

TITLE: FUNCTIONAL SCREENING FOR NOVEL OIL DISPERSANTS FROM MANGROVE METAGENOMIC LIBRARY

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ABSTRACT:

Application of chemical dispersants has become one of the main strategies employed to handle offshore oil spill accidents. The unprecedented surface and subsurface use of chemical dispersants during the Deepwater Horizon oil spill into Gulf of Mexico in 2010 raised scientific, public, and political concerns regarding their effectiveness and efficacy, and their potential ecological consequences to aquatic resources. In that way, the hazardous effects of chemical dispersants have seized researchers' attention leading to a growing demand for "green" and non-toxic biological dispersants. Mangroves are ecosystems with intense biological activity believed to be a valuable source for the discovery of novel genes e bioactive compounds that can be widely explored by metagenomics technology, which access the total diversity potentiality including uncultivable microbes. Thus, the objective of the currently study was to evaluated the oil dispersant activity of clones from a metagenomic library derived from Jaguaribe's mangrove sediments, Ceará, Brazil. For that, DNA was extracted and fragmented with *EcoRV* and the inserts ranging from 1 to 8 Kb were inserted in the vector pJET1.2/*blunt* and used to transform *Escherichia coli* TOP 10F'. A total of 384 clones were cultured in LB medium supplemented with ampicillin (100 µg/ml) and subjected to a 96 well microplate screening, containing 150 µL of artificial sea water (pH 7.62, salinity 24%), 20 µL of oil and 5 µL of culture. The formation of a halo indicated positive activity. Positive clones were subsequently tested in Petri dishes with a diameter of 4 cm, containing 3 mL of artificial sea water, 20 µL of oil, and 20 µL of culture, performed in triplicate. The diameter of the halo was measured after 5 min and 24 h. Dispersant produced by a strain of *Bacillus subtilis* and sterile broth were used as positive and negative controls, respectively. The first screening resulted in eight positive clones that were further confirmed in larger trials. Seven clones presented non-stable dispersant activity within 24 h, and one clone stood out by its instantaneous activity, producing a full 4 cm stable oil dispersant halo after application of the culture. These results are very encouraging and show the great potential of mangroves sediments as a source of novel dispersant molecules. The next steps involve sequencing and further characterization of these molecules.

Keywords: mangrove, metagenome, clone, oil dispersant.

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