BRAZILIAN JOURNAL OF MICROBIOLOGY xxx (2018) xxx–xxx

BRAZILIAN JOURNAL OF MICROBIOLOGY

http://www.bjmicrobiol.com.br/

Environmental Microbiology

# Petroleum contamination and bioaugmentation in bacterial rhizosphere communities from *Avicennia schaueriana*

*Simone Dealtry*[a,b,*], *Angela Michelato Ghizelini*[b,c], *Leda C.S. Mendonça-Hagler*[b,c], *Ricardo Moreira Chaloub*[d], *Fernanda Reinert*[b,e], *Tácio M.P. de Campos*[a], *Newton C.M. Gomes*[f], *Kornelia Smalla*[g]

[a] *Pontifícia Universidade Católica do Rio de Janeiro, Departamento de Engenharia Civil, Rio de Janeiro, RJ, Brazil*
[b] *Universidade Federal do Rio de Janeiro, Programa de Pós-Graduação em Biotecnologia Vegetal, Rio de Janeiro, RJ, Brazil*
[c] *Universidade Federal do Rio de Janeiro, Instituto de Microbiologia Paulo de Góes, Rio de Janeiro, RJ, Brazil*
[d] *Universidade Federal do Rio de Janeiro, Instituto de Química, Departamento de Bioquímica Rio de Janeiro, RJ, Brazil*
[e] *Universidade Federal do Rio de Janeiro, Instituto de Biologia, Laboratório de Fisologia de Planta, Rio de Janeiro, RJ, Brazil*
[f] *Universidade de Aveiro, Departamento de Biologia, Centro de Estudos do Ambiente e do Mar (CESAM), Aveiro, Portugal*
[g] *Julius Kühn-Institut (JKI) Bundesforschungsinstitut für Kulturpflanzen, Institut für Epidemiologie und Pathogendiagnostik, Braunschweig, Germany*

ARTICLE INFO

*Article history:*
Received 12 May 2017
Accepted 14 February 2018
Available online xxx

Associate Editor: Vânia Melo

*Keywords:*
Phytoremediation
Mangrove
IncP-9 plasmids
*ndo* genes

ABSTRACT

Anthropogenic activity, such as accidental oil spills, are typical sources of urban mangrove pollution that may affect mangrove bacterial communities as well as their mobile genetic elements. To evaluate remediation strategies, we followed over the time the effects of a petroleum hydrocarbon degrading consortium inoculated on mangrove tree *Avicennia schaueriana* against artificial petroleum contamination in a phytoremediation greenhouse experiment. Interestingly, despite plant protection due to the inoculation, denaturing gradient gel electrophoresis of the bacterial 16S rRNA gene fragments amplified from the total community DNA indicated that the different treatments did not significantly affect the bacterial community composition. However, while the bacterial community was rather stable, pronounced shifts were observed in the abundance of bacteria carrying plasmids. A PCR-Southern blot hybridization analysis indicated an increase in the abundance of IncP-9 catabolic plasmids. Denaturing gradient gel electrophoresis of naphthalene dioxygenase (*ndo*) genes amplified from cDNA (RNA) indicated the dominance of a specific *ndo* gene in the inoculated petroleum amendment treatment. The petroleum hydrocarbon degrading consortium characterization indicated the prevalence of bacteria assigned to *Pseudomonas* spp., *Comamonas* spp. and *Ochrobactrum* spp. IncP-9 plasmids were detected for the first time in *Comamonas* sp. and *Ochrobactrum* spp., which is a novelty of this study.

© 2018 Sociedade Brasileira de Microbiologia. Published by Elsevier Editora Ltda. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

* *Corresponding author at*: Civil Engineering Department, Catholic University of Rio de Janeiro, Rua Marquês de São Vicente 225/301-L, Gávea, 22453-900 Rio de Janeiro, RJ, Brazil.
E-mail: simonedgs@yahoo.com.br (S. Dealtry).
https://doi.org/10.1016/j.bjm.2018.02.012
1517-8382/© 2018 Sociedade Brasileira de Microbiologia. Published by Elsevier Editora Ltda. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Please cite this article in press as: Dealtry S, et al. Petroleum contamination and bioaugmentation in bacterial rhizosphere communities from *Avicennia schaueriana*. *Braz J Microbiol*. (2018), https://doi.org/10.1016/j.bjm.2018.02.012

## Introduction

Mangroves are tropical and subtropical region coastal ecosystems located in transitional zones between terrestrial environments and sea and rivers.[1] Several studies have demonstrated the role of the mangrove archaeal,[2] bacterial[3] and fungi[4] communities in important biogeochemical cycles. Moreover, mangroves ecosystems have a particular and great microbial diversity. Different microorganisms inhabiting mangroves provide support for mangrove trees to survive under extreme conditions, likely by producing enzymes which are also of considerable industrial interest.[5–10]

In view of the current severe mangrove degradation, mainly due to the anthropogenic activity impacts, the present study is focusing on a pilot scale study for recovering these ecosystems through the adoption of bioaugmentation by using natural polycyclic aromatic hydrocarbon (PAH) degrading bacteria from the rhizosphere of mangrove plants, which might be a promising strategy to be applied in oil-polluted mangroves.[11–15]

The mangrove studied herein is located at Guanabara Bay, Rio de Janeiro, Brazil, near the Duque de Caxias Oil Refinery (REDUC), which has a long history of oil spill accidents and an intense raw sewage discharge into the bay.[16,17] Therefore, such pollution makes the surrounding mangrove a hotspot for microbe-assisted degradation of petroleum components, such as polycyclic aromatic hydrocarbons (PAHs).[18] Herein we target PAHs because they are well known to form a group of priority organic pollutants, due to the risk to the human health, high toxicity and persistence in the environment.[19]

Many studies have demonstrated that mobile genetic elements (MGEs), such as plasmids, play an important role in bacterial adaptation to oil biodegradation within a bacterial population, by promoting the assembly and spread of PAH catabolic gene clusters within the community.[20] Among the known catabolic plasmids involved in PAH compound degradation, the narrow host range IncP-9 plasmids, which belongs to the incompatibility (Inc) plasmids group[21] are of particular importance, as they often carry PAH degrading genes, such as the naphthalene dioxygenase (*ndo*) genes clusters.[22–24] The *ndo* genes were target in the present study instead of others PAH degrading genes, due to their important role to initiate PAH degradation.[25,26]

We studied and tested a previously isolated petroleum hydrocarbon degradative consortium (PHDC) derived from the rhizosphere of the mangrove tree *Avicennia schaueriana* which showed a high ability for PAH removal and high abundances of IncP-9 plasmids and *ndo* genes.[27] Herein the PHDC was characterized in order to test potential for bioremediation and application as inoculum in *A. schaueriana* artificially contaminated with oil in a phytoremediation greenhouse microcosm experiment. Due to the high ability for PAH removal, and high abundances of IncP-9 plasmids and *ndo* genes previously shown by the PHDC isolated by Gomes et al.[27] *A. schaueriana* was the mangrove tree of choice for the current microcosm experiment. This mangrove tree is typically growing in the intertidal regions of sheltered tropical and subtropical coasts,[28] 15–20 m in size and very tolerant to high salinity.[29] Microcosm experiments are often performed in the laboratory or under greenhouse conditions in order to mimic natural environment conditions as much as possible to study biodegradation pathways of different pollutants and the interactions between microorganisms and organic compounds under well controlled conditions.[30]

We hypothesized that the isolation of bacterial communities which have been regularly exposed to high oil concentrations might show accelerated hydrocarbon degradation, which can be explained by the proliferation of adapted bacteria involved in biodegradation pathways.[31] Therefore, we expected that the PHDC inoculation to *A. schaueriana* grown in sediment artificially contaminated with oil, in comparison to treatments without PHDC would: (i) protect plants against deleterious oil effects, (ii) cause changes in the composition of bacterial populations, and (iii) increase the abundance of plasmids potentially carrying catabolic genes, such as *ndo* genes, due to the proliferation of indigenous or introduced bacteria carrying catabolic plasmids such as IncP-9 plasmids, or their spread through horizontal gene transfer.

In order to prove our hypothesis, the assessment of changes in the bacterial communities over time and the detection of functional genes and plasmids involved in PAH degradation, denaturing gradient gel electrophoresis (DGGE) analysis of bacterial 16S rRNA gene and *ndo* fragments, detection of *ndo* gene and IncP-1, IncP-7 and IncP-9 by polymerase chain reaction (PCR) and Southern blot hybridization (SBH) were performed.

## Materials and methods

### *Petroleum hydrocarbon degrading consortium (PHDC) bioaugmentation inoculum source*

Tree sampling, rhizosphere processing and PHDC enrichment were performed according to Gomes et al.[27] *A. schaueriana* was selected for the phytoremediation microcosm experiment based on previous gas chromatography (GC)-based data, which indicated a significant decrease in the total polycyclic hydrocarbon (PH) concentrations ($129.6 \pm 62\,mg\,L^{-1}$) in comparison with the control flasks ($301 \pm 109.3\,mg\,L^{-1}$) ($p < 0.05$). This PHDC also showed a high abundance of naphthalene dioxygenase (*ndo*) genes genotypes and plasmids belonging to the incompatibility (Inc) group.[27] In this study, the *A. schaueriana* rhizosphere PHDC were further characterized, as described below, and were used as the bioaugmentation inoculum in the phytoremediation microcosm experiment.

### *Petroleum hydrocarbon degrading consortium (PHDC) bacteria characterization*

A cultivation-based approach combined with molecular characterization was performed, in order to characterize the bacterial community present in the inoculum, previously used in Gomes et al.,[27] applied in the microcosm experiment.

Bacterial strains were isolated from the enriched inoculum applied in the microcosm experiment described below. First, 4 replicates of the inoculum were incubated in a liquid Minimum Salt Medium (MSM)[32] with the addition of 1% of Arabian Light crude oil (cordially provided by Petrobras S.A.) as the only

Please cite this article in press as: Dealtry S, et al. Petroleum contamination and bioaugmentation in bacterial rhizosphere communities from *Avicennia schaueriana*. Braz J Microbiol. (2018), https://doi.org/10.1016/j.bjm.2018.02.012

Download English Version:

https://daneshyari.com/en/article/11013155

Download Persian Version:

https://daneshyari.com/article/11013155

Daneshyari.com