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Microbial functional responses to long-term anthropogenic impact in mangrove soils

Lucélia Cabral[a,*], Sanderson Tarciso Pereira de Sousa[a,b], Gileno Vieira Lacerda Júnior[a,b], Erik Hawley[c], Fernando Dini Andreote[d], Matthias Hess[e], Valéria Maia de Oliveira[a]

[a] Research Center for Chemistry, Biology and Agriculture (CPQBA), University of Campinas (UNICAMP), Campinas, São Paulo, Brazil
[b] Institute of Biology (IB) - University of Campinas (UNICAMP), Campinas, São Paulo, Brazil
[c] Washington State University, Pullman, WA, USA
[d] Department of Soil Science, "Luiz de Queiroz" College of Agriculture, University of Sao Paulo, Piracicaba, São Paulo, Brazil
[e] University of California, Davis, Department of Animal Science, Davis, CA, USA

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ABSTRACT

Mangroves are coastal ecosystems of transition between terrestrial and marine environments, that have been particularly contaminated in the last decades. Organic compounds are part of these contaminants, which have increased in the environment due to industrial activities and accidental oil spills. These contaminants are toxic to higher organisms, but microorganisms can metabolize most of these compounds and thus offer a tool for bioremediation purposes. The aim of the present study was to characterize the microbial potential and activity for degradation of aromatic compounds in sediment samples from mangroves using metagenomic and metatranscriptomic approaches. Sediment samples were collected for DNA and RNA extraction from each of the mangrove sites: highly oil-impacted (*Oil Mgv*), anthropogenically impacted (*Ant Mgv*) and pristine (*Prs Mgv*) mangrove. Hydrocarbon concentrations in *Oil Mgv* sediments were higher than those observed in *Ant Mgv* and *Prs Mgv*. Genes and transcripts associated with aromatic compound degradation, particularly the meta and ortho-pathways, were more abundant in *Oil Mgv* and *Ant Mgv* suggesting that many of the aromatic compounds are being aerobically degraded by the microbiome in these sites. Functions involved in the degradation of aromatic compounds were also found in pristine site, although in lower abundance. Members of the genera *Aromatoleum*, *Desulfococcus, Desulfatibacillum, Desulfitobacterium* and *Vibrio* were actively involved in the detoxification of sediments affected by the oil spill. Results obtained from this study provided strong evidence that microbial degradation of aromatic compounds plays an active role in the biological response to mangrove sediment pollution and subsequent ecosystem recovery.

1. Introduction

Aromatic compounds are commonly found in nature in the form of BTEX (benzene, toluene, ethylbenzene and xylene) and petroleum derivatives (Fuchs et al., 2011). Industrial activities and oil spills resulted in increased loads of aromatic compounds in various ecosystems. Since these compounds possess low solubility and high hydrophobicity, they can easily accumulate in mangrove sediments and therefore represent a significant ecological challenge for this ecosystem (Lu et al., 2011; Ostling et al., 2009).

Microbial degradation of organic compounds has been used for bioremediation of areas contaminated with hydrocarbons (Fuentes et al., 2014; Xue et al., 2015) and gained renewed attention after the recent oil spill in the Gulf of Mexico in 2010 (Kostka et al., 2011). Aromatic compounds serve as carbon source for a variety of microbes and several bacteria, archaea, fungi and microbial consortia have been identified as being capable of either mineralize or partially degrade hydrocarbons to less hazardous products (Fathepure, 2014). The less toxic intermediates can eventually reenter and be further converted within the global geochemical cycles (Joutey et al., 2013).

Marine coastal ecosystems comprise intertidal zones that promote daily fluctuations in environmental conditions, including oxygen availability. Such physico-chemical heterogeneity, together with biotic and abiotic ecological interactions, can affect microbial degradation processes, leading microbial communities to alternate between aerobic and anaerobic metabolism for the degradation of hydrocarbon compounds (Cravo-Laureau and Duran, 2014; Robins et al., 2015) The aerobic pathway for hydrocarbon degradation is considered faster than

* Corresponding author.
E-mail address: luc.g.cabral@gmail.com (L. Cabral).

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the anaerobic one, however the rate of this process depends mostly on the hydrocarbon class and the microbe physiology (Kleindienst et al., 2015).

The initial step of degradation of many environmental pollutants, including hydrocarbons, under aerobic conditions is catalyzed by an oxygenase (Olajire and E, 2014). In the case of aromatic hydrocarbons, essential degradation pathways can be divided into peripheral (upper) and central (lower) pathways. In the upper pathways, a diversity of aromatic compounds is transformed into intermediates that are then subjected to dioxygenases that cleave the aromatic ring during a reaction of the lower pathway. In aerobic environments, microorganisms can use a diversity of aromatic compounds and transform them into compounds such as: catechol, protocatechuate and benzoyl-CoA via the peripheral (upper) pathways. After that, in the central (lower) pathways dioxygenases can help to cleave the aromatic ring and convert it into intermediary metabolites such as: acetyl-CoA, succinyl-CoA and pyruvate (Fuchs et al., 2011).

The limited ability to cultivate the “unculturable” microbes in the laboratory has biased a better understanding of the microbial role in the environment and their technological potential (Chikere et al., 2011). However, the advances of molecular microbial ecology techniques allowed the development of experimental procedures to address the ecological role of microbial communities involved in hydrocarbon degradation (Cravo-Laureau and Duran, 2014). Field studies have been performed in marine sediments addressing the impact of oil on microbial communities and it is already known that sulfate-reducing bacteria affiliated primarily with the Deltaproteobacteria (such as *Desulfosarcina* spp. and *Desulfococcus* spp.) are key players in the hydrocarbon biodegradation process (Kleindienst et al., 2015). In the last decades, techniques (i.e. metagenomics and metatranscriptomics) have been developed that allow to study the genetic potential and to determine the actively expressed genes of an organism or a mixture of organisms without the need to grow them in the laboratory (Bikel et al., 2015; Sharpton, 2014). This approach has been revealed as extremely powerful, helping to unveil the composition and functioning of a number of poorly accessed and explored microbial communities, including the ones of mangrove sediments. Thus, this study aimed to enhance our understanding of the genes and transcripts, as well as their microbial hosts, actively involved in the biodegradation of aromatic compounds in sediments exposed to anthropogenic activity and oil spill by comparative metagenomics and metatranscriptomics.

2. Material and methods

2.1. Mangrove sites and sampling

Three different mangrove sites on the coast of São Paulo State (SP), Brazil, were selected for sediment sampling based on the level of contamination. The location map of mangroves and sampling details were previously described by Andreote et al. (2012) and Cabral et al. (2016). Briefly, mangrove sites located on the coast of São Paulo State, Brazil, were chosen based on their level of contamination: 1) *Oil Mgv* - area highly impacted by oil, contaminated with approximately 35 million liters of oil in 1983 (Andreote et al., 2012); 2) *Ant Mgv* - mangrove close to Bertioga city and moderately impacted by sludge and other urban waste. 3) *Prs Mgv* - a pristine mangrove, located in the region of Canaéia. The sampling was performed using the following design: within each site, triplicate samples were collected, yielding a total of 27 samples (three mangroves x three subregions x three replicates). Also, the collection of samples were perpendicularly to the mangrove transect (approximately 300 m in total) from three sites separated by at least 30 m (Andreote et al., 2012). The samples were immediately transport to the lab in a cooler at 4 °C for immediate processing.

2.2. Analytical procedures for hydrocarbon determination

The analytical procedures used to determine hydrocarbon concentration were described in Lima (2012). Briefly, 10 g of sediment were extracted for eight hours with 80 mL of a mixture of dichloromethane (DCM) and n-hexane (1:1, 8 h). A blank control was added in each extraction. Before extraction, a solution with cupper and internal standards (naphthalene-d8, acenaphthene-d10, phenanthrene-d10, chrysene-d12 and perylene-d12) were added to all samples and blank control. The extract was concentrated in rotary evaporator, and the sample was then purified by column chromatography with 5% deactivated alumina and silica. The column was eluted with 20 mL of n-hexane (Fraction 1) and 15 mL of 30% dichloromethane solution in n-hexane (Fraction 2). Sample extracts of fraction 2 were concentrated to 1 mL before gas chromatographic analysis. Concentrations of hydrocarbons were analyzed using a Fisons 8000 GC interfaced directly to a V.G. Masslab-Fisons quadrupole mass spectrometer; model Trio 1000 (GC-MS).

Table 1
Concentration of most abundant hydrocarbons in the sediment samples from *Oil Mgv*, *Ant Mgv* and *Prs Mgv* sites using a Fisons 8000 GC interfaced directly to a V.G. Masslab-Fisons quadrupole mass spectrometer (mg/Kg^{-1}) (Lima 2012). The numbers in parentheses correspondto the standard error.

Hydrocarbons	*Oil Mgv* [mg/kg]	*Ant Mgv* [mg/kg]	*Prs Mgv* [mg/kg]
H-27: Methylchrysene;	119.2 ± 53.1	6.35 ± 2.3	1.25 ± 0.08
H-34: Perylene	61.8 ± 36.4	23.89± 1.4	43.35 ± 28.7
H-28: Dimethylchrysene	145.5 ± 57.7	2.6 ± 0.54	1.2 ± 0.00
H-38: Benzo (g, h, i) perylene	22.6 ± 4.0	13.1 ± 4.8	1.4 ± 0.20
H-35: Indene [1,2,3-c, d] pyrene	23.2 ± 11.3	12.9 ± 3.2	1.08 ± 0.08
H-26: Chrysene	34.6 ± 26.6	4.7 ± 1.4	1.21 ± 0.01
H-23: Methylpyrene	25.5 ± 22.6	2.3 ± 0.7	1.30 ± 0.00
H-32: Benzo (e) pyrene	25.4 ± 21.2	5.61 ± 1.7	1.30 ± 0.00
H-20: Pyerene	9.6 ± 3.4	6.4 ± 1.8	1.28 ± 0.11
H-5: Dimethylnaphthalene	6.4 ± 3.8	6.3 ± 3.5	2.60 ± 0.00
H-19: Fluoranthene	8.6 ± 2.45	8.6 ± 1.8	1.55 ± 0.35
H-2: Methylnaphthalene	1.7 ± 0.55	1.41 ± 0.1	1.30 ± 0.00

2.3. DNA extraction

The DNA extraction was performed from each of the 27 samples using 400 mg of drained mangrove sediment and the PowerSoil DNA Isolation kit (MoBio, Inc. Solana Beach, USA), according to the manufacturer's protocol. After extraction, DNA quantity and integrity were evaluated by agarose (1% w/v) gel electrophoresis.

2.4. RNA extraction and mRNA enrichment

Total RNA was extracted in triplicate from 2 g of drained mangrove soil using Total RNA Isolation PowerSoil® kit (Mobio Labs, Inc. Solana Beach, USA), according to the manufacturer's instructions. To enrich samples for mRNA, 1 µg of total RNA was treated with the Ribo-Zero™ Magnetic Kit*-Bacteria (Epicenter, Madison, WI, USA). The total RNA (1 µg) was mixed with Ribo-zero rRNA removal solution and incubated at 68 °C for 10 min, followed by another incubation at room temperature for 15 min. The RNA/rRNA reactions were incubated with magnetic beads to remove the hybridized rRNA molecules from the mRNA. The solution was vigorously vortexed and incubated twice: first at room temperature for 5 min, followed by another incubation for 5 min at 50 °C. Qiagen RNeasyTM MinElute® Cleanup Kit (Qiagen, Hilden, Germany) was used to further purify the rRNA-depleted RNA. Purified mRNA was used for cDNA library construction with the Script Seq™ mRNA-Seq Library Preparation Kit (Epicenter, Madison, WI, USA) (Carvalhais et al., 2012).