Science of the Total Environment 557–558 (2016) 888–900

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Metagenomic analysis of sediments under seaports influence in the Equatorial Atlantic Ocean

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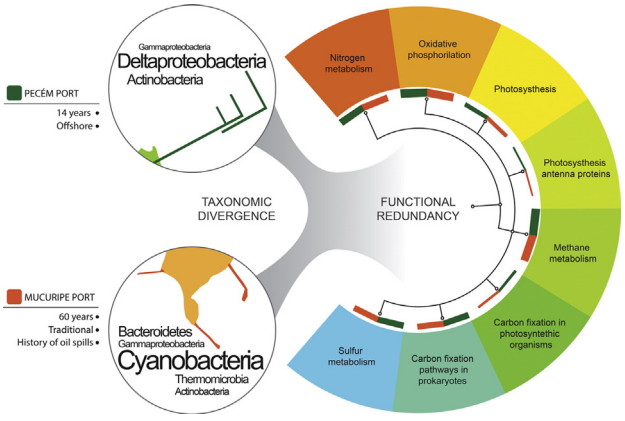
HIGHLIGHTS

- Seaports history of operation and structure could affect benthic microbiomes.
- DGGE and metagenomics evidenced taxonomic alterations in the study areas.
- Cyanobacteria enrichment seems to be related to the record of oil spills.
- Similar metabolic profiles suggest functional redundancy and ecosystem resistance.
- *In silico* analysis pointed regional determinants as key factors for the metagenomes.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 15 September 2015
Received in revised form 18 March 2016
Accepted 19 March 2016
Available online xxxx

Editor: D. Barcelo

Keywords:
Seaports
Microbiomes
Pyrosequencing
Bacteria

ABSTRACT

Maritime ports are anthropogenic interventions capable of causing serious alterations in coastal ecosystems. In this study, we examined the benthic microbial diversity and community structure under the influence of two maritime ports, Mucuripe (MUC) and Pecém (PEC), at Equatorial Atlantic Ocean in Northeast Brazil. Those seaports differ in architecture, time of functioning, cargo handling and contamination. The microbiomes from MUC and PEC were also compared *in silico* to 11 other globally distributed marine microbiomes. The comparative analysis of operational taxonomic units (OTUs) retrieved by PCR-DGGE showed that MUC presents greater richness and β diversity of Bacteria and Archaea than PEC. In line with these results, metagenomic analysis showed that MUC and PEC benthic microbial communities share the main common bacterial phyla found in coastal environments, although can be distinguish by greater abundance of Cyanobacteria in MUC and Deltaproteobacteria in PEC. Both ports differed in Archaea composition, being PEC port sediments dominated by Thaumarchaeota. The microbiomes showed little divergence in their potential metabolic pathways, although shifts on the microbial taxonomic signatures involved in nitrogen and sulphur metabolic pathways were observed. The comparative

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http://dx.doi.org/10.1016/j.scitotenv.2016.03.141

Archaea
Marine sediments

analysis of different benthic marine metagenomes from Brazil, Australia and Mexico grouped them by the geographic location rather than by the type of ecosystem, although at phylum level seaport sediments share a core microbiome constituted by Proteobacteria, Cyanobacteria, Actinobacteria, Tenericuteres, Firmicutes, Bacteriodetes and Euryarchaeota. Our results suggest that multiple physical and chemical factors acting on sediments as a result of at least 60 years of port operation play a role in shaping the benthic microbial communities at taxonomic level, but not at functional level.

1. Introduction

Marine coastal ecosystems are among the most diverse and productive on Earth. Such huge biodiversity is essential to their proper functionality and stability, *i.e.* resistance and resilience to natural and anthropogenic perturbations (Johnston and Roberts, 2009).

Maritime ports are considered very altered and stressed environments with significant levels of pollutants in water, air and sediments (Ingole et al., 2009; Nipper, 2000). Common contaminants include hydrocarbons, detergents, surfactants and anti-fouling compounds, mainly heavy metals and biocides (Nogales et al., 2011). Therefore, there is considerable interest in investigating the distribution and magnitude of these impacts in marine environments, particularly in coastal zones (Port et al., 2012). This task is particularly important in countries with long coastlines, such as Brazil (~7500 km of coastal zone), that have experienced an increase in industrial plant installations and ports (Carvalho, 2011).

Mitigation of the anthropogenic threats is one of the biggest scientific challenges nowadays and there is increasing interest in the development of new strategies for monitoring or controlling impacts in marine sediments (Ager et al., 2010). Thus, detecting and quantifying microbial diversity in marine sediments is a prerequisite of any environmental mitigation issue as it can lead to a better understanding of the ecosystem, its services and recovery potential (Kisand et al., 2012).

Microorganisms can be very useful for diversity surveying, as they are virtually everywhere and perform key roles in biogeochemical cycles (Kisand et al., 2012; Zinger et al., 2011). It is now possible to identify the core microbiome of a given environment, *i.e.* the microorganisms that are critical to its proper functionality, which is the first step in defining the health status of an environment and predicting how the community will respond to anthropogenic changes (Shade and Handelsman, 2012).

Nevertheless, knowledge on benthic microbiome under seaports activity influence is still limited (Gomes et al., 2013; Iannelli et al., 2012; Tal et al., 2005; Zhang et al., 2008) and there is no study particularly at Equatorial Atlantic Ocean.

Thus, this study aimed to access and compare the microbiomes of surface sediments in two port zones at Equatorial Atlantic Ocean intending to test the hypothesis that the ports activities influence the diversity and structure of benthic microbial community. Both port sediment metagenomes were also compared *in silico* with marine metagenomes from other world geographical regions in order to detect any biogeographic pattern assigned to seaports.

2. Materials and methods

2.1. Study sites

Surface sediments were collected in two seaports, Pecém (PEC) (03°32′52.03″S; 38°48′46.14″W) and Mucuripe (MUC) (03°42′45.55″S; 38°28′26.80″W), located at Equatorial Atlantic Ocean in Northeast Brazil.

PEC is a modern offshore port constructed 2000 m from the shoreline and connected to the land by a pillar-supported bridge, which is intended to preserve the shoreline and minimize the negative effects on coastal currents and sediments transport (Buruaem et al., 2012). It has been active for 14 years and it is located in a port-industrial complex at Ponta do Pecém, municipal district of São Gonçalo do Amarante, state of Ceará, distant approximately 60 km west of Fortaleza, the State's capital.

MUC is located within Mucuripe bay, in the metropolitan region of Fortaleza. It was constructed between two traditional breakwaters over 60 years ago. The port zone is considered chronically polluted, mainly due to several oil spill records (IBAMA, 2006, 2007, 2008, 2011) and industrial and domestic activities discharges in its surroundings. Over the years, MUC architecture has contributed to a marked alteration in the coastal sediments balance, with deposition of fine sediments in the port area (Maia et al., 1998).

2.2. Sample collection and processing

Sediment sampling was conducted in November 2011 in PEC at 10 different stations (P_1-P_{10}) and in MUC at 15 stations (M_1–M_{15}) (Fig. 1; Supplementary file 1). Distances between the sampling stations in each port were about 500 m in order to cover the whole area of the ports operation. Surface sediments sampling was conducted with a stainless steel Van Veen grab sampler at water depths ranging from 9 m to 27 m. After collection, samples were transferred to sterile plastic flasks and transported in a refrigerated container to the laboratory. Depth and water transparency (Secchi disk) were measured *in situ* while the other physicochemical variables were measured in the laboratory.

2.3. Physicochemical characterization of sediments

The sediments were submitted to analysis of grain size, organic matter (OM), carbonate, toxic metals, polycyclic aromatic hydrocarbons (PAH), benzene, toluene, ethyl benzene, and xylene (BTEX), biphenyls, total organic carbon (TOC), nitrate, nitrite and sulphate. Particle size distribution was assessed by dry sieving of sand-gravel fractions (Suguio, 1973), after previous separation of the silt-clay by wet sieving. OM was measured according to the procedure described by Schulte and Hopkins (1996), in terms of weight loss on ignition in a muffle. Carbonate content was estimated by gravimetry following 4 N HCl digestion of the sediments with several washes to eliminate HCl (Gross, 1971). The other chemicals were analyzed according to United States Environmental Protection Agency (US EPA) protocols. All assays were conducted in triplicate.

2.4. Environmental DNA extraction

DNA extractions were performed following two protocols. Firstly, aliquots of sediments from each sampling station (25 samples) were extracted using the Power Soil DNA Isolation kit (MOBIO, USA), following the manufacturer's protocol. DNA samples were used to analyze the structure of Bacteria and Archaea assemblages in each sampling station by denaturing gradient gel electrophoresis (DGGE).

For metagenomic shotgun analysis, the sediments from each sampling station in each port were pooled, homogenized and subjected to DNA extraction using the protocol described by Zhou et al. (1996) to obtain total-community DNA of each port. DNA samples were resuspended in DNAse-free water, purified with chloroform:isoamyl alcohol (24:1) and precipitated with cold isopropanol. The samples were then washed with 70% ethanol and resuspended in Tris-EDTA buffer with RNAse after evaporation of ethanol. DNA quality was