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Archaeal diversity and the extent of iron and manganese pyritization in sediments from a tropical mangrove creek (Cardoso Island, Brazil)

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ABSTRACT

Even though several studies on the geochemical processes occurring in mangrove soils and sediments have been performed, information on the diversity of *Archaea* and their functional roles in these ecosystems, especially in subsurface environments, is scarce. In this study, we have analyzed the depth distribution of *Archaea* and their possible relationships with the geochemical transformations of Fe and Mn in a sediment core from a tropical mangrove creek, using 16S rRNA gene profiling and sequential extraction of different forms of Fe and Mn.

A significant shift in the archaeal community structure was observed in the lower layers (90–100 cm), coinciding with a clear decrease in total organic carbon (TOC) content and an increase in the percentage of sand. The comparison of the archaeal communities showed a dominance of methanogenic *Euryarchaeota* in the upper layers (0–20 cm), whereas *Crenarchaeota* was the most abundant taxon in the lower layers. The dominance of methanogenic *Euryarchaeota* in the upper layer of the sediment suggests the occurrence of methanogenesis in anoxic microenvironments. The concentrations of Fe-oxyhydroxides in the profile were very low, and showed positive correlation with the concentrations of pyrite and degrees of Fe and Mn pyritization. Additionally, a partial decoupling of pyrite formation from organic matter concentration was observed, suggesting excessive Fe pyritization. This overpyritization of Fe can be explained either by the anoxic oxidation of methane by sulfate and/or by detrital pyrite tidal transportation from the surrounding mangrove soils. The higher pyritization levels observed in deeper layers of the creek sediment were also in agreement with its Pleistocenic origin.

1. Introduction

Studies on intertidal environments have shown that the geochemical conditions of their soils and sediments depend on the combination of many physical, chemical and biological factors, as well as on the nature of the substrates (Gleason et al., 2003; Ferreira et al., 2007a,b). The resulting spatial variability is largely regulated by the microtopography, e.g. presence of creeks, microdepressions, and microelevations (Ferreira et al., 2010), which modifies the flooding patterns and accelerates the sedimentation processes. Even though the factors controlling the local geochemical conditions in temperate-climate marshes (Otero and Macías, 2002, 2003) and tropical mangroves (Ferreira et al., 2007a, b; Otero et al., 2009) have been extensively studied, the microbial ecology and the functional roles of different groups of microorganisms in these environments need further understanding (Lambais et al., 2008; Mitterer, 2010).

It has been estimated that approximately 4–6% of all prokaryotic cells occur in soils, 2–3% in aquatic habitats and the vast majority in subsurface, i.e. under 8 m in terrestrial environments and 10 cm in marine sediments (Whitman et al., 1998). Most of the prokaryotic species in soils and sediments remain to be described and isolated in pure cultures (Lambais et al., 2008). The knowledge of bacterial and archaeal community organization and functioning in subsurface environments is relatively modest, despite their significant influence and roles on global biogeochemical processes (Pesaro and Widmer, 2002; Lambais et al., 2008), and enormous source of genetic and functional diversity (Polymenakou et al., 2005).

Tropical mangroves represent one the most productive ecosystems of the world and play an important role in the regulation of the biogeochemical cycles of C, Mn, S and Fe in costal environments

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(Otero and Macías, 2011). These ecosystems are characterized by oxygen-poor, nutrient-rich and constantly flooded saline soils containing high amounts of organic matter, whose decomposition usually occurs under anaerobic conditions. Due to the high concentration of sulfate in seawater, the oxidation of organic matter in these environments is mainly coupled to the bacterial reduction of sulfate (Mackin and Swider, 1989). Most of the reduced forms of sulfur (H_2S) generated by the sulfate reducing bacteria (SRB) will generally precipitate as iron sulfides (FeS and FeS_2) in the presence of dissolved Fe^{2+} (Moeslund et al., 1994).

Despite the existence of several studies on geochemical processes in mangrove soils and sediments, little is known on the archaeal diversity in these ecosystems, especially in subsurface environments (e.g., Gouvêa et al., 2010, Dias et al., 2011). Studies concerning the occurrence and distribution of archaeal communities in mangrove soils and sediments may contribute to a better understanding of the roles of these microorganisms in the biogeochemical processes taking place in these environments, as well as to the development of new isolation and cultivation approaches. The goals of this study were: (1) to determine what changes occur in the structure of archaeal communities along a sediment core from a mangrove creek in São Paulo (Brazil) using PCR-DGGE and sequencing of 16S rRNA clones and, (2) to establish the relationships between these communities and the Fe, Mn and S geochemical cycles, especially their effects on pyritization through the anaerobic oxidation of methane coupled to sulfate reduction.

2. Materials and methods

2.1. Site location description and sampling

The Ipaneminha Creek is located in a pristine mangrove estuarine system at the Cardoso Island State Park (25°04′44″ S and 47°56′14″ W; Fig. 1A, B). Tidal ranges oscillate between 0.13 m at neap tide to 0.83 m at spring tide (Miyao and Harari, 1989). Due to the concave profile and to the creek inlet being partially closed by a sand barrier, the Ipaneminha mangrove soils and creek are subjected to long periods of inundation, which favors prolonged periods of tidal water stagnation, resulting in the development of an anoxic environment (Fig. 1C,D, Ferreira et al., 2007b).

Sampling was performed (Fig. 1) using a 5 cm i.d. polycarbonate tube coupled to a sediment sampler (Eijkelkamp auger; Fig. 1C). Approximately 120 cm long sediment cores were collected from the bottom of the creek (Fig. 1C), at 3 m from the creek edge and the adjacent mangrove forest (Fig. 1). The average water depth in the creek was 1 m. Once in the laboratory, the undeformed sediment cores were extruded from the sampler by pressure, and one set of subsamples, used for geochemical analysis, was taken from thinner core sections (0–3, 3–6, 6–10, 10–15, 15–20, 20–25, 30–40, 40–60, 60–80, 80–90, 90–100 cm) to achieve better depth resolution, and another set of subsamples, used for microbiological analysis, was taken from larger sections (thickness of 10 cm) in order to minimize the number o samples. DNA from sediment samples from depths 10–20 (upper layer) and 90–100 cm (lower layer), two contrasting layers identified by DGGE analyses, were used for detailed analyses of the archaeal community using partial sequencing of 16S rRNA gene.

2.2. Archaeal community analyses

2.2.1. DNA extraction and denaturing gradient gel electrophoresis (DGGE) analyses

Total DNA was extracted from 500 mg of the same sediment sections reserved for chemical analyses, using the Fast DNA kit (Qbiogene, Irvine, CA, USA), according to Lambais et al. (2008). The V3 region of the archaeal 16S rRNA gene was amplified by PCR using 50 ng of the total soil DNA extracted as template and primers ARCH21f (5′ TTC YGG TTG ATC CYG CCI GA 3′) and ARCH958r (5′ YCC GGC GTT GA (I/C) TCC AAT T 3′) (Moyer et al., 1998). Amplification was performed in 1X recombinant Taq DNA polymerase buffer containing 0.2 mM dNTPs, 3 mM $MgCl_2$, 1 U Taq DNA polymerase (Life Technologies, São Paulo, Brazil), 5 pmol of each primer and 50 ng of total DNA. PCR amplification conditions were 5 min at 95 °C; 30 cycles of 1 min at 95 °C, of 1 min at 55 °C, of 1 min at 72 °C, and final extension for 10 min at 72 °C. Resulting PCR products (amplicons) were used as templates for nested PCR amplification with ARCH340fGC (5′ CGC CCG CCG CGC GCG GGC GGG GCG GGG GCA CGG GGG GCC CTA CGG GGY GCA SCA G 3′) and ARCH519r (5′ TTA CCG CGG CKG CTG 3′) primers (Øvreås et al., 1997). The concentrations of amplicons were determined by densitometry, as described above. Negative controls for PCR amplifications were performed using reaction mixes without DNA. Amplicons (300 ng) were analyzed by DGGE using 8% (w/v) acrylamide:bisacrylamide (37.5:1, m:m) gels containing a 15–55% linear gradient of formamide and urea (100% denaturing solution containing 40% formamide and 7 M urea), according to Lambais et al. (2008).

DGGE banding patterns representing the archaeal community structure in the sediment were analyzed as discrete data (presence or absence of bands with the same mobility in the gel, *Rf*) using Hierarchical Clustering Analysis based on simple matching similarity matrices calculated with Ward's algorithm and Euclidian distances (Systat, SPSS Inc.).

2.2.2. Archaeal 16S rRNA gene cloning and sequencing

Based on the contrasting differences observed in the DGGE and chemical analyses of the top and bottom core layers, sub-samples from depths 0–20 and 90–100 cm were used for DNA extraction and nested PCR amplification with primers ARCH21f and ARCH958r, and ARCH21f and ARCH519r, as described above. Amplicons were purified and sequenced as described previously (Lambais et al., 2008).

2.2.3. Sequence analyses, operational taxonomic units (OTU) definition and richness and diversity indices estimations

Nucleotide sequences (reads) were trimmed for the removal of low quality bases (quality parameter > 20, i.e., less than one error in 100 nucleotides) and vector sequences using the Phred program (Ewing and Green, 1998). Valid sequences were aligned using ClustalX2 software, setting gap opening penalty to 10 and gap extension penalty to 0.1 for pairwise and multiple alignments (Thompson et al., 1997). A Jukes-Cantor distance matrix was calculated using DNAdist algorithm (Felsenstein, 1985). The similarities between the archaeal community structures were compared using S-Libshuff 1.22 (Schloss et al., 2004). The number of sequences for each OTU was computed, considering a cut-off evolutionary distance of 0.03, and the Richness (ACE and Chao1) and diversity (Shannon and Simpson's reciprocal) indices calculated using Mothur (Schloss et al., 2009).

Taxonomical affiliation of the sequences was performed using Mothur with Naïve Bayesian algorithm (Wang et al., 2007), Silva archaeal 16S rRNA reference alignment (Quast et al., 2013) and Greengenes reference taxonomy database and nomenclature (DeSantis et al., 2006). A neighbor-joining phylogenetic tree with the most representative OTUs was constructed using the Kimura-2 algorithm (1000 replicates) in MEGA5 (Tamura, 2011). The best sequence match of each OTU in Megablast (database NT/NR) was included in the phylogenetic tree as a reference (Altschul et al., 1990).

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