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Metagenome enrichment approach used for selection of oil-degrading bacteria consortia for drill cutting residue bioremediation☆

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ABSTRACT

Drill cuttings leave behind thousands of tons of residues without adequate treatment, generating a large environmental liability. Therefore knowledge about the microbial community of drilling residue may be useful for developing bioremediation strategies. In this work, samples of drilling residue were enriched in different culture media in the presence of petroleum, aiming to select potentially oil-degrading bacteria and biosurfactant producers. Total DNA was extracted directly from the drill cutting samples and from two enriched consortia and sequenced using the Ion Torrent platform. Taxonomic analysis revealed the predominance of *Proteobacteria* in the metagenome from the drill cuttings, while *Firmicutes* was enriched in consortia samples. Functional analysis using the Biosurfactants and Biodegradation Database (BioSurfDB) revealed a similar pattern among the three samples regarding hydrocarbon degradation and biosurfactants production pathways. However, some statistical differences were observed between samples. Namely, the pathways related to the degradation of fatty acids, chloroalkanes, and chloroalkanes were enriched in consortia samples. The degradation colorimetric assay using dichlorophenolindophenol as an indicator was positive for several hydrocarbon substrates. The consortia were also able to produce biosurfactants, with biosynthesis of iturin, lichnysin, and surfactin among the more abundant pathways. A microcosms assay followed by gas chromatography analysis showed the efficacy of the consortia in degrading alkanes, as we observed a reduction of around 66% and 30% for each consortium in total alkanes. These data suggest the potential use of these consortia in the bioremediation of drilling residue based on autochthonous bioaugmentation.

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1. Introduction

Several methods have been developed for the treatment of environments contaminated by petroleum and its derivatives, which include incineration, stabilization and solidification, oxidation, and bioremediation (Hu et al., 2013). Among the wastes generated by the oil industry, drill cuttings are an environmental liability of major proportions, due to lack adequate disposal treatment (API, 2000). Drill cuttings are a mixture of drilling fluids and rock pieces generated during the drilling process. The contaminants present in drill cuttings include heavy metals, aliphatic hydrocarbons, polycyclic aromatic hydrocarbons (PAHs), and polychlorinated biphenyls (Neff, 2005).

In general, the physical-chemical methods used for treating drill cuttings are expensive and not environmentally safe (Ball et al., 2012; Hu et al., 2013). Bioremediation, widely accepted as being an efficient and environmentally sustainable treatment technology,

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has therefore emerged as a cleaner and lower cost alternative (Ball et al., 2012; Boopathy, 2000; Iwamoto and Nasu, 2001; Ma et al., 2015).

The bioremediation process occurs naturally through reduction of the contaminant concentration (bioattenuation). Nonetheless, this is a slow process and often requires enhancement by a nutrient supply (biostimulation). In this case, the toxic effects on the environment must be taken into account. When the environment to be treated does not have appropriate microorganisms, a bacterial inoculum with desired catalytic capabilities (bioaugmentation) can be added. However, this implementation should also be done carefully because of the unknown effects on the ecosystem, especially when using allochthonous (i.e., from another environment) organisms (Iwamoto and Nasu, 2001).

Autochthonous bioaugmentation, using well-characterized indigenous microorganisms, has been proposed and is considered a promising option to remediate hydrocarbon-contaminated sites and to reduce contamination risks (Wu et al., 2013). The isolation of pure cultures or consortia of these environments is important in providing an inoculum for bioaugmentation. Owing to the complexity of crude oil, a microbial consortium with different microorganisms endowed with diverse metabolic capacities should work better than a pure culture (Wu et al., 2013).

Biosurfactants (e.g., glycolipids, lipopeptides, and lipoproteins) are also usually produced during microbial degradation. They act as dispersants by emulsifying oil in water to form small droplets, thereby rendering the oil potentially available for biodegradation (Bailey and Ward, 1997) and consequently enhancing microbial growth (Lindstrom and Braddock, 2002).

The identification and characterization of the microorganisms and genes involved in hydrocarbon degradation and biosurfactant production are important to develop bioremediation strategies. Although information about the microorganisms involved in these processes is available (Desai and Banat, 1997; Rojo, 2009), little is known about the changes in the microbial community to be isolated and the selectivity of the culture medium, which influences the diversity of the existing community when cultivation is directed towards bioremediation.

In this work, metagenomics approaches, defined as collective microbial genome analysis (Handelsman et al., 1998), were used for determining the microbial diversity in samples from drill cuttings residue and consortia obtained by enrichment in presence of petroleum. Metagenomes were obtained from DNA extracted directly from drill cuttings residue samples and consortia culture growth in different enrichment media. In order to evaluate the microbial community diversity, metagenome sequences were analysed by several bioinformatics tools aiming the identification of taxa and genes involved in the hydrocarbon degradation and biosurfactant production pathways.

2. Materials and methods

2.1. Sample

The samples of drill cuttings were kindly provided by Brasil Química e Mineração Industrial Ltda, a private company located in the city of Mossoró, Rio Grande do Norte, one of the oil-producing states in Brazil. The residue properties of the drill cuttings are provided in Table 1.

2.2. Obtaining undefined microbial consortia

The undefined consortia from the drill cuttings were obtained through the enrichment technique according to Wu et al. (2013), with minor modifications. Two culture media were used for enriching the microbial communities: lysogeny broth (LB; composition in g/L: tryptone, 10; yeast extract, 5; and NaCl, 10) and yeast extract peptone dextrose (YPD; composition in g/L: yeast extract, 10; peptone or tryptone, 20; and glucose, 20). In brief, 8 g of drill cuttings were sieved through 2 mm sterile sieves and then transferred to Erlenmeyer flasks containing 50 mL of LB or YPD medium (pH 7) with 1% (v/v) petroleum as the additional carbon source. The flasks were kept in a rotary incubator at 180 rpm and 37 °C for 7 days. Thereafter, an aliquot (1%) was transferred to new LB or YPD medium containing 1% (v/v) petroleum and incubated under the same conditions. The initial enrichment step was repeated for a total of three cycles (every cycle corresponds to 7 days).

Table 1
Physical and chemical characteristics of the drill cuttings.

Characteristics of drill cuttings	
pH (1:2.5)	7.53
Organic carbon ($g.dm^{-3}$)	60.12
Organic matter ($g.dm^{-3}$)	120.25
N ($g.dm^{-3}$)	1.06
P ($mg.dm^{-3}$)	1
Sand ($g.Kg^{-1}$)	690
Silt ($g.Kg^{-1}$)	290
Clay ($g.Kg^{-1}$)	20
Calcium ($cmol_c.dm^{-3}$)	59.75
Sodium ($mg.dm^{-3}$)	3090
Potassium ($mg.dm^{-3}$)	793

After the three cycles, two samples were obtained by centrifugation (4500 × g, 15 min). The pellet was resuspended in the Bushnell-Haas (BH; composition in g/L: $MgSO_4$, 0.2; $CaCl_2$, 0.02; KH_2PO_4, 1; K_2HPO_4, 1; $(NH_4)_2SO_4$, 1; and $FeCl_3$, 0.05) medium and 2% of BH culture was transferred to 50 mL of the same BH culture medium containing 1% (v/v) petroleum as the sole carbon source. The samples were incubated for 10 days under the same conditions described previously. After three cycles (every cycle corresponds to 10 days), two independent consortia were obtained, designated L consortium (pre-enriched in the LB medium) and Y consortium (pre-enriched in the YPD medium). These consortia were stocked in glycerol (50%) at −20 °C until further use.

2.3. Growth of undefined microbial consortia

To investigate the growth of the microbial community in the presence of petroleum, 1% each of the L and Y consortia was transferred to 250-mL Erlenmeyer flasks containing 50 mL of BH medium with 1% petroleum as the sole carbon source (pre-inoculation). After 48 h, 1% of the grown culture stock was transferred to new Erlenmeyer flasks containing 50 mL of BH medium with 1% petroleum. The optical density at 600 nm was measured every 24 h, from time 0–96 h. The controls were prepared under the same conditions described previously but without petroleum or without bacterial consortia.

2.4. Metagenome sequencing

DNA from the drill cuttings was extracted and purified using the PowerSoil DNA Kit (MoBio Laboratories, Inc., Carlsbad, CA, USA), following the manufacturer's recommendations. The UltraClean Microbial DNA Isolation Kit (MoBio Laboratories) was used for the extraction and purification of DNA from each consortium obtained in this work.

The DNA quality and quantity were estimated using the Qubit 2.0 fluorometer. Subsequently, sequencing was performed using the Ion Torrent Personal Genome Machine (PGM) system (Thermo Fisher Scientific, Waltham, MA, USA). Each sample was sequenced

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