

Isolation and Morphological Characterization of Petroleum Crude Oil Degrading Bacteria from Contaminated Sites in Sri Lanka

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The objective of the present study was to isolate petroleum hydrocarbon degrading indigenous bacteria from chronically contaminated sites. Sludge and water samples collected aseptically from an area (Gampaha district) subjected to chronic discharges of petroleum hydrocarbons were separately inoculated into Bushnell Haas minimal (BHMS) salt broth amended with 2% filter sterilized Murban light crude oil. Cultures were incubated at 28 °C for seven days as the primary enrichment along with an uninoculated BHMS broth as the control. Crude oil was used as the model petroleum hydrocarbon and sole source of carbon and energy. After five successive enrichment cycles, six pure cultures of bacterial isolates were obtained from the discrete colonies observed in the spread plates. The isolated bacteria were grown on BH agar amended with crude oil and nutrient agar (NA) medium. Colony morphological characterization based on colour, size, form, texture, elevation and opacity and Gram staining were performed on bacterial cultures grown on both media. Two of the isolates were Gram positive while other isolates were Gram negative. The ability to degrade crude oil was assessed by inoculating the bacterial isolates into BH culture broths amended with crude oil. The observations showed different degrees of disintegration of crude oil layers through visual observations as well as different degrees of growths through OD₆₂₀ measurements over a seven day period of incubation at 28 °C. Three isolates showed relatively high growth while other three isolates showed a comparatively lower growth as indicated by OD₆₂₀ measurements. The three isolates that showed higher growth capacities have a higher potential to utilize crude oil as the sole source of carbon and energy and thus, may be employed for the bioremediation of contaminated sites. However, the species should be subjected to molecular identification and the respective crude oil degradation capacities should be further studied.

Keywords: Bacteria, biodegradation, petroleum hydrocarbon, sludge and water