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Bacterial community characterization and biogeochemistry of sediments from a tropical upwelling system (Cabo Frio, Southeastern Brazil)

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ABSTRACT

The Cabo Frio Upwelling System is one of the largest and most productive areas in southeastern Brazil. Although it is well-known that bacterial communities play a crucial role in the biogeochemical cycles and food chain of marine ecosystems, little is known regarding the microbial communities in the sediments of this upwelling region. In this research, we address the effect of different hydrological conditions on the biogeochemistry of sediments and the diversity of bacterial communities. Biogeochemistry profiles of sediments from four sampling stations along an inner-outer transect on the continental shelf were evaluated and denaturing gradient gel electrophoresis (DGGE) of PCR-amplified 16S rRNA gene fragments was used to study the bacterial community composition in these sediments. Our sequencing analysis of excised bands identified *Alpha-* and *Gammaproteobacteria*, *Bacteroidetes* and bacteria belonging to the *Firmicutes* phyla as the phylogenetic groups, indicating the existence of great diversity in these marine sediments. In this multidisciplinary study, the use of multivariate analysis was crucial for understanding how biogeochemical profiles influence bacterial community distribution. A Principal Component Analysis (PCA) indicated that the biogeochemical variables exhibited a clear spatial pattern that is mainly related to hydrological conditions. A Correspondence Analysis (CA) revealed an important association between certain taxonomic groups and specific sampling locations. Canonical Correspondence Analysis (CCA) demonstrated that the biogeochemistry influences the structure of the bacterial community in sediments. Among the bacterial groups identified, the most taxonomically diverse classes (*Alphaproteobacteria* and *Gammaproteobacteria*) were found to be distributed regardless of any studied biogeochemical variables influences, whereas other groups responded to biogeochemical conditions which, in turn, were influenced by hydrological conditions. This finding was observed for members of the two classes in the *Bacteroidetes* phylum, which were associated with either proteins or carbohydrates.

1. Introduction

Marine sediments are complex systems affected by the interaction of geological, hydrological, physicochemical and biological factors (Chen et al., 2011) and are host to the largest reservoir of organic carbon and the largest number of microorganisms in the world (Jorgensen et al., 2012; Parkes et al., 2005). Technological developments in marine geochemistry and microbiology have revealed that prokaryotes form highly complex communities and have a metabolic activity that profoundly affects global biogeochemical cycles (Fry et al., 2008; Jorgensen et al., 2012). Understanding their function and structure is crucial for predicting the fate of carbon and other essential elements in the marine system (Jorgensen et al., 2012). Approximately 5 to 10 billion tons of particulate organic matter sinks in the oceans each year, and most of this is deposited on the continental shelves, which constitute only 8.6% of the total ocean floor. Furthermore, continental shelves are important sites for biogeochemical cycles in the ocean because only a small percentage of organic matter is permanently buried, and most of the organic matter is re-mineralized in the shelf sediment (Hong and Cho, 2012).

A large fraction of the total benthic biomass is dominated by sedimentary bacteria that represent a critical component of marine ecosystems (Zheng et al., 2014); however, studies of benthic bacterial diversity have typically been confined to the sediment surface.

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Conversely, a limited number of studies have been conducted on sediments from −1 m to hundreds of meter below the surface (Inagaki et al., 2003; Orcutt et al., 2011). Since the relationship between microbial communities and the biogeochemical impact in subsurface sediments remains poorly defined, more studies are needed to explore and understand how microbial communities respond to different geochemical properties at different depths.

Distribution patterns of bacterial communities in coastal environments have been well documented (Crump et al., 1999; Scala and Kerkhof, 2000) and studied in-depth at sites with Arctic and Antarctic sediments (Bowman et al., 2003; Bowman and McCuaig, 2003; Li et al., 2006; Ravenschlag et al., 2000), deep sea sediments (Heijs et al., 2008; Polymenakou et al., 2005), shallow-water hydrothermal vents (Giovannelli et al., 2013) and on the continental shelf (Breuker et al., 2013; Hunter et al., 2006; Johnson and Hill, 2003). These studies have shown that the predominant groups of marine sediments are often highly similar and more diverse than those from any other environment (Zinger et al., 2011). Furthermore, studies focusing on the importance of bacterial turnover diversity in order to understand diversity and to identify the driving factors have demonstrated that bacterial communities are strongly affected by factors such as temperature, light, salinity, dissolved oxygen, pH, hydrostatic pressure, water movement and sediment type (Crump et al., 2004; Ikenaga et al., 2010; Mahmoudi et al., 2015), organic matter content and the quality of organic matter (Bissett et al., 2007; Giovannelli et al., 2013; Powell et al., 2003). However, the effect of hydrological conditions and the associated changes in biogeochemical parameters in sedimentary bacterial community remains underrepresented in these studies. Therefore, new studies are crucial for obtaining more information about the diversity and distribution of microbial communities in marine sediments in complex systems and how they respond to different biogeochemical properties.

In southeastern Brazil, the continental shelf of Cabo Frio (Rio de Janeiro State) has gamered particular interest due to its special features (Diaz et al., 2012). This area is characterized by a coastal upwelling system dominated by two principal physiographic, oceanographic and geomorphological systems (Sanders et al., 2014). One is the Brazil current (BC), which moves away from the coast due to winds predominantly from the NE. The other is marked by the upwelling of South Atlantic Central Water (SACW), which is cold and rich in nutrients and considered the main source of nutrients for primary production. The continental shelf is characterized by two distinct bathymetric surfaces, one approximately 50 m deep and extending 5 km offshore and a second ranging from 90 m to 130 m deep and extending from 5 to 100 km off shore to the shelf break (Campos et al., 2000). Furthermore, the Cabo Frio region is characterized by cyclonic and anticyclonic vortices from the change in orientation of the coastline and the gradient of the bottom topography (Cruz et al., 2013). The likely interaction between upwelling events and vortices favors the high productivity that is in agreement with other upwelling regions in the world (Baumgart et al., 2010; Niggemann et al., 2007), demonstrating that this region has a complex hydrodynamic system (Calado et al., 2008).

The geochemistry of this region was been previously researched (Cordeiro et al., 2014; Diaz et al., 2012; Figueiredo et al., 2013; Sanders et al., 2014; Souto et al., 2011); however, little is known about the microbial community in this upwelling area. To our knowledge, only one culture-independent study was performed by Cury et al. (2011), which characterized the microbial community in water samples of the Brazilian upwelling region. In their study, bacterial communities were found to be dominated by *Proteobacteria* and *Bacteroidetes* phyla (Cury et al., 2011).

In the present work, molecular characterization of the sedimentary bacterial community obtained from different locations in Cabo Frio's upwelling area was analyzed with a culture-independent methodology based on 16S ribosomal RNA (rRNA) gene using Denaturing Gradient Gel Electrophoresis (DGGE) and a sequencing analysis. Moreover, the biogeochemistry of the sediments was also studied. DGGE profiles and the biogeochemical properties of the sediments were analyzed using a variety of statistical approaches, including cluster analysis and ordination methods, such as principal component analysis (PCA) and canonical correspondence analysis (CCA), in order to provide a way to better understand the relationship between microbial community distribution and biogeochemistry. Our results significantly contribute to the knowledge of ecological and biogeochemical aspects of bacterial communities in this dynamic region.

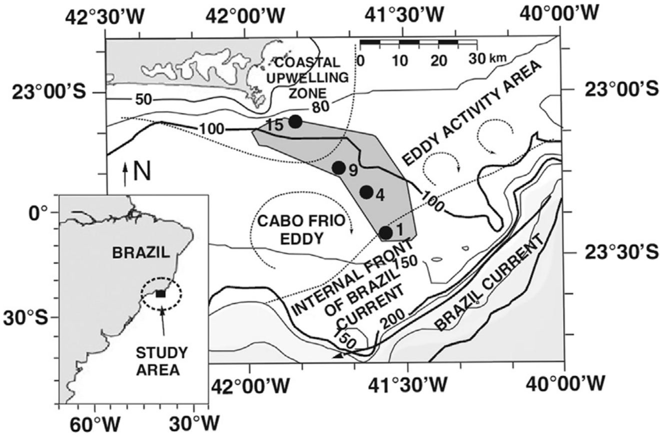

Fig. 1. Sampling stations in the Cabo Frio Upwelling region. The shaded area represents the mud bank. Adapted from Diaz et al., 2012.

2. Materials and methods

2.1. Sampling stations and general sampling methods

Four sediment cores were collected using a gravity core sampler (Kullenberg type) between April 24th and May 3rd, 2010, from the AV.Pq. Oc. Diadorim vessel of the Instituto de Estudos do Mar Almirante Paulo Moreira – IEAPM/Navy of Brazil. PVC core tubes were inserted into the sediment at four stations across the shelf within a 680 km^2 mud bank in the Cabo Frio region (Fig. 1) and were identified according to the location of the sampling stations (Table 1). The stations were selected based on hydrological differences in the system. The CF10-01 core was collected at 142 m water depth at Station 1 in the outer shelf, which was mainly influenced by the Brazil Current. The CF10-04 and CF10-09 cores were sampled at 120 m and 117 m water depths from Station 4 and Station 9, respectively, which were both located on the middle-shelf area and influenced by stationary and quasi-stationary cyclonic and anti-cyclonic eddy activity (Calado et al., 2008; Diaz et al., 2012). The fourth core (CF10-15) was retrieved at 80 m water depth at Station 15, which was located on the inner-shelf and influenced by wind-driven coastal upwelling (Fig. 1, Table 1). Since environmental factors such as temperature, salinity and primary productivity were not evaluated in this work, data from water samples in previous studies of this area were included and are shown in Table 1.

Each core was carefully transported to the laboratory under refrigeration and stored at −20 °C. The cores were opened in a camera and then, 10 cm sections were taken from the center of the tube (to avoid contamination) and were sliced at different depths (40, 140, 240, 340 cm in all of the cores and, additionally, 440 cm in cores from Stations 4, 9 and 15, and 540 cm in the core from Station 9). Subsamples of molecular analysis were stored at −20 °C while subsamples for biogeochemical analysis were freeze-dried prior to use.

2.2. Sediment analysis and organic matter composition

The total organic carbon (TOC) was determined in order to measure