COMPARISON OF MICROBIAL COMMUNITY STRUCTURE IN COASTAL MARINE SEDIMENTS OF THREE AUSTRAL SUMMERS IN ADMIRALTY BAY, KING GEORGE ISLAND

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Abstract: *This study was conducted to characterize and compare the microbial community diversity in marine sediments from four different sites at Admiralty Bay (CF, Comandante Ferraz Antarctic Station; BP, Botany Point; PU, Ullman Point; and R2, Refuge 2) by means of 16S rRNA pyrosequencing. The results revealed differences between all samples and indicated predominance of the Phylum Proteobacteria in all sites. Shifts in Proteobacteria classes (Alpha, Beta, Gamma, Delta and Epsilonproteobacteria) accounted for most of the variation among stations, and these were more evident in pristine sites (BP, PU and R2) than in CF.*

Keywords: Microbial Diversity, Sediment, 16S rRNA, Pyrosequencing

Introduction

Microbial diversity in Antarctic Peninsula has been assessed in different ecosystems, including soils, sediments, lakes, hydrothermal vents, volcanoes, glaciers, permafrost, among others. Research has demonstrated that microorganisms at benthic biosphere can represent one tenth to one third of the total biomass on Earth and about 70% of the total prokaryotic biomass (Parkes *et al.*, 1994; Whitman *et al.*, 1998).

The abundance of microbial cells in marine sediments is related to sedimentation rates and distance from the coast. Cell densities in surficial sediments increase toward continental margins and decrease toward the open sea (Kallmeyer *et al.*, 2012). Marine sediments and their microbiota play a fundamental role in the Antarctic region, since they act as indicators of environmental changes that take place in response to different processes occurring in the water column.

Several studies have been carried out in marine sediments worldwide (Arctic, Atlantic and Pacific Oceans, as well as Baltic and Mediterranean Seas) with the objective of determining the composition of microbial communities, using next generation sequencing techniques, and obtaining data that allow for phylogenetic and functional analysis of biogeochemical processes. Sequences from the Bacteria Domain described in these studies belong mostly to the Phylum Proteobacteria, especially in Gamma and Delta-Proteobacteria classes. On the other hand, sequences from the Archaea Domain are mostly related to Euryarchaeota Phylum (Hamdan *et al.*, 2012; Zinger *et al.*, 2011; Lyra *et al.*, 2013; Li *et al.*, 2009; Danovaro *et al.*, 2010; Sogin *et al.*, 2006).

In this context, the objective of the present work was to assess, through next generation sequencing techniques, the variation in microbial community structure in coastal marine sediment of Martel and McKellar Inlets at Admiralty Bay, King George Island.

Material and Methods

Sediment samples were collected in the Austral Summers 2007, 2010 and 2012 (Brazilian Antarctic Expeditions XXV, XXVIII and XXX) in four stations (Table 1) at 20-30m isobaths and kept at -20°C until analysis in Brazil. Total DNA in the samples was extracted with the *PowerSoil DNA Isolation Kit* (MoBio, USA) and amplified with the Prokaryotic-Universal primers U519F and U1068R (Wang & Qian, 2009), corresponding to V4-V6 regions of the rRNA16S gene in *Escherichia coli*. The amplicons were sequenced using a 454 GS FLX Pyrosequencing (Roche).

Sequences were analyzed on Mothur v.1.30 (Schloss *et al.* 2009) and on RDP Classifier (Wang *et al.* 2007).

Results

About 80,000 sequences of ~90-570 pb were obtained. Bioinformatic analysis showed higher abundance of Bacteria when compared to Archaea. The most abundant bacterial Phyla were Proteobacteria, Firmicutes, Actinobacteria, Verrucomicrobia, Bacteroidetes and Planctomycetes, which are commonly found in marine sediments (Figure 1).

Table 1. Sample location (geographic coordinates) and sampling depth (m).

SampleID	Coordinates		Depth (m)	Inlet
	Lat	Long		
Comandante Ferraz (CF)	62° 05. 131" S	58°23.369" W	30	Martel
Botany Point (BP)	62° 05. 701" S	58° 19.849" W	30	Martel
Ullman Point (PU)	62° 05. 015" S	58° 23.987" W	30	Martel
Refuge 2 (R2)	62° 04. 210" S	58° 25.335" W	30	Mackellar

Figure 1. Main bacterial phyla and Proteobacteria classes found in sediments from Admiralty Bay across three austral summers.

Discussion

Preliminary analysis of the pyrosequencing results showed variation in community composition with time (Figure 1) and indicated predominance of the Phylum Proteobacteria in all sediments. Shifts in Proteobacteria classes (Alpha, Beta, Gamma, Delta and Epsilonproteobacteria) also accounted for most of the variation between samples in all stations, and were more evident in pristine sites (BP, PU and R2) than in CF. These changes are probably related to natural variation in environmental conditions (organic and inorganic substrates, oxygen availability, bioturbation, ice scours' interference). On the other hand, CF communities remained relatively stable across the time, what might be related to human activities around the scientific station Comandante Ferraz. It is well known that anthropogenic perturbations such as increase in organic content, sediment load and fuel combustion can affect marine ecosystems (Nogales *et al.*, 2011). However, further sequence analyses and their relation to physico-chemical parameters are necessary to determine the drivers of these variations.

Results also revealed different trends in variation between sites. In BP, a decrease in Alphaproteobacteria was followed by an increase in Gammaproteobacteria from 2007 to 2012. Gammaproteobacteria was an important group in all samples and most of its presence was explained by the high number of *Psychrobacter* sequences (14 to 64% of the Gammaproteobacteria sequences found). *Psychrobacter* is a genus of strictly aerobic chemoheterotrophic, cold-adapted and halotolerant bacteria. It has been found in many cold marine habitats, from sea ice to marine sediments, and has been cultivated from Admiralty Bay sediment (Rodrigues *et al.*, 2009).

Increases in Deltaproteobacteria frequencies were also observed, mostly in PU (2010) and R2 (2010 and 2012),

followed by BP (2012) and CF (2012). Deltaproteobacteria are traditionally associated to sulfur metabolism under anaerobic conditions and play an important role in carbon mineralization in marine sediments (Jørgensen, 1982). An increase in sulfate-reducing bacteria may indicate a higher input of carbon in the Admiralty Bay from 2007 to 2010-2012, what could lead to higher emissions of H₂S and CO₂ in the water column and atmosphere (Muyzer & Stams, 2008).

Conclusions

Pyrosequencing analysis of sediment microbial communities revealed high prevalence of the phylum Proteobacteria in all samples, but subsequent taxa within this Phylum varied across the time and between sites, indicating that different combinations of environmental and geographical factors contribute to defining the microbial community structure. CF community structure showed less variability with time than the other sites and this might be related to some kind of impact, but further analysis of physical, chemical and biological data are necessary confirm this stability is not due to natural factors.

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