TITLE: DIESEL OIL DETOXIFICATION BY MICROORGANISMS ISOLATED FROM MARINE ENVIRONMENTS

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ABSTRACT:
The diesel oil is the petroleum derivative most commercialized in Brazil. This fuel is a complex mix of hydrocarbons and, due to its recalcitrance and frequent spill accidents, it is worldwide recognized as a threat to aquatic and terrestrial ecosystems. Bioremediation is considered a promising alternative to the remediation of sites contaminated with hydrocarbons since it is more sustainable, efficient and cost-effective when compared to other remediation technologies. In previous studies carried out by our research group a microbial consortium composed by two marine-derived fungi, two bacteria from oil reservoir (off-shore), and one yeast from marine Antarctic sample, was able to efficiently detoxify the diesel oil after 21 days of submerged cultivation. The aims of this study were to analyze the detoxification of diesel oil by the microorganisms of the consortium in a separated way and to evaluate the production of ligninolytic enzymes during the detoxification process. Microbial isolates were grown in artificial seawater (ASW) containing 10 g L^{-1} of malt extract (ME) and 2% of diesel oil at 28 ºC for 7 days. The isolates with the best performance were evaluated in Mineral Medium (MM) containing 2% of diesel as the only carbon source. After incubation, cultures of filamentous fungi were filtered through a filter paper while the cultures of yeast and bacteria were centrifuged in order to determine biomass and diesel detoxification. The detoxification was measured by toxicity analysis using *Artemia* sp. Enzymatic assays were performed by oxidation of ABTS (Lac), malonate complex formation (MnP), and veratryl alcohol oxidation (LiP). The fungi *Cladosporium cladosporioides* CBMAI 857 and *Aspergillus sclerotiorum* CBMAI 849 showed the best results of diesel detoxification after 7 days of incubation in ME with 2% of diesel oil and ASW: 82,8 and 74,8 % of *Artemia* sp. survivors, respectively. The detoxification capacity of these two fungi isolates was reduced in MM containing only diesel as carbon source: 38,8% (*C. cladosporioides* CBMAI 857) and 11,1 % (*A. sclerotiorum* CBMAI 849) of *Artemia* sp. survivors. The enzymatic activities were very low or absent. The results of the present study suggest the potential use of these microorganisms in bioremediation processes of saline areas contaminated with diesel oil and opening perspectives for new studies involving distinct strategies for detoxification/degradation of soils contaminated with this pollutant.

Keywords: marine biotechnology, bioremediation, diesel oil, microbial detoxification

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