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Anthropogenic impact on mangrove sediments triggers differential responses in the heavy metals and antibiotic resistomes of microbial communities☆

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ABSTRACT

Mangroves are complex and dynamic ecosystems highly dependent on diverse microbial activities. In the last decades, these ecosystems have been exposed to and affected by diverse human activities, such as waste disposal and accidental oil spills. Complex microbial communities inhabiting the soil and sediment of mangroves comprise microorganisms that have developed mechanisms to adapt to organic and inorganic contaminants. The resistance of these microbes to contaminants is an attractive property and also the reason why soil and sediment living microorganisms and their enzymes have been considered promising for environmental detoxification. The aim of the present study was to identify active microbial genes in heavy metals, i.e., Cu, Zn, Cd, Pb and Hg, and antibiotic resistomes of polluted and pristine mangrove sediments through the comparative analysis of metatranscriptome data. The concentration of the heavy metals Zn, Cr, Pb, Cu, Ni, Cd, and Hg and abundance of genes and transcripts involved in resistance to toxic compounds (the cobalt-zinc-cadmium resistance protein complex; the cobalt-zinc-cadmium resistance protein *CzcA* and the cation efflux system protein *CusA*) have been closely associated with sites impacted with petroleum, sludge and other urban waste. The taxonomic profiling of metatranscriptome sequences suggests that members of Gammaproteobacteria and Deltaproteobacteria classes contribute to the detoxification of the polluted soil. *Desulfobacterium autotrophicum* was the most abundant microorganism in the oil-impacted site and displayed specific functions related to heavy metal resistance, potentially playing a key role in the successful persistence of the microbial community of this site.

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1. Introduction

Brazil has one of the largest areas of mangrove forests in the world, covering more than 20,000 km^2 (Food and Agriculture and Organization, 2007). Mangroves are defined as tropical ecosystems located in the flood plains of the tides, which is the transition zone between land and sea (Feller et al., 2010). These forests have high biodiversity (including aquatic animals, birds, reptiles, mammals and microorganisms) and a great ecological importance, representing one of the most productive ecosystems in the world (Sheaves, 2005).

Anthropogenic activity has resulted in the contamination of many environments, and mangroves have suffered from diverse human activities with economic and agricultural purposes, such as fishing and waste disposal (Peixoto et al., 2011; Waycott et al., 2009). Some studies have reported that approximately 35% of the world's mangroves have been destroyed over the last two decades (Alongi, 2002; Feller et al., 2010) and that the remaining mangroves worldwide are often affected by oil spills, industries, refineries, coal mining, urban and industrial garbage disposal and deforestation (Van Lavieren et al., 2012). Recent studies have now focused on the effects of pollutants on the microbiota of the mangrove ecosystem.

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Gomes et al. (2011) investigated the bacterial diversity in the sediment of three urban mangroves and concluded that contamination might shape the structural composition of microbial communities (Gomes et al., 2011). Moreover, the anthropic impact affecting surface mangrove sediments might result in diverse bacterial populations that degrade polycyclic aromatic hydrocarbons (PAH) (Zhou et al., 2008).

The cleanup of environmental sites contaminated by heavy metals constitutes a great challenge. In many cases, these contaminants originate from sewage sludge, industrial and animal residues and synthetic fertilizers. These contaminants also naturally occur in soils and rock but at low concentrations and therefore represent no environmental risk (Li et al., 2008). In contrast, recent studies have revealed high concentrations of Cu and Pb in petroleum products (Adeniyi and Afolabi, 2002) and increased concentrations of Cu, Pb and Zn in surface soils contaminated with petroleum (Grujić et al., 2004). As a result of the toxic effect of elevated concentrations of heavy metals on prokaryotes and eukaryotes, the biodiversity of the ecosystems could be significantly affected.

The quality of an ecosystem, such as sediments, is strongly influenced by microbial processes, which in turn are influenced by the microbiome diversity (Dos Santos et al., 2011; Peixoto et al., 2011). Reflecting their short life cycle and genetic organization, microorganisms rapidly accumulate random genomic changes favorably selected by contaminated environments, conferring on these organisms the metabolic capability to transform or even remove pollutants from their habitats (de Menezes et al., 2012; Silver and Phung, 2005). Therefore, the microbial community structure, metagenome and expression of specific genes in response to particular environmental stimuli are sensitive indicators of changes in the overall characteristics of the ecosystem (Maphosa et al., 2012; Vieites et al., 2009).

The aim of the present study was to assess the microbial taxonomy and functional responses encoded by heavy metals, i.e., Cu, Zn, Cd, Pb and Hg, and antibiotic resistance genes, collectively referred to as the resistome, expressed in response to the anthropogenic impact in mangrove sediments using a metatranscriptomics approach.

2. Material and methods

2.1. Sampling sites and sample collection

Three different mangrove sites on the coast of São Paulo State (SP), Brazil, were selected for sediment sampling based on the level of contamination (Fig. 1). The first site was an area highly impacted by petroleum located close to the city of Bertioga (SP) (*Oil Mgv*). This area was contaminated with approximately 35 million liters of oil in 1983 (Andreote et al., 2012). The second sampling site, also located close to Bertioga but different from the first site, was moderately impacted by sludge and other urban waste (*Ant Mgv*). The third sampling site was located in a pristine area in the region of Cananéia City (SP) in an open sea area with vegetation comprising typical mangrove species (*Prs Mgv*). The distance between *Oil Mgv* and *Ant Mgv* is approximately 5 km, while the distance between these areas and *Prs Mgv* is approximately 280 km. Regarding the vegetation, the *Oil Mgv* site presented a low density of *Rhizophora mangle*, *Ant Mgv* exhibited abundant vegetation and the presence of other species, in addition to those typically observed in mangroves. However, *Prs Mgv* presented abundant vegetation exclusively comprising mangrove species. The samples were collected perpendicularly to the mangrove transect (approximately 300 m in total) from three sites separated by at least 30 m, according to Andreote et al. (2012). Sampling was performed in the morning (to avoid the high tidal regime), and soil cores (30 cm deep) were collected using a cylindrical sampler (7 cm diameter). Within each site, triplicate samples were collected, yielding a total of 27 samples (three mangroves × three subregions × three replicates).

2.2. Heavy metal determination

The concentration of the heavy metals Zn, Cr, Pb, Cu, Ni, Cd, and Hg in the sediment samples were determined following digestion using method SW-846, 3051a (Usepa, 2007). The digests were conducted using a method suitable for coupled plasma mass spectrometry (ICP-MS). The concentration of mercury (Hg) in the sediment was determined following digestion using the EPA method 245.1 (Epa, 1994). The mercury remaining in the digestion products was analyzed using atomic absorption spectrophotometry/cold vapor (Teledyne Leeman Labs Hudson, NH, USA). The heavy metal determinations were performed in triplicate.

2.3. DNA extraction

For the metagenome analysis, DNA was extracted from 0.4 g of drained soil obtained from each of the 27 samples. Total DNA was extracted from each sample using the PowerSoil DNA Isolation kit (MoBio, Inc. Solana Beach, USA), according to the manufacturer's protocol. The DNA quantity and integrity was determined through agarose gel electrophoresis (1% w/v).

2.4. RNA extraction and mRNA enrichment

For the metatranscriptome analysis of each of the core sediment samples (n = 27), RNA extraction was performed using the total RNA Isolation PowerSoil® Kit (Mobio Labs, Inc. Solana Beach, USA), using 2 g of sampled mangrove sediment, according to the manufacturer's instructions. Approximately, 1 μg of total RNA was used for the removal of rRNA using the Ribo-Zero™ Magnetic Kit*-Bacteria (Epicenter, Madison, WI, USA). In this procedure, RNA (1 μg) was mixed with Ribo-zero rRNA removal solution, followed by incubation (68 °C for 10 min and 15 min at room temperature). To remove the hybridized rRNA molecules from the mRNA, the RNA/rRNA reactions were incubated with magnetic beads. The solution was vigorously mixed and incubated at room temperature for 5 min, followed by vortexing and incubation for 5 min at 50 °C. The supernatant of the rRNA-depleted sample was purified using the Qiagen RNeasyTM MinElute® Cleanup Kit (Qiagen, Hilden, Germany). The purified mRNA was used to construct cDNA libraries using the ScriptSeq™ mRNA-Seq Library Preparation Kit (Epicenter, Madison, WI, USA) (Carvalhais et al., 2012).

2.5. DNA and cDNA sequencing

Quantification of DNA or cDNA suitable for sequencing in each library was verified prior to the injection of the samples into the sequencing device. The library concentrations were measured through quantitative qPCR using the KAPA library quantification kit for Illumina (KAPA Biosystems). The DNA and cDNA libraries were sequenced on an Illumina® HiSeq 2500 platform using the V3 kit at the Laboratory for Functional Genomics Applied to Agriculture (Luiz de Queiroz College of Agriculture – ESALQ, University of São Paulo, Piracicaba, SP, Brazil). Raw sequences were de-multiplexed (total DNA = 86 ± 14; mRNA = 98 ± 5) according to their tags, and low-quality sequences were filtered (score limit of 0.05; maximum 1 ambiguous nucleotide allowed; minimum sequence length of 100 nt) using the CLC's Workbench software version 6.5.1 (CLC Bio-Qiagen, Aarhus, Denmark). The resulting sequences were

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