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Bacterial interactions and implications for oil biodegradation process in mangrove sediments

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ABSTRACT

Mangrove sediment harbors a unique microbiome and is a hospitable environment for a diverse group of bacteria capable of oil biodegradation. Our goal was to understand bacterial community dynamics from mangrove sediments contaminated with heavy-oil and to evaluate patterns potentially associated with oil biodegradation is such environments. We tested the previously proposed hypothesis of a two-phase pattern of petroleum biodegradation, under which key events in the degradation process take place in the first three weeks after contamination. Two sample sites with different oil pollution histories were compared through T-RFLP analyses and using a pragmatic approach based on the Microbial Resource Management Framework. Our data corroborated the already reported two-phase pattern of oil biodegradation, although the original proposed explanation related to the biophysical properties of the soil is questioned, opening the possibility to consider other plausible hypotheses of microbial interactions as the main drivers of this pattern.

1. Introduction

Microbial communities in contaminated ecosystems tend to be dominated by the organisms that can degrade or tolerate the contaminant. Since contamination is a strong selection force, these communities are typically less diverse than those in non-stressed ecosystems. Several studies on oil contamination reported a drastic short-term reduction in the diversity of the bacterial communities, which could be accounted for by oil toxicity and strong selection for particular hydrocarbonoclastic bacteria, such as *Alcanivorax* spp. and *Cycloclasticus* spp. (Hazen et al., 2010; Jurelevicius et al., 2013; Kimes et al., 2012; Kostka et al., 2011; Sutton et al., 2012).

It has been reported that oil biodegradation follows a two-phase pattern, characterized by a first phase of fast petroleum degradation with high abundance of few species, followed by a second phase of slower petroleum degradation with high richness of low abundant species. This two-phase pattern has been related to the bioavailability of free total petroleum hydrocarbons (TPH) in the first phase and with a slower desorption rate of soil-sequestered TPH in the second phase (Kaplan & Kitts, 2004).

Several studies suggest that mangrove is a hospitable environment for the growth of a diverse group of bacteria capable of oil biodegradation (Brito et al., 2006; Gomes et al., 2008; Jurelevicius et al., 2013; Liu et al., 2011; Ramsay et al., 2000; Santos et al., 2010; Tian et al., 2008). Mangroves are intertidal ecosystems along the coastlines of tropical and subtropical regions, with unique features such as high primary productivity, abundant detritus, rich organic carbon content and anoxic/reduced (Ghizelini et al., 2012). In tropical mangroves, bacteria and fungi constitute 91% of the total microbial biomass, whereas algae and protozoa represent only 7% and 2%, respectively (Alongi, 1987). Microbial structure and function of mangroves are directly responsible for this ecosystem functioning (Holguin et al., 2001).

Mangrove sediments harbor a unique microbiome and metabolic reconstructions suggest that ecological processes may be modulated by the prevailing conditions found in mangrove (Andreote et al., 2011). We conducted a laboratory oil contamination experiment using sediments from two mangroves with different oil contamination histories, aiming to test the two-phase pattern of oil biodegradation hypothesis (Kaplan & Kitts, 2004). We approached this goal by performing an ecological survey (Marzorati et al., 2008) aiming at understanding bacterial community dynamics from mangrove sediments under heavy-oil contamination stress, and at looking for common patterns that may be associated with oil biodegradation is such environments. This ecological survey is a key step in the decision flow of the Microbial Resource

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Management Framework (Read et al., 2011; Verstraete et al., 2007), which was developed with the goal of finding sustainable solutions to global challenges, through the use of microorganisms.

2. Materials and methods

2.1. Sampling sites and sample collection

Four sampling sites were chosen with respect to their different hydrocarbon pollution history. Sampling sites GBA (22°41′14.5″S; 43°05′6.83″O) and GBB (22°41′1.55″S 43°05′9.21″O) were located in the Guanabara Bay, in the city of Rio de Janeiro, Brazil, and sampling sites GR (21°36′27.85″S 41°03′05.74″O) and GV (21°35′9.11″S 41°03′39.70″O) were located in Gargaú, in the city of São Francisco do Itapaboana, in the northern part of the state of Rio de Janeiro, Brazil (Fig. 1). Physicochemical parameters of the four sampling sites are shown in Table 1. The organic carbon (OC) and total nitrogen (TN) were measured in an elemental analyzer (Flash 2000) and the values were expressed in percentage of dry weight (%). Organic carbon was analyzed after direct acidification in silver vials and total nitrogen in bulk sample. Analytical coefficients of variation for elemental compositions were below 5% for individual samples and accuracy was determined using a certificate material (Low Organic Content Soil, Elemental Microanalysis) with a 97% of recovery.

Guanabara Bay is notorious for its chronically polluted conditions, with a history of oil spill accidents (Ghizelini et al., 2012). The mangrove in Gargaú is located in the estuary of Rio Paraíba do Sul, the biggest estuary in the northern region of the state of Rio de Janeiro. The degradation of this mangrove is primarily related to selective logging and deforestation for the implantation of pastures for cattle ranching, raw sewage, urban runoff, industrial waste release, and construction of roads and landfills (Bernini et al., 2010). There is no record of oil spill in this area.

For each site, three composite samples consisting of five sediment cores each (c. 10 cm of top sediment with 8 cm diameter) were randomly collected. The samples were at least 10 m apart from each other and within each sample the cores were at least 1 m distant from each other. Within each site, the composite samples were collected at the same time, during the low tide. After collection, they were transported to the laboratory in an insulated container with ice, where they were thoroughly homogenized to one representative sample per locality and immediately processed.

Table 1
Physicochemical parameters of the four sampling sites (GBA, GBB, GV, and GR) considered in this study.

		GBA	GBB	GV	GR
pH		7,7	7,6	6,8	6,1
Salinity		24	24	4	3
Granulometry (%)	Sand	30	76	14	12
	Clay	13	6	18	20
	Silt	57	18	68	68
OC (%)		5,72	0,75	5,86	7,56
TN (%)		0,24	0,04	0,39	0,43

OC (organic carbon); TN (total nitrogen).

2.2. Artificial oil contamination

Heavy oil contamination was performed using fresh sediment samples from each locality and the oil biodegradation process was monitored weekly during the first month and then monthly during the four following months, when the oil was visually degraded. This strategy was based in the reported two-phase pattern of petroleum degradation, where key events in the degradation process take place in the first three weeks after the contamination (Kaplan & Kitts, 2004). Fifty grams of fresh samples were incubated at 28 °C in an Erlenmeyer flask with 450 ml of mineral medium (K_2HPO_4 0,1%; KH_2PO_4 0,1%; NH_4Cl, 0,1%; $MgSO_4 \cdot 7H2O$ 0,05%, $CaCl_2$ 0,001%, $FeSO_4$ 0,001%) and 2% oil. Samples were kept shaking at 120 rpm. Aliquots of sediment were taken weekly for DNA extraction, at days 7, 14, 21 and 28 of incubation. The samples were kept under the same conditions until the oil was visibly degraded, which happened after 5 months. Mineral medium was added monthly. Aliquots of sediment for DNA extraction were taken monthly, at 60, 90, 120, and 150 days of incubation.

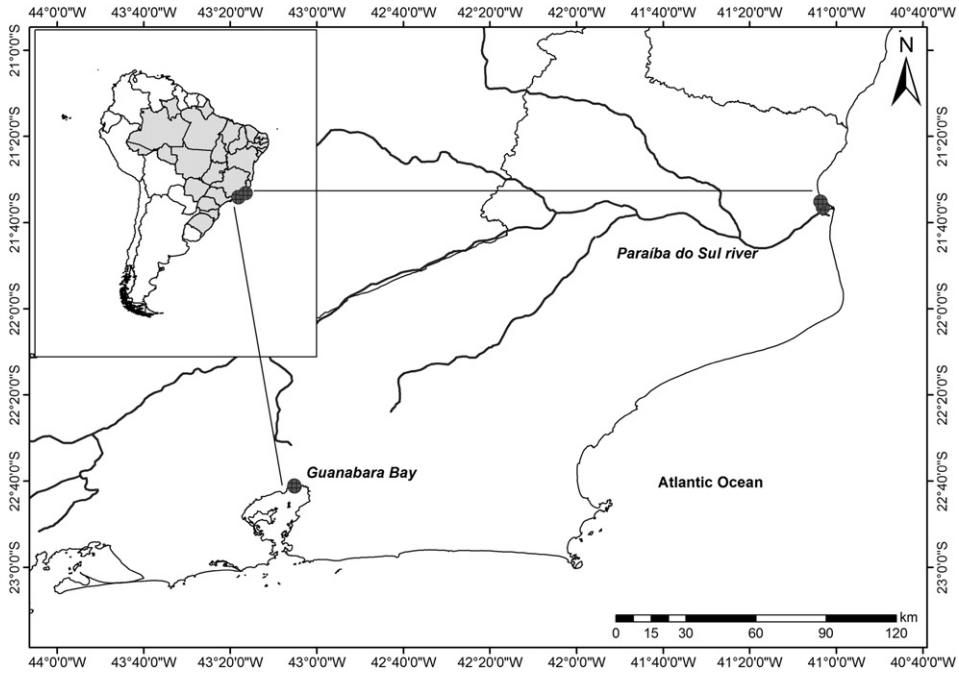

Fig. 1. Location of the sampling sites considered in this study.