed with 2% paraformaldehyde (Gasol and del Giorgio 2000; Andrade et al. 2003). Microbial activity was analyzed by a ^{3}H-leucine incorporation method (Kirchman et al.1985; Smith and Azam 1992). Specific production (SP) was calculated as the ratio MP MA^{-1} (Urbach et 2001).

DNA extraction from environmental samples

DNA was prepared according to Somerville et al. (1989). Briefly, water filtration on a 3.0 µm ester-cellulose filter (Millipore) was carried out to capture prokaryotic symbionts of microalgae, zooplankton or those attached to particulate organic materials. The free-living planktonic microbes were concentrated on a 0.22µm filter (Sterivex-Millipore). A volume of 50µl of freshly prepared lysozyme (1 mg/ml) was added to filter units containing 1.8 ml of lysis buffer and incubated at 37°C for 45 min. Then, 50µl of freshly prepared proteinase K (0.2 mg/ml) and 200µl of 10% sodium dodecyl sulfate (SDS) were added to the filter and incubated at 55°C for 1 h. Lysates were removed from the filter units with sterile 3 ml syringes, each rinsed with 1 ml of lysis buffer and incubated for 15 min. The rinse buffer and lysates were pooled. Crude lysates were extracted twice with phenol-chloroform-isoamyl alcohol (25:24:1, pH 8.0) and once with chloroform-isoamyl alcohol (24: 1). The nucleic acids in the aqueous phase were precipitated with two volumes of ethanol at −20°C overnight, centrifuged at 7,000 rpm for 15 min, washed with 70% ethanol, dried and then dissolved in 100µl TE (10 mM Tris–HCl, 0.1 mM EDTA, pH 8.0). The quality of the DNAs was checked in agarose gels stained with Syber Green after electrophoresis and the image digitalized with Storm Image Scanner (GE Health Care).

PCR amplification conditions

PCR was performed in 50 µl reaction mixtures (2.5 mM MgCl, 0.2 mM dNTPs, 50 pmol of each primer, 2.5 U of Platinum Taq DNA polymerase, and PCR buffer (Invitrogen) for approximately 50 ng of environmental genomic DNA extracted for each sample. Three universal forward primers were used, Bacteria 27BF (5′-AGAGTTTGATCATGGCTCAG-3′) (Lane 1991), archaea 21AF (5′-TTCCGGTTGATCCTGCCGGA-3′) (DeLong 1992), or Cyanobacteria 517CyanF (5′-TTATTGGGCCTAAAGCRTC-3′) (this work). Primer design was performed based on multiple alignments of cyanobacterial 16S rRNA sequences available in the public database. The sequences were downloaded from GenBank and aligned using ClustalX (Thompson et al. 1997). Consensus regions were used for primer design with amplicon sizes ranging from 800 to 1,000 bp.

Two prokaryotic universal reverse primers for archaea and bacteria 907ABR (5′-TTTGAGTTTMTTAATGCC-3′) (Lane et al. 1985) and cyanobacteria 1492ABR (5′-GGTTACCTTGTTACGACTT-3′) were used (Lane 1991). PCR amplification began with a 5-min denaturing step at 94°C; this was followed by 30 cycles of 94°C for 1.30 min, 50°C for 1.30 min, and 72°C for 2 min. The final cycle was an extension at 72°C for 10 min.

The *nifH*F (5′-GGHAARGGHGGHATHGGNAARTC-3′) and *nifH*R (5′-GGCATNGCRAANCCVCCRCANAC-3′) primers recognize *nif*H and its homologous *chl*L, *bch*L and *bch*Y genes (Mehta et al. 2003). For PCR, the DNA was denatured for 2 min at 94°C, followed by 35 cycles of 1 min at 94°C, 1 min at 60°C and 1 min at 72°C. A final extension step was carried out for 10 min at 72°C. Positive controls were obtained from *Rhizobium meliloti* ATCC 9930, *Agrobacterium tumefaciens* ATCC 4720, *Klebsiella pneumoniae* ATCC 13883 and *Bradyrizobium japonicum* ATCC 10324. *Escherichia coli* ATCC 11775 was used as negative control for *nifH* amplifications. Two libraries of these genes were constructed, one from P1 and one for P2.

Cloning and sequencing of 16S rRNA PCR products

PCR bands with the expected molecular weight were excised from a 1% agarose gel and eluted using the GFX-DNA and gel band purification kit (GE Health Care). Amplicons were cloned into the plasmid vector pGEM-T (Promega), according to the manufacturer's recommendations and transformed into *Escherichia coli* DH-10b cells. Two archaeal, two bacterial and two cyanobacterial 16S rRNA libraries were constructed from free-living planktonic prokaryotes collected at the two sampling sites (P1 and P2). The other two libraries for *nif*H/protochlorophyllide genes were also constructed. Clones from each library were submitted to sequence analysis. DNA from each clone was prepared (400 ng) and sequences were obtained with primer 21F for archaea and 27F for bacteria. The nucleotide sequence of the inserts was determined by cycle sequencing with the Big Dye reagent (Applied Biosystems, Foster City, CA, USA) and run in an Applied Biosystems ABI Prism 3730 automated DNA sequencer and by capillary electrophoresis on a MegaBace 1000 using the DYENamic dye terminator cycle sequencing kit (GE Healthcare). Chromatograms were transformed into Fasta format sequences with Phred software (Ewing et al. 1998). A total of 246 valid sequences were compared with sequences in the Ribosomal Database Project II (RDP II) (Cole et al. 2003). Chimeric sequences were identified and

removed using CHECK-CHIMERA (Cole et al. 2003). Alignments with representative sequences obtained at GenBank databases were carried out using ClustalX program (Thompson et al. 1997) to compare sequences.

Sequence trimming and analysis

Plasmid vector sequences and regions with quality below 20, according to Phred scores, at the 3′- and 5′-ends of the 16S rRNA and NifH genes were removed. Thus, only high quality sequences were used in the analyses. Sequences with less than 300 bp were excluded from subsequent analyses. The sequences obtained were compared with those in GenBank by the BLAST search tool. Phylogenetic trees were calculated by the Kimura two-parameter model (Kimura 1980) and the neighbor-joining algorithm (Saitou and Nei 1987) using the MEGA software (Kumar et al. 2001). A total of 1,000 bootstraps were performed to assign confidence levels. The diversity of the phylotypes was further examined using the DOTUR software, LIBSHUFF statistics and rarefaction analysis (Singleton et al. 2001). The 246 partial 16S rRNA, *nif*H and protochlorophyllide gene sequences reported in this study were submitted to GenBank/NCBI database under accession numbers EF598848 - EF599094.

Results

Abiotic and microbiological parameters

The temperatures were 31°C in the lagoon and 37°C in the saline saltern pond at midday, typical of winter superficial waters in this system. The salinity in Araruama Lagoon was 5.5% in contrast to salt saturated water (nearly 37%) in the saline pond. Waters showed pH values varying from 8.0 for lagoon to 8.5 for saline saltern. Microbial abundance (MA), production (MP) and specific production (SP) were evaluated to characterize the sampling sites P1 and P2 (Fig. 1, Table 1). Prokaryotic counts varied from 4.11×10^7 cells ml^{-1} in the lagoon water (P1) to 1.09×10^8 cells.ml^{-1} in the hypersaline water in P2. Prokaryotic productivity varied from 3.43 ngC l^{-1} h^{-1} in P2 to 7,583.90 ngC l^{-1} h^{-1} in P1. We observed higher specific productivity (SP) in the P1 station, probably due to abundant available organic matter, nutrients and dissolved oxygen. The lower SP observed in P2 indicated very low microbial metabolic activity, attributed to abiotic stress of high salt waters to lagoon microbes in saline ponds. The low SP observed in saline pond water can be related to the presence of halophilic archaea and bacteria. Those are known for their low metabolic rates and long periods of cell duplication (Maturrano et al. 2006).

Table 1 Microbiological parameters of sampling sites at Araruama Lagoon

Sites	P1	P2
Microbial abundance (cells.ml^{-1})	4.11×10^7	1.09×10^8
Microbial production (ng C l^{-1} h^{-1})	7,583.90	3.43
[a]Specific production (ag C l^{-1} h^{-1})	184.52	0.03

[a] Calculated as the ratio MP/MA

Table 2 Species richness estimates of 16S gene sequences determined by the DOTUR program

Sites	Archaea		Bacteria		Cyanobacteria	
	P1	P2	P1	P2	P1	P2
[a]TNS	55	13	41	43	29	55
[b]CS	2	1	6	4	3	4
SG	53	12	35	39	26	51
[c]OTUs	28	11	30	32	11	20
BRE	35.8	14.6	43.6	41.9	14.1	24.3
ACE	95.7	66	365	89.1	31	36.4
Chao1	75.5	35.5	199	69.5	20.3	47.5
SW	2.98	2.37	3.45	3.41	1.73	2.68

SG Sequences deposited at GenBank; *BRE* bootstrap richness estimators; *ACE* abundance-based coverage estimator; *SW* Shannon-weaver index of diversity

[a] Total number of sequences with more than 300 bp with Phred score >20

[b] Chimeric sequences (CS) identified at RDP II and removed

[c] Number of unique OTUs defined by using the furthest neighbor algorithm in DOTUR at 97% similarity

Free-living prokaryotic clone libraries, sequencing and diversity

The free-living microbial DNA recovered at each site was amplified with archaea, bacteria and cyanobacteria 16S rRNA-specific primers and cloned (Table 2). A total of 96 archaeal, 96 bacterial, 96 cyanobacterial and 48 clones for *nif*H/protochlorophyllide genes from each habitat were randomly selected and sequenced. Chimeric sequences were identified and removed using the CHECK-CHIMERA software at RDP II (http://www.rdp.cme.msu.edu). A total of 246 selected sequences, comprising 65 archaeal, 74 bacterial, 77 cyanobacterial and 30 *nif*H/protochlorophyllide genes sequences were analyzed. Valid sequences with phred score ≥20 were used for database query with online BLAST search in GenBank (http://www.ncbi.nim.nih.gov).

Phylogenetic analysis indicated that a great number of archaea (A), bacteria (B) and cyanobacteria (C) clones were related to uncultivated environmental microorganisms (Fig. 2). The majority of archaeal clones were related to uncultured environmental Euryarchaeota. In the lagoon

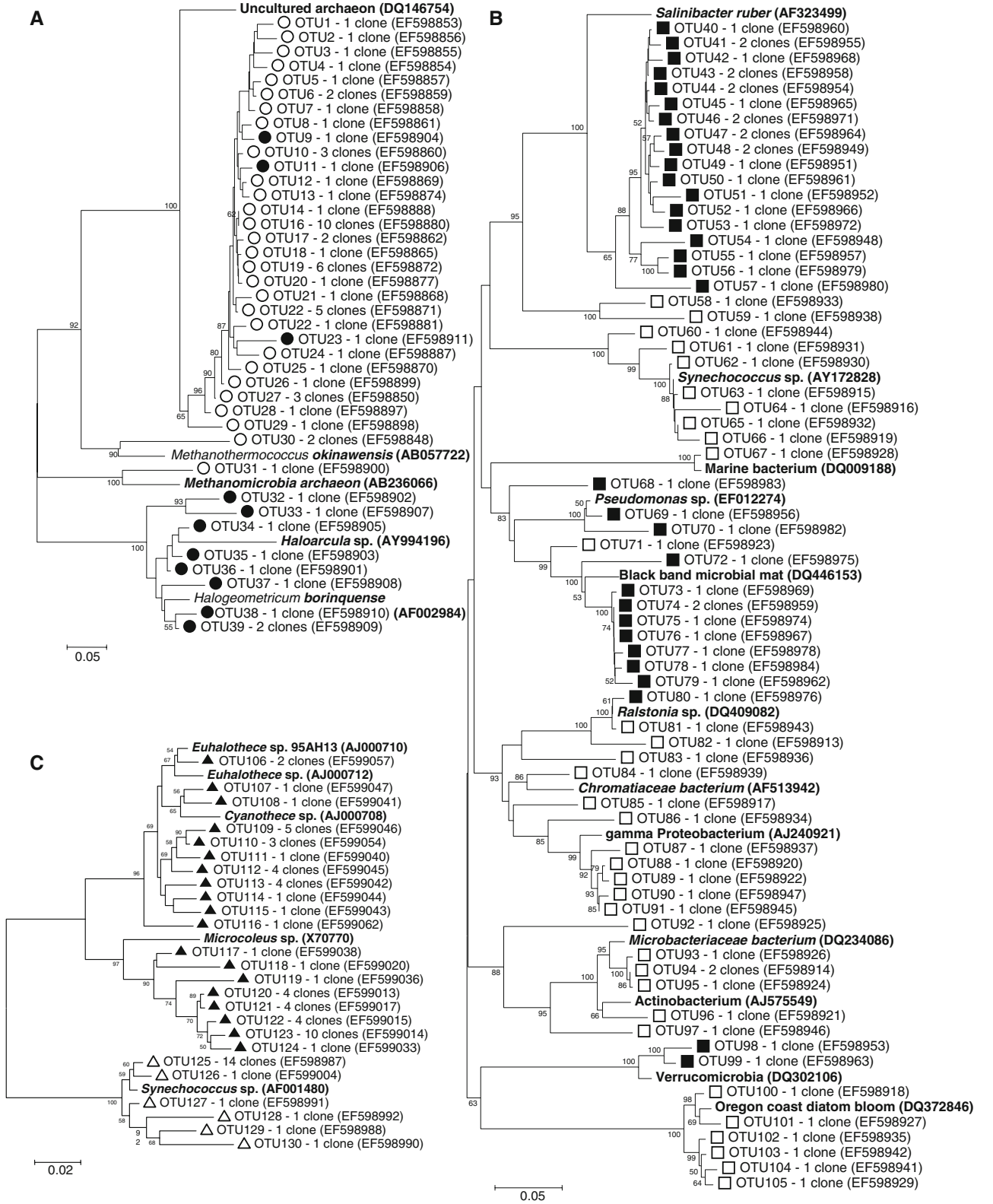

Fig. 2 Phylogenetic relationship of communities from the Araruama Lagoon. **a** Archaea (*open circle*) lagoon water, (*filled circle*) saline pond. **b** Bacteria (*open square*) lagoon water, (*filled square*) saline pond. **c** Cyanobacteria (*open triangle*) lagoon water, (*filled triangle*) saline pond. The trees were constructed by the neighbor-joining method. Access number for each OTU is indicated at the end of the corresponding sequence typed in parentheses. Bootstrap values (1,000 replicates) higher than 50% are shown. *Scale bar* represents the substitution percentage

water, we found relatives of *Methanomicrobia* and *Methanothermococcus* groups, while in the saline pond water clones related to members of the genus *Haloarcula* were obtained (Fig. 2a).

Bacterial phylotypes were specific for each habitat. In Araruama Lagoon we observed several clones related to *Gamma-proteobacteria*, *Actinobacteria*, and *Synechococcus*. Groups distantly related to uncultured environmental bacteria were observed. On the other hand, saline pond waters presented a characteristic bacterial community dominated by *Salinibacter ruber* relatives. We could also detect the presence of *Alpha-proteobacteria*, *Pseudomonas*-like bacteria and *Verrucomicrobia.* Only representatives of the genus *Ralstonia* were cosmopolitan, being observed in both systems (Fig. 2b).

We analyzed the distinct genera of cyanobacteria in each habitat. Lagoon water was dominated by *Synechococcus* phylotypes. This genus seems to be very sensitive to the high salt environment of saline ponds. However, this NaCl-saturated water presented representatives related to the halophilic genera of *Euhalothece*, *Cyanothece* and *Microcoleus*. We also observed two potential candidates for a new group of halophilic cyanobacteria that is phylogenetically related to the genera *Cyanothece* and *Microcoleus* (Fig. 2c).

Rarefaction analysis at 97% cutoff stringency indicated that different levels of diversity were displayed for the archaea, bacteria and cyanobacteria analyzed in the two environments (Fig. 3). The number of sequenced clones from each library was insufficient to cover the total environmental microbial diversity. Thus, we performed a quantitative comparison between the lagoon and the saline pond habitats for archaea (Fig. 4a), bacteria (Fig. 4b) and cyanobacteria (Fig. 4c) libraries by the LIBSHUFF statistic. This procedure uses Monte Carlo methods to generate homologous and heterologous coverage curves from the 16S rRNA clone libraries. Sequences were randomly shuffled 999 times between samples prior to the distance between the curves being calculated using the Cramér-von Mise statistic test. The analysis showed that compared libraries were significantly different ($P = 0.001$) between the lagoon saline versus NaCl-saturated water in salt crystallizers.

Additionally, two molecular markers with ecological relevance were analyzed, one is linked to nitrogen fixation, the *nif*H gene, and the other is linked to carbon fixation by bacterial photosynthesis, the protochlorophyllide genes (Fig. 5). We observed that *nif*H genes were related to marine microorganisms and cyanobacteria. Some clones were similar to clones observed in other ecosystems such as Falls Lake (Dyble et al. 2002) and Chesapeake Bay (Chen et al. 2006). The *chl*L, *bch*L and *bch*X genes (Oz et al. 2005) were related to photosynthetic cyanobacteria and *Roseobacter* populations.

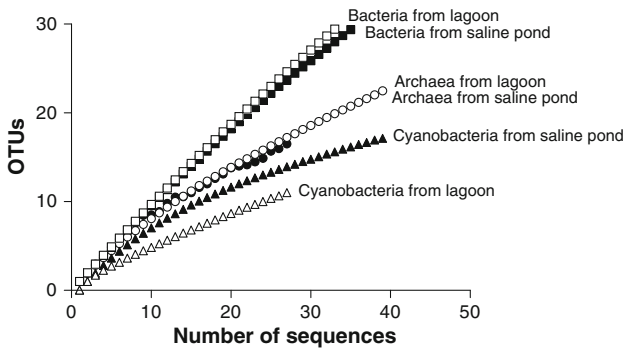

Fig. 3 Comparative rarefaction curves representing expected diversity of 16S rRNA clones for each library with clusterization stringency at 97%

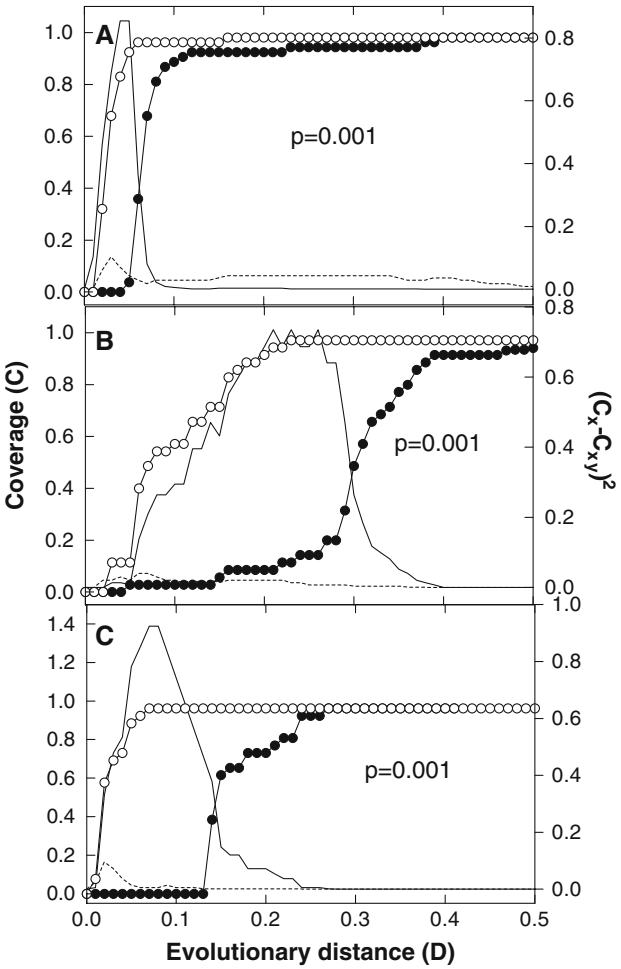

Fig. 4 LIBSHUFF comparisons. Homologous (*open circle*) and heterologous (*filled circle*) coverage curves for 16S rRNA gene libraries from (**a**) archaea, (**b**) bacteria and (**c**) cyanobacteria. *Solid lines* indicate the value of $(C_x - C_{xy})^2$ for the original samples at each value of evolutionary distance (D). Broken lines indicate the 950th value (or $P = 0.05$) of $(C_x - C_{xy})^2$ for the randomized samples

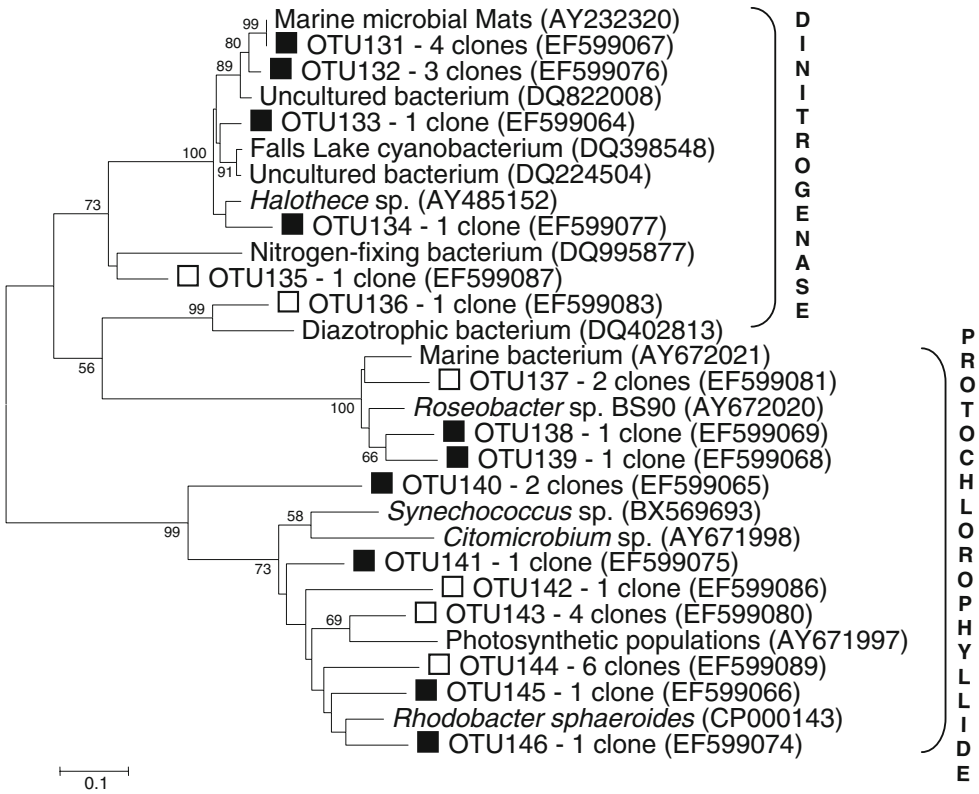

Fig. 5 Phylogenetic relationship of *nif*H and protochlorophyllide genes from Araruama lagoon. (*open square*) lagoon water; (*filled square*) saline pond

Discussion

Hypersaline coastal lagoons result from seawater confinement in shallow depressions with a negative water balance, where evaporation exceeds precipitation. These thalassohaline systems typically have the same major cation composition as seawater, although with much higher ionic activity (Grant 2004). The effect of high salt concentrations can be dangerous or lethal to many components of the biota. However, microbial life can be found in hypersaline environments with NaCl concentrations in the saturation limits displaying mainly *Arthemia* sp. and *Dunaiella* sp. as eukaryotic life forms.

Either way, hypersaline conditions subject the biota to severe osmotic stress and an overall decrease in physiological activities and growth rates (Souza et al. 2003). Studies along salinity gradients showed a decreasing trend in the diversity of bacterial, archaeal and eukaryotic communities when the salinity increases (Jungblut et al. 2005; Abed et al. 2007). Colonization of salt lakes and saline ponds by halophilic organisms can frequently lead to the establishment of microbe climax ecosystems (Ley et al. 2006).

Recently, we analyzed archaeal and bacterial diversity in natural and impacted areas in and around Guanabara Bay (Clementino et al. 2007; Vieira et al. 2007; Vieira et al. 2008), an eutrophic estuarine system located in a humid tropical region surrounded by the second largest metropolitan area of Brazil. Since there were no previous reports on the microbial diversity of the halophilic and halotolerant prokaryotic communities present in the waters of the Araruama Lagoon, we undertook this study to evaluate, for the first time, the microbiota of this biosystem. By understanding and evaluating the microbial diversity of this environment, we can obtain insights into the composition, dynamics and the roles played by this microbiota.

Prokaryotic microorganisms are quite abundant in the lagoon, probably due to high nutrient availability. Specific production showed that microbial metabolic rates were higher in lagoon communities and lower in salt-saturated assemblages. This indicates that tolerance to high salt levels may be linked to low metabolic rates for halophilic microbes. *Salinibacter ruber was* one of these organisms that also has been found repeatedly in significant numbers in saltern crystallizer communities (Antón et al. 2002).

The microbial diversity of hypersaline environments seems to be very low (Oren 2002). This aspect was observed in the saline pond analyzed here, as well as in the analysis of a crystallizer pond from a solar saltern located in Santa Pola, Alincate, Spain (Benlloch et al. 2001). Meanwhile, at 97% stringency, we can see high microdiversity, at strain level, in the co-existence of several closely related organisms in the uncultured archaeon (Fig. 2a) and *Salinibacter ruber* phylotypes (Fig. 2b). Most archaeal

clones identified in lagoon waters were related to uncultured environmental Euryarchaeota, while in the saline pond water they were closely related to halobacteria, an archaeal class that is abundant in hypersaline settings (Oren 2002). The most common cyanobacterial taxa found in hypersaline environments include *Aphanothece halophytica* and a variety of *Lyngbya*, *Microcoleus*, *Phormidium*, *spirulina*, and Synechococcal-like species (Kirkwood et al. 2007). A great number of environmental clones from the P2 site clustered with *Euhalothece, Cyanothece* and *Microcoleus* (Fig. 2c). Burns et al. (2004) revealed a number of sequences clustering with *Euhalothece*, a group of cyanobacteria that has been also identified in hipersaline microbial mats in other geographical locations (Nubel et al. 2000).

Rarefaction analysis is a useful procedure to compare biodiversity from different habitats with distinct sample numbers (Hurlbert 1971; Simberloff 1978). The curves for the 16S rRNA clone libraries show that more clones have to be sequenced and analyzed in order to achieve saturation and to make a visible representation of the total environmental microbial diversity (Fig. 3). On the other hand, rarefaction curves suggest that we obtained a reasonable initial coverage of the main representative groups of each habitat investigated. Archaea diversity is higher than cyanobacteria and lower than bacteria in the two habitats studied. The analysis of archaea, bacteria and cyanobacteria libraries by LIBSHUFF quantitative statistics shows that there are significant differences ($P = 0.001$) between the two environments. This result is in accordance with our phylogenetic analyses.

The phylum Cyanobacteria comprises oxygenic photoautotrophic microorganisms and many species are also capable of diazotrophic growth (Fay 1992). They contribute with a significant portion of phytoplankton carbon absortion in many ecosystems (DuRand et al. 2001) and constitute a diverse group of microorganisms that are found in an array of habitats (Kirkwood et al. 2007). Although this phylum belongs to the bacteria domain, it was analyzed separately due to its presence in high concentrations in the Araurama Lagoon. At hypersaline environments, cyanobacteria and anoxygenic phototrophs may be responsible for primary productivity (Hess 2004). Heterocyst-forming species are specialized in nitrogen fixation as ammonia (NH_3), nitrites (NO_2^-) or nitrates (NO_3^-), which can be absorbed by algae and plants.

Protochlorophyllide reductase catalyzes the reductive formation of chlorophyllide from protochlorophyllide during biosynthesis of chlorophylls and bacteriochlorophylls. The light-independent (dark) form of protochlorophyllide reductase plays a key role in the ability of algae and green photosynthetic bacteria to form chlorophyll. Given the strong similarities between *chl*L, *bch*L, *bch*X and nitrogenase iron proteins, one can use the same set of primers to study the occurrence of these two microbial functions (carbon and nitrogen fixation) encoded by different genes occurring in different organisms. Since Chl-based photosynthesis is unique to bacteria and chloroplasts, it probably originated in bacteria after the divergence of archaeal and bacterial lineages (Nomata et al. 2006). In spite of high levels of water eutrophycation by nitrogenous compounds, we were able to identify diazotrophic organisms by *nif*H gene surveys in the studied sites, suggesting a diverse distribution of this group. The nitrogen cycle in the lagoon involves the production of ammonia by fermentative anaerobes. Ammonia is utilized by methanotrophs and nitrifiers producing nitrate. Nitrate, in turn, is utilized by the chemoorganotrophs, creating a link between the nitrogen and carbon cycles (Rees et al. 2004).

Perhaps one of the most interesting aspects of hypersaline environments is the effect of salt living strategy on the cell biology and ecology of halophilic prokaryotes. Since there were no previous reports on the microbial diversity of the halophilic and halotolerant prokaryotic communities present in water from Araruama Lagoon and a salted pond near it, we undertook this study to evaluate, for the first time, the biodiversity and biogeography of prokaryotes in a tropical extreme saline environment. According our clone libraries, few species were related to taxonomically identified microorganisms, as is the case for *Salinibacter ruber* relatives that dominated the bacterial clone library. This result is in agreement with FISH counts, in which over 90% of the prokaryotes stained by the eubacterial probe were also stained with a *Salinibacter* probe (Antón et al. 2000). They are among the most halophilic bacteria known, requiring at least 15% salt for growth and are bright red-colored and rod shaped (Antón et al. 2002). The presence of a high number of sequences related to incultured prokaryotes suggests the possibility of isolation and characterization of new species. It has been speculated that alloctonous halophilic microorganisms could be used in biotechnological processes (Ventosa et al. 1998). This and other similar studies may provide a better understanding of nitrogen and carbon cycles and reveal the intriguing biodiversity of indigenous halotolerant and halophilic microbiota living in the tropical hypersaline lagoon ecosystems in Brazil.

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