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ORIGINAL PAPER

Archaeal and bacterial communities of heavy metal contaminated acidic waters from zinc mine residues in Sepetiba Bay

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Abstract Mining of metallic sulfide ore produces acidic water with high metal concentrations that have harmful consequences for aquatic life. To understand the composition and structure of microbial communities in acid mine drainage (AMD) waters associated with Zn mine tailings, molecular diversity of 16S genes was examined using a PCR, cloning, and sequencing approach. A total of 78 operational taxonomic units (OTUs) were obtained from samples collected at five different sites in and around mining residues in Sepetiba Bay, Brazil. We analyzed metal concentration, physical, chemical, and microbiological parameters related to prokaryotic diversity in low metal impacted compared to highly polluted environments with Zn at level of gram per liter and Cd–Pb at level of microgram per liter. Application of molecular methods for community structure analyses showed that Archaea and Bacteria groups present a phylogenetic relationship with uncultured environmental organisms. Phylogenetic analysis revealed that bacteria present at the five sites fell into seven known divisions, *α-Proteobacteria* (13.4%), *β-Proteobacteria* (16.3%), *γ-Proteobacteria* (4.3%), *Sphingobacteriales* (4.3%), *Actinobacteria* (3.2%) *Acidobacteria* (2.1%), *Cyanobacteria* (11.9%), and unclassified bacteria (44.5%). Almost all archaeal clones were related to uncultivated Crenarchaeota species, which were shared between high impacted and low impacted waters. Rarefaction curves showed that bacterial groups are more diverse than archaeal groups while the overall prokaryotic biodiversity is lower in high metal impacted environments than in less polluted habitats. Knowledge of this microbial community structure will help in understanding prokaryotic diversity, biogeography, and the role of microorganisms in zinc smelting AMD generation and perhaps it may be exploited for environmental remediation procedures in this area.

Keywords Acidophiles · Systematics · Ecology · Phylogeny · Archaea · Biodiversity · Ecology · Molecular phylogeny and molecular biology · Zinc mine

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Introduction

Zinc ions are essential for life as they are required for proper function of a large number of proteins. Concentrations of Zn in natural seawater range from 3.9 to 4.9 μg L^{-1}. About 37% of this Zn is in organic form and the main inorganic ion species are $ZnOH^{+}$, Zn^{2+}, and $ZnCO_3$ (Maeda and Sakaguchi 1990). Waters with high Zn

concentrations are toxic to most organisms but some acidophilus microbes tolerate and accumulate heavy metals (Dopson et al. 2003; Johnson 1998).

Acid mine drainage (AMD) water is a worldwide environmental problem caused by active and abandoned mines (Johnson and Hallberg 2003). The acidic drainage is originated from exposure of sulphidic minerals to O_2, which results in the formation of soluble sulphates, and H^+. The low pH enhances metal mobilization and toxicity in mine wastewaters (Edwards et al. 2000). High Zn levels can be dangerous or lethal to components of the biota, such as phytoplankton, zooplankton, benthos, mangroves, fish, birds, mammals, and finally to humans (Amado Filho et al. 1997; Correa Junior et al. 2000; Junior et al. 2002).

Sepetiba Bay is a semi-enclosed 520 Km^2 water body separated from the Atlantic Ocean by a natural sand barrier known as Restinga da Marambaia. Its salinity ranges between 2.0 and 3.4% and it has a shallow basin with depths going from 2 to 12 m. The bay is connected to sea in the West by three large channels and in the East by a small channel in Barra de Guaratiba (Fig. 1). Tidal movement regulates water circulation with a residence time around 4.17 days. In the North and in the East Sepetiba Bay supports 40 km^2 of mangrove forests that play a role in providing nursery and feeding for the bay's fauna (SEMADS 2001). Industrialization processes in this area have increased pollution significantly over the past 40 years (Lacerda et al. 1987; Molisani et al. 2004). Of great concern is the impact from large heavy metal loads being discharged from industrial emissions into the bay (SEMADS 2001). Acidic and highly metal contaminated water generated by dissolution of sulphidic minerals are a source of Sepetiba Bay pollution. Emission rate estimates suggest that the main Zn and Cd sources to Sepetiba Bay are effluents from the Ingá zinc refinery processing plant (Fig. 1), which added 24 t $year^{-1}$ of Cd and 3,660 t $year^{-1}$ of Zn into the bay's basin until 1998, when the plant closed (Molisani et al. 2004).

Acidic mining tanks, which are part of the disabled Ingá zinc processing plant, located in Ilha da Madeira near Itaguaí city, are an extreme environment characterized by a low pH and very high-metal concentrations. When the plant was active, AMD water, after being partially treated, was actively pumped and discharged in the bay. When government funding was withdrawn all treatment ceased and the rising mine water, with a pH 2.8 and high zinc

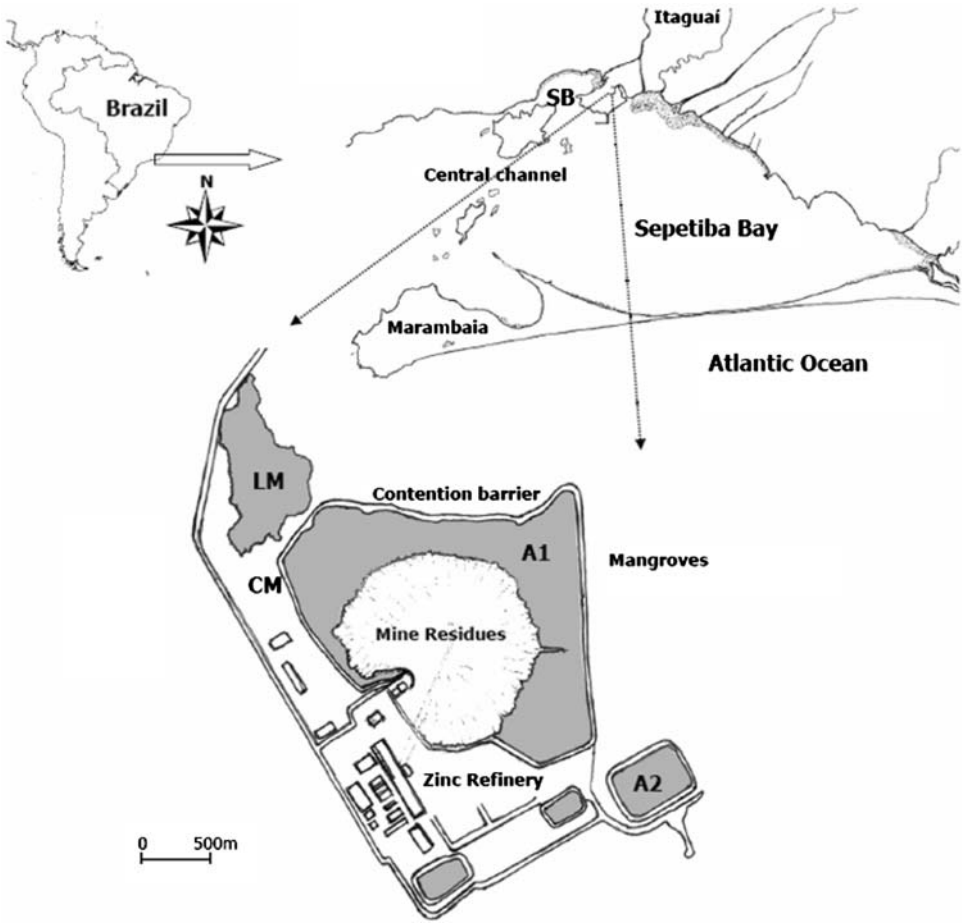

Fig. 1 Study area with detail to the zinc smelter plant and sampling locations: South America; Brazil and Sepetiba Bay sampling site (SB). Sketch of the zinc smelter plant and surrounding environment sampling sites A1, A2, CM, and LM

content (at a level of $g.L^{-1}$), was passively discharged. Consequently, high concentrations of heavy metals can be observed in the bay's compartments (animal and plant tissues, water and sediments) (Amado Filho et al. 1997; Correa Junior et al. 2000; Junior et al. 2002). The abandoned industrial area accumulated three million tons of solid residues (Molisani et al. 2004). Fisheries and about 100 industries including metallurgical, textile, chemical and also touristy businesses, are active around the bay's area. Studies in the bay have identified significant changes in sedimentation rates, concentration of inorganic pollutants, and eutrophycation (SEMADS 2001).

Microbial community structure has been described only in a few tailing categories of AMDs, such as iron, sulfidic, cooper, lead, zinc and coal mine waste, by culture-independent methods (Hallberg et al. 2006; He et al. 2007, 2008; Nicomrat et al. 2006). Therefore, a comprehensive study of the prokaryotic composition in mine tailings still remains to be done due to the great chemical and physical heterogeneity of tailings over the world (Johnson 1998; Johnson et al. 2001; Nordstrom and Alpers 1999; Tan et al. 2006). Due to limited types of substrates available in mining environments, biodiversity of Archaea and Bacteria were initially expected to be extremely low. However, cultivation and culture-independent methods revealed a great diversity of microbial community in AMDs (Johnson et al. 2001; Zang et al. 2007). The presence of various bacterial species including common prokaryotic chemolithotrophs such as *Acidithiobacillus ferrooxidans*, *A. thiooxidans*, *A. caldus*, and *Leptospirillum ferrooxidans* has been reported in mining environments. The presence of archaea including a group of sulfur and/or iron-oxidizers, such as *Sulfolobus, Acidianus*, *Metallosphaera*, *Sulfurisphaera*, and *Ferroplasma* has also been reported in acidic environments (Bond et al. 2000; Edwards et al. 2000; Tyson et al. 2004).

The present study describes for the first time the archaeal and bacterial diversity in water samples from highly Zn, Cd, and Pb contaminated settlement tanks and surrounding environments in Sepetiba Bay (Fig. 1), through sequencing of 16S rDNA genes in clone libraries.

Materials and methods

Site description and sampling

The Ingá zinc smelter plant (22°54′S and 43°54′W) is located at the margin of Sepetiba Bay in Itaguai City, Rio de Janeiro State, Southeastern Brazil (Fig. 1). The climate in this region is typically hot-humid tropical, with an annual mean precipitation of 1,400 mm, which contribute to fluvial load of heavy metal in Sepetiba Bay (Molisani et al. 2004). Superficial water samples (0.5 m deep) were collected at five different sites: highly contaminated (A1 and A2) mining residues tanks and an at underground leakage point from the main tank (CM) at Ingá plant on 4 October 2005 and 16 March 2006. In addition, two surrounding habitats were sampled at the same time: one sample was collected at neighbor freshwater environment, Lake Marrecas (LM), which is affected by spillage from Inga's tanks and one marine sample in Coroa Grande, Sepetiba Bay (SB) during low tide (Fig. 1). Samples were immediately kept on ice for transport to the laboratory and processed for analysis.

Physicochemical and microbiological parameters

Physical and chemical parameters were determined according to standard oceanographic methods (Grasshoff et al. 1999). Electric conductivity, pH and Eh were measured in situ using specific electrodes. Heavy metal measurements were carried out after hydrolysis with nitric acid in an atomic absorption spectrometer (Perkin Elmer 3100). Prokaryotic abundance was determined by flow cytometry after nucleic acid staining with Syto13 fluorochrome at 2.5 μM in samples fixed with 2% paraformaldehyde (Andrade et al. 2003; Gasol and del Giorgio 2000). Prokaryotic production was analyzed by a ^{3}H-leucine method (Smith and Azam 1992).

DNA extraction

Water was collected on 16 March 2006, filtered, and DNA was prepared by a standard method (Somerville et al. 1989). Briefly, water filtration in 3.0 μm ester–cellulose filter was performed to capture particulate materials. The free-living planktonic microbes were concentrated on a Sterivex-filter (0.22 μm) (Millipore). Then, 50 μl of freshly prepared lysozyme (1 mg ml^{-1}) was added to filter units containing 1.8 ml of lyses buffer, and incubated at 37°C for 45 min. 50 μl of freshly prepared proteinase K (0.2 mg ml^{-1}) and 200 μl 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 distilled water. 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 with 100 μl TE (10 mM Tris–HCl, 0.1 mM EDTA, pH 8.0). DNA quality was checked in agarose gels stained with SYBR Green after electrophoresis and the image digitalized with Storm Image Scanner (GE Health Care).

PCR amplification of partial 16S rDNA gene

Four archaeal from sites A1, A2, LM and SB and three bacterial 16S rDNA gene libraries from sites A1, A2, and LM were constructed from free-living planktonic prokaryotes. PCR was performed in 50 μL reaction mixtures (2.5 mM $MgCl_2$, 0.2 mM dNTPs, 50 pmol of each primer, 2.5 U of high fidelity Platinum *Taq* DNA polymerase, and PCR buffer (Invitrogen) for approximately 100 ng of environmental genomic DNA extracted for each sample. Three oligonucleotides were used: universal prokaryotes reverse primer 907ABR (5′-TTTGAGTTTMTTAATGCC-3′) (Lane 1991), and either universal Bacteria forward primer 27BF (5′-AGAGTTTGATCATGGCTCAG-3′) (Amann et al. 1992) or universal Archaea forward primer 21AF (5′-TTCCGGTTGATCCTGCCGGA-3′) (Delong 1992). 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.

Cloning and sequencing of PCR products

PCR bands with the expected molecular weight (about 880 bp) were excised from a 1% agarose gel, and eluted using GFX-DNA and gel band purification kit (GE Health Care). Amplicons were cloned into plasmid vector pGEM-T (Promega), according to manufacturer's recommendations, and transformed into DH-10b *Escherichia coli* cells. Clones from each library were submitted to sequence analysis. Plasmid DNA from each clone was prepared (400 ng) and sequences were obtained after sequencing reactions with vector primer (T7) for Archaea and 27BF for Bacteria by capillary electrophoresis on a MegaBace1000 using DYENamic dye terminator cycle sequencing kit (GE Healthcare). Chromatograms were transformed into Fasta format sequences with Phred software (Edwing et al. 1998).

Sequence trimming and analysis

Plasmid vector sequences and regions with quality below 20, according to Phred scores at 3′ and 5′ ends of 16S rDNA gene inserts were manually removed. Chimeric sequences were identified and removed using online CHECK-CHIMERA at Ribosomal Database Project II (RDP II) (Cole et al. 2003). Alignments with representative archaeal and bacterial sequences obtained at GenBank databases were carried out using ClustalX program (Thompson et al. 1997). The sequences were compared with those in GenBank by BLAST search tool (Altschul et al. 1990). Phylogenetic trees were calculated by Kimura 2 parameter model (Kimura 1980) and neighbor joining algorithm (Saitou and Nei 1987) using MEGA software (Kumar et al. 2001). One thousand bootstraps were performed to assign confidence levels. The diversity of the phylotypes was further examined using DOTUR software, LIBSHUFF statistics (Singleton et al. 2001), and rarefaction analysis (Heck et al. 1975; Hurlbert 1971). The partial 16S rDNA gene sequences reported in this study were submitted to GenBank database under accession numbers (EF463932 to EF464065 and EF517812 to EF517814).

Results

Abiotic and microbiological parameters

Abiotic data were determined to monitor nutrient concentration and chemical characteristics of each site in Sepetiba Bay area. Temperature values ranged from 27 to 30°C, and were typical of superficial waters in this region (Table 2). All highly metal polluted acidic waters (A1, A2, and CM) showed low pH values varying from 1.5 to 3.5, in contrast to the more neutral pH of Lake Marrecas (LM) and Sepetiba Bay (SB) estuarine water. Nutrient concentrations were high and ammonia was observed with tenfold higher concentration in tanks A1, A2, and CM, when compared to LM freshwater. At sites A1, A2, and CM the acidic water presented elevated levels of zinc (1,950.0–3,950.0 mg L^{-1}), cadmium (10–27 mg L^{-1}) and lead (2.1–4.5 mg L^{-1}). LM, which is affected by leakage and spillage from Ingá's tanks, presented lower metal contamination level with 130 mg L^{-1} of Zn and cadmium and lead in the μg L^{-1} level (Table 3).

Prokaryotic abundance (PA), production (PP), and specific production (SP) were evaluated to characterize microbiological parameters and specific metabolic activity in the five sampled sites: A1, A2, CM, LM, SB (Table 2). Prokaryotic counts varied from 4.4×10^5 cells mL^{-1} in LM to 7.5×10^6 cells mL^{-1} in the main tank A1. The heavily polluted tanks A1 and A2 presented low-prokaryotic production ranging from 32.4 to 59.2 ng C L^{-1} h^{-1} in opposition to the high values of 176 ng C L^{-1} h^{-1} found in LM and 760 ng C L^{-1} h^{-1} observed in SB. Low levels, going from 7.8 to 32.6 agC $cell^{-1}$ h^{-1} of specific production were found in acidic and highly metal contaminated A1, A2, and CM waters in contrast to high SP 396.6–481.1 ag C $cell^{-1}$ h^{-1} in the natural habitats LM and SB.

Clone libraries, phylogenetic analysis, and biodiversity

A total of 192 bacterial clones and 96 archaeal clones were randomly selected and sequenced. Sequences with less than 300 bp were excluded from subsequent analyses and a total of 140 (98 bacterial and 41 archaeal) valid sequences with

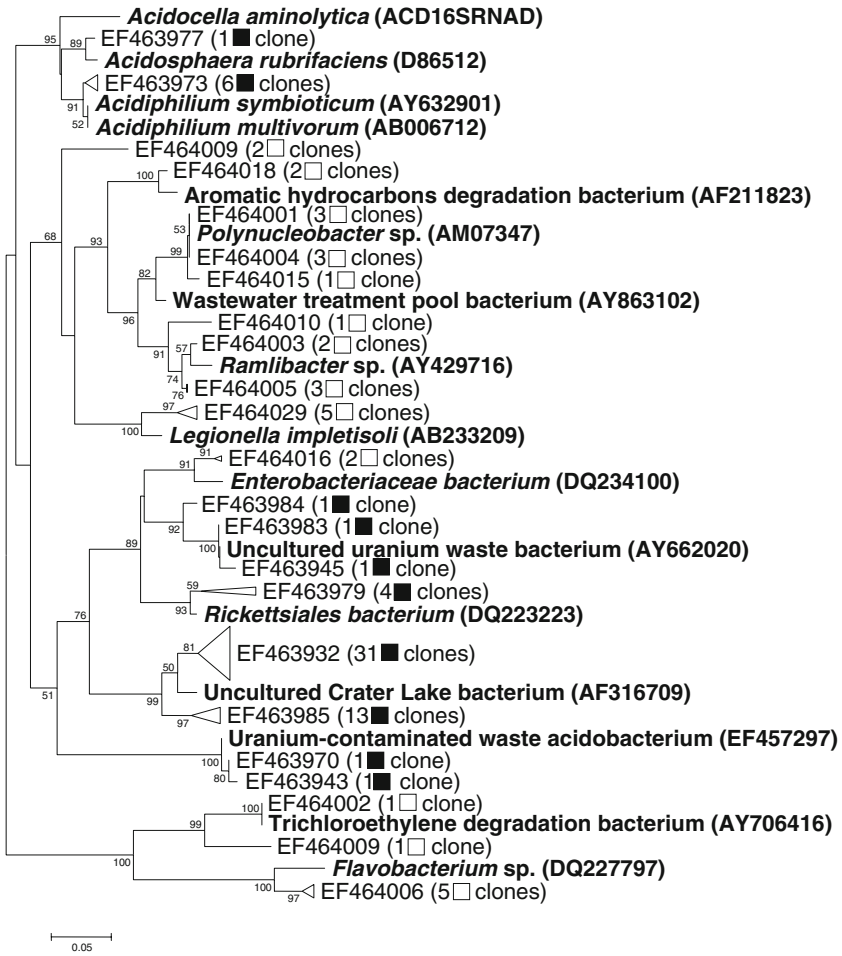

Fig. 2 Neighbor-joining Bacterial phylogenetic tree construction from partial 16S rDNA sequences. (*Filled square*) Clones from acidic and highly metal impacted environments A1 and A2. (*Open square*) Clones from low metal impacted neutral LM. One access number for each OTU is shown on the tree. Bootstrap values (1,000 replicates) higher than 50% are shown. Scale bar represents the 5% substitution percentage

Phred score $\geq$ 20 were obtained. Five chimeric sequences were identified and removed using CHECK-CHIMERA software at RDP II (http//rdp.cme.msu.edu) (Cole et al. 2003). A total of 135 sequences with Phred score $\geq$ 20 were used for database query with online BLAST (Altschul et al. 1990) search in GenBank (http://www.ncbi.nim.nih.gov).

Phylogenetic analysis indicated that most of bacterial clones were related to uncultivated environmental microorganisms (Fig. 2). Several clones found in metal contaminated waters were related to environmental freshwater bacterial groups. Others were related to acidophilic species like *Acidocella, Acidosphaera* and *Acidiphilium*. The LM bacterial community presented members of the CFB group (Cytophaga, Flavobacterium and Bacteriodetes) and *Legionella* genera, which were not found in tanks A1 and A2. Most of archaeal clones were related to environmental uncultivated Crenarchaeota species, with exception of four clones of the Euryarchaeota *Methanoregula boonei* found only in LM (Fig. 3). Interestingly, the Zn contaminated tanks A1 and A2 showed distinct bacterial communities when compared to LM, while members of the archaeal communities were shared between highly impacted tanks and lowly impacted LM freshwater (Figs. 2, 3).

Rarefaction curves for 16S rDNA at 97% cutoff stringency showed that the number of sequenced clones from each library was insufficient to cover Archaea and Bacteria diversity at species level and to obtain a representation of the total environmental microbial diversity (Fig. 4). Rarefaction analysis at this stringency also indicated that different levels of diversity were displayed for Archaea and Bacteria communities (Fig. 4a). However, rarefaction analyses at 80% cutoff stringency suggested that we reached a reasonable coverage of the main representative groups (Fig. 4b). At this stringency, the prokaryotic communities of the acidic highly metal impacted environments (A1 and A2) show low biodiversity when compared to Archaea and Bacteria communities of neutral and less impacted habitats LM and SB (Fig. 4c).

We also performed a quantitative comparison between archaeal and bacterial libraries obtained in highly and lowly impacted habitats by a LIBSHUFF statistic procedure. This procedure uses Monte Carlo methods to generate homologous and heterologous coverage curves from the 16S rDNA clone libraries. Sequences were randomly

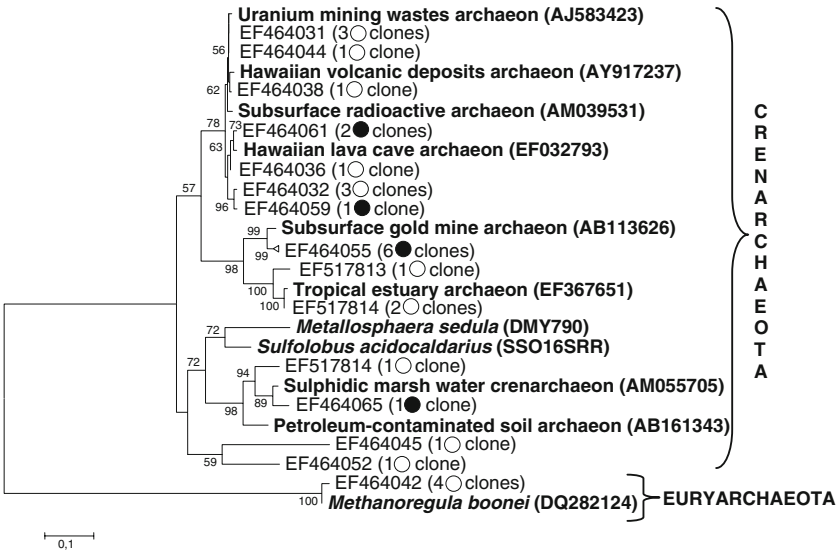

Fig. 3 Neighbor-joining Archaeal phylogenetic tree construction from partial 16S rDNA sequences. (*Filled square*) Clones from acidic and high metal impacted environments A1 and A2. (*Open square*) Clones from low metal impacted neutral LM and SB. One access number for each OTU is shown on the tree. Bootstrap values (1,000 replicates) higher than 50% are shown. Scale bar represents the 10% substitution percentage

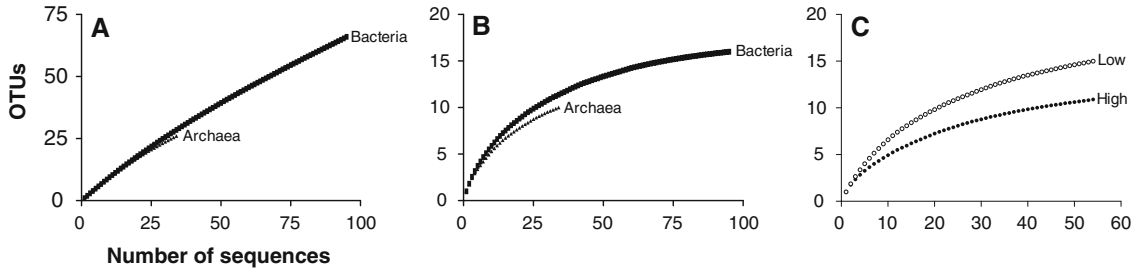

Fig. 4 Rarefaction curves comparative analysis. **a**, **b** Archaea (*Filled triangle*) and Bacteria (*Filled square*) (**c**) low metal impacted natural, LM, and BS (*Open circle*) versus acidic and high metal impacted environments A1 and A2 (*Filled circle*). Clusterization stringency in **a** 97% or (**b**, **c**) 80% identity

shuffled 999 times between samples prior to distance between curves being calculated using the Cramér–von Mise statistic test (Singleton et al. 2001). The LIBSHUFF analysis indicated that the bacterial libraries are significantly different ($P = 0.001$) and archaeal libraries are less different ($P = 0.046$) between highly metal impacted A1 and A2 versus lowly impacted LM and SB habitats (data not shown).

Discussion

Prokaryotes can be found in acidic waters containing high levels of heavy metals (Dopson et al. 2003) which originate naturally by volcanic activity (Cánovas et al. 2007; Lohr et al. 2006; Wendt-Potthoff and Koschorreck 2002) or artificially by mining (Hallberg et al. 2006; He et al. 2007, 2008; Johnson et al. 2001; Kamjunke et al. 2005; Peplow and Edmonds 2005; Tan et al. 2006). Perhaps one of most intriguing aspects of mining problems is that most AMD formed is a direct result of microbial activity (Edwards et al. 2000; Hallberg et al. 2006; Johnson and Hallberg 2003). Acidic drainage is originated from the exposure of sulphidic minerals to O_2, resulting in the formation of soluble sulphates. Minerals with high ferrous iron content become oxidized in contact with water producing ferric ions and H_2 (Edwards et al. 2000; Johnson and Hallberg 2003). These ions when leached into streams cause water to become more acidic, normally reaching pH values below 3. Additionally, other metal ions such as Zn, Hg, Ni, Cr, Cd, Cu, Mn, Al, As and Pb occur in AMD waters at final concentrations far above permissible levels (Hallberg et al. 2006; Molisani et al. 2004; Takai et al. 2001; Tan et al. 2006).

The diversity and roles that microorganisms play in situ remain largely unknown as normally only a small proportion of microbiota present in environment are readily cultivable (Giovannoni and Stingl 2005; Rappe and Giovannoni 2003). There is only limited literature available for description of microbial in AMD, especially in impacted environments with high zinc and cadmium concentrations. Our study is the first description of Archaea community structure by 16S gene libraries in an environment with the highest Zn concentrations ever reported

(Table 1). We observed higher specific productivity (SP) in LM and SB environments, possibly by abundant available organic matter, nutrients, and dissolved oxygen (Table 2). The lower SP observed in A1, A2 and CM indicate low microbial metabolic activity, probably due to acidity and high metal concentration imposed on active microbes in this environment (Table 2). These waters impacted with metals did not display macroscopic forms of life, but prokaryotic cells were quite abundant, due to high nutrient availability and low grazing. However, specific production showed that microbial metabolic rates are higher in LM and SB communities and lower in the assemblages of highly metal impacted environment. Furthermore, tolerance to high Zn, Cd and Pb levels can be linked to reduced metabolic rates in these microbial assemblages. It has been shown that the impact of metals on the microbial cell are decreased decomposition of organic matter, reduced respiration, lower diversity, and decreased activity of several enzymes (Rühling and Tyler 1973; Tyler 1974).

The metal resistant bacterial groups seem to be exclusive of impacted habitats, while archaeal groups appeared to have a wider distribution in the studied environment. Some bacterial sequences showed relationship to *Acidithiobacillus* or *Leptospirilium* genera, which are well known contributors to AMD generation (Dopson et al. 2003; Tyson et al. 2004). Several bacterial clones of the impacted tanks were phylogenetically affiliated with the Proteobacteria, belonging to species capable of ferric iron and sulfate reduction as the predominant physiological trait (Bond et al. 2000; Johnson et al. 2001). In addition, several sequences of the microbial communities investigated were closely related to acidophilic bacteria (*Acidocella, Acidosphaera* and *Acidiphilium*). Organisms of the genera *Acidiphilium* and related to uncultured Crater Lake bacterium (Urbach et al. 2001) were dominant in the extremely acidic and heavy metal contamined waters.

Environmental Crenarchaeota dominated impacted sites A1, A2, as well as the less impacted habitats LM and BS. We found four Euryarchaeota clones affiliated to a candidatus *Methanoregula boonei* (Brãuer et al. 2006) only in the fresh water of LM. We identified three marine Crenarchaeota in SB plankton that are affiliated to an environmental crenarchaeon phylotype retrieved in a previous study in Guanabara Bay (Vieira et al. 2007). This uncultured planktonic crenarchaeon appears to be widely distributed in the coastal zone of Rio de Janeiro state.

Table 1 16S gene libraries in different AMDs with high zinc concentration

Tailing/ Country	Zn[a]	Cd[a]	Pb[a]	16S[b]	pH	Ref
[c]Zn; Brazil	2,462.5	13.9	2.6	AB	1.5–3.5	This study
Cu; China	8.8	ND	0.7	B	1.5–3.0	(Yin et al. 2008)
Pb/Zn; China	408.4	ND	90.0	B	1.5–2.0	(He et al. 2008)
S; China	404.0	ND	ND	B	2.5	(He et al. 2007)
Cu; UK	50.0	ND	ND	B	2.5–2.9	(Hallberg et al. 2006)
Pb/Zn; China	97.0	ND	0.7	B	1.9–2.1	(Tan et al. 2006)
Cu; Norway	25.4	0.02	0.05	B	2.7–3.7	(Johnson et al. 2001)

[a] Zinc, Cadmium and Lead (mg L^{-1}), maximum value from each study

[b] 16S libraries for (A) Archaea and (B) Bacteria

[c] Average values from the Brazilian tanks (A1 and A2)

ND, not determined

Table 2 Abiotic and microbiological parameters of zinc mine and Sepetiba Bay sampling sites

[a]Parameters	Sites				
	A1	A2	CM	LM	SB
Temperature (°C)	29	30	30	28	27
pH	3.5	2.8	1.5	6.6	7.5
Salinity (S)	0.17	0.16	0.55	0.20	2.37
Chlorophyll (μg L^{-1})	1.02	1.26	7.38	2.78	4.01
Feofitin (μg L^{-1})	0.74	1.74	16.7	2.16	3.44
Orthophosphate (μM)	0.89	0.09	0.92	0.11	0.78
Phosphorous (μM)	2.61	0.27	7.48	0.92	1.28
Ammonia (μM)	1.62	3.70	2.38	0.25	1.11
Nitrite (μM)	0.58	0.18	3.58	0.16	0.45
Silicate (μM)	630	387	30.6	51.2	51.7
[b]SPM (mg L^{-1})	18.8	12.2	32.4	40.00	49.8
[c]PA	7.58×10^6	1.31×10^6	3.01×10^6	4.45×10^5	1.58×10^6
[d]PP	59.28	32.48	98.40	176.51	760.26
[e]SP	7.82	24.79	32.69	396.66	481.18

[a] Values are average from two collection data except for SB 4 October 2005 and 15 March 2006

[b] Suspended particulate material

[c] Prokaryotic abundance (cells mL^{-1})

[d] Prokaryotic production (ngC L^{-1} h^{-1})

[e] Specific production (agC $cell^{-1}$ h^{-1}), calculated as the ratio PP PA^{-1}

Table 3 Dissolved metals and electrical data of zinc mine sampling sites

[a]Parameters	Sites			
	A1	A2	CM	LM
Zn (mg L^{-1})	1,950	2,975	3,950	130
Cd (mg L^{-1})	10.4	17.5	27.5	0.5
Pb (mg L^{-1})	2.1	3.2	4.5	0.1
Ca (mg L^{-1})	430	560	775	ND
Mg (mg L^{-1})	750	810	1,100	ND
Eh (mV)	703	765	790	660
Conductivity (μS)	6,575	9,450	13,700	1,363

[a] Data were taken as triplicates on 18 June 2005 and 15 March 2006. Values are average from two collection data

ND not determined

Rarefaction analysis is a procedure to compare biodiversity from different habitats with distinct species or clone numbers (Heck et al. 1975; Hurlbert 1971). Our analysis showed that bacterial diversity is higher than archaeal. The diversity of Bacteria in acidic, heavy metal impacted waters of A1 and A2 is lower than in Lake Marrecas (LM). We are studying the linkage between the geochemistry of AMD formation and its microbial community structure to understand diversity of prokaryotic organisms that tolerate high Zn concentrations in acidic environments. The presence of a high number of sequences related to uncultivated prokaryotes suggests the possibility to isolate new species. We can speculate that the allochtonous metal resistant microbiota could be applied in AMD remediation processes. In conclusion, this study has shown an intriguing biodiversity of indigenous Archaea and Bacteria living in the highest reported Zn concentrations from AMD in an abandoned mining area.

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