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Isolation and morphological characterization of petroleum crude oil degrading fungi from contaminated site in Sri Lanka

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Intentional and unintentional release of hydrocarbon contaminants into the environment might pose a range of detrimental impacts on the exposed biota including humans. Bioremediation is a promising tool for decontaminating petroleum hydrocarbon contaminated environments. The objective of the present study was to isolate petroleum hydrocarbon degrading indigenous fungi from a chronically contaminated site. Sludge and water samples were collected aseptically from an area subjected to long term intermittent discharges of petroleum hydrocarbons. Aliquots from the collected samples were separately inoculated into Bushnell Haas minimal (BHMS) salt broth amended with 2% filter sterilized Murban light crude oil and incubated at 28^0C for 7 days in a rotary shaker at 130 rpm. Crude oil was used as the model petroleum hydrocarbon and sole source of carbon and energy. After five successive enrichment cycles, three pure cultures of fungal isolates were obtained. The isolated fungi were grown on both BH agar amended with crude oil and potato dextrose agar (PDA). The microscopic morphological characterization of the fungal isolates were carried out using lacto phenol cotton blue stain based on the macroscopic characteristics of the colonies grown on PDA. The three isolated fungi were identified as *Penicillium* spp. based on morphological observations under the microscope. Further, the colony diameters of the three isolates grown on BH agar after a 5 day incubation period showed no significant differences ($p>0.05$) reflecting similar radial growth rates. The three fungal isolates grown on BH agar plates were inoculated into BH culture broths amended with crude oil and a disintegrated crude oil layer with the developed mycelial strands was observed after a 28 day incubation period. Further microscopic observations of the oil particles confirmed an extensive growth of mycelial strands on the oil particles along with conidia bearing conidiophores. In conclusion, the three *Penicillium* species isolated in this study, are capable of utilizing crude oil as the sole source of carbon and energy, and could be considered as potential crude oil degraders. Further molecular identification of the fungal isolates and their relative crude oil degrading capacities under different optimization conditions should be carried out in future studies.

Keywords: Petroleum hydrocarbon, indigenous fungi, *Penicillium* sp.

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