Mycofloral Diversity of Hydrocarbon Contaminated Soils

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Abstract

This study was conducted to generate information regarding the diversity of marine fungi inhabiting soils contaminated with hydrocarbons. The soil employed in this study was clayey-sand, accidentally contaminated due to a crude oil spill. Microbial isolation was realized from polluted soil samples, by soil dilution and plate technique using Sabouraud Agar (SA) and Potato Dextrose Agar (PDA) with the purpose of offering different nutritional options for the fungal species that could have been established in the contaminated samples.

Hydrocarbon-tolerant strains were isolated and identified. The dominant genera were *Aspergillus*, *Penicillium*, *Rhizopus* and *Fusarium* and at species level, *Aspergillus* showed maximum diversity. The isolated strains were further screened for their hydrocarbon tolerance level and growth capability.

Because these fungi are adapted to the marine environment, they can be attractive agents for the bioremediation of saline environments, such as ocean and marine sediments that are contaminated by hydrocarbons.

KEYWORDS: Marine Fungi, diversity, Hydrocarbon-tolerant, biodiversity, crude oil, contaminated

INTRODUCTION

Oil spills along the coastal region is one of the most common problems all over the world. Oil spillage is the accidental discharge or pouring of crude oil into the environment. It involves the contamination of any part of the environment with any liquid hydrocarbon. These spills endanger public health, imperil drinking water, devastate natural resources, and disrupt the economy (Gesinde et al., 2008)

Although oil spills from tankers and pipelines release crude oil particles to the water surface and move it to the beaches and contaminates living and nonliving organisms, microorganisms specially fungi have a higher tolerance to the toxicity of hydrocarbons due to their physiology and adaptation to such variations in the environment and have the mechanism for the elimination of spilled oil from the environment (Dibble et al., 1979 & Atlas 1995). The abundance and persistence of hydrocarbons in several polluted environmental areas have been reported.

Cleaning up of the hydrocarbon contaminated soil is a severe challenge. Fungi are of interest because of their ability to synthesize relatively unspecific enzymes involved in cellulose and lignin decay that can degrade high molecular weight, complex and more recalcitrant toxic compounds, including aromatic structures (Adekunle et al., 2007). Certain microbes show increase in population due to use of petroleum hydrocarbons as

nutrients (Westlake et al., 1974). Such species are commonly being used for remediation of contaminated site. These organisms are directly involved in biogeochemical cycles of the degradation of many carbon sources, including petroleum hydrocarbons (Santos et al. 2011). The inputs of various condition related to indigenous microbial communities at contaminated sites are also required for their use in bioremediation approaches (Desai et al., 2010).

Fungi play a central role in the biodegradation or decomposition of organic compounds and are producers of an array of extracellular enzymes. In particular, filamentous fungi have been implicated in the biodegradation of a wide range of aromatic hydrocarbons and thus they could contribute significantly to bioremediation efforts (Hughes et al., 2007& 2009).

The previous studies have focused on few fungal species like white-rot fungi, (Farnet et al., 2009; Lu et al., 2009; Valent´ın et al., 2007; Zhou et al., 2007) although this species does not dominate contaminated and natural soil. It would be interesting to be able to find new indigenous fungal isolates that have the capability to degrade a wide range of hydrocarbons. Recently, there were a few studies being conducted to isolate and characterize the hydrocarbon degrading fungi. These studies revealed the potential of the fungi for use and application in the bioremediation of hydrocarbon (Lu et al., 2009; Cajthaml et al., 2001; & Mancera-L´opez et al., 2008). The present work hence focuses on this approach, aiming to isolate and identify fungi capable of hydrocarbon degradation.

MATERIALS AND METHODS

The soil sample used in this study was clayey-sand, near a beach, accidentally contaminated due to a crude oil spill. Sample was transferred into sterile bottles. Stones and other unwanted soil debris were removed. Soil was analyzed for the presence of hydrocarbon by GC-MS.

Soil fungi were estimated by soil dilution plate count method. Serial dilution (1:10) was made in sterile water using 1 gm of contaminated soil sample. 1ml of each dilution was mixed with Potato dextrose agar and Saboraud's agar each and poured in petri plates. Plates were incubated for a week at a room temperature of approximately 37^{o} C. Streptomycin (500 mg/L) as antibiotic inhibit bacterial growth was added to the media after sterilization process. The isolated fungal strains were identified morphologically by slide culture technique. Fungal colonies were counted for percentage frequency.

Bushnell-Haas broth medium was used for the screening test which composed of: $MgSO_4$ (0.2 g/L), $CaCl_2$ (0.02 g/L), KH_2PO_4 (1 g/L), K_2HPO_4 (1 g/L), $FeCl_2$ (0.05 g/L) and NH_4NO_3 (1 g/L). From 7 days fungal isolate old two plugs (1 cm^2 for each plug) were picked from the peripheral area of Petri dish and transferred carefully to inoculate into 50 ml Bacto Bushnell - Haas broth medium using 250 ml Conical flask. 0.1% (v/v) Tween 80 and 1% (v/v) n-octane. The utilization of 0.1% of Tween 80.The Bacto Bushnell - Haas broth medium was prepared using sterilized sea water. All flasks incubated in room temperature for seven to ten days.

Those which survived were further tested for their tolerance level and growth capability. These strains were inoculated in four concentrations of n-octane contaminated Bushnell-Haas broth medium (5%, 10%, 15% and 20%). One control media (non-oil contaminated) was maintained. After 10 days of incubation, the colonies were observed for growth. Fungal biomass was estimated on the basis of mycelia dry weight.

RESULTS AND DISCUSSION

Results of this study showed the presence of eight fungi, as shown in the table 1. Fungi isolated from the hydrocarbon contaminated soil were *Aspergillus*, *Penicillium*, *Rhizopus Curvularia* and *Fusarium* and at species level, *Aspergillus* showed maximum diversity. Among the Aspergilli, *A. niger* topped the list with 24.65%. Other species of this genus included *A.flavus* 16.43% and *A. terreus* 23.28%. Abundance of aspergilli in soil contaminated with various pollutants is a common observation (Kumari et al., 2010) and its wider reach in the soil has made the species of this genus a favourable biological system for various applications. Actinomycetes also showed its existence in the soil contaminated with hydrocarbons with a 19.17%.

Table 1: Percent frequency of fungi in Hydrocarbon contaminated soil.

No	Name of the organism	No. of colonies	(%) Percentage frequency
1	*Aspergillus niger*	18	24.65
2	*Aspergillus flavus*	12	16.43
3	*Aspergillus terreus*	17	23.28
4	*Penicillium*	04	5.47
5	*Rhizopus*	03	4.10
6	*Fusarium*	04	5.47
7	*Curvularia*	02	2.73
8	*Actinomycetes*	14	19.17

The screening method used in the present study depends on developing a mass of fungal growth in the bottom of the culture medium.

In the present work, out of the eight genera, *Aspergillus* species had the highest growth rate and demonstrated perfect hydrocarbon biodegradation ability. *A. niger* and *A. terreus* demonstrated active ability of hydrocarbon degradation, this result agree with results of Gesinde et al. (2008) who indicated that *Aspergillus niger* have very active degradation capabilities. Bartha & Atlas (1981) listed 14 genera of fungi isolated from an aquatic environment which had been demonstrated to contain members which utilize petroleum hydrocarbon. The evolution of the hydrocarbon mixture depends on the nature of the oil, microbial community, and environmental factors which impact microbial activities.

Table 2: Growth response of fungi in different concentrations of n-octane

FUNGI	Growth in different concentrations (%) of n-octane			
	5%	10%	15%	20%
Aspergillus niger	+	+	+	+
Aspergillus terreus	+	+	+	+
Aspergillus flavus	+	+	+	-

The above results indicate the potentiality of fungi to degrade a wide spectrum of hydrocarbon pollutants. It also indicates biological treatments as the promising alternative to reduce the environmental impact caused by oil spills.

Microbial remediation process is successful and safe way to enhance environment health in particular with low cost, technique and high public acceptance to cleaning up aquatic and terrestrial ecosystems from oil spills. Further research is needed on biochemical and genetic aspects of hydrocarbon degrading fungi for the bioremediation of hydrocarbon contaminated environments.

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