

Isolation and Identification of Novel Bacteria that Could Potentially Capable of Degrading Crude Oil from a Heavily Contaminated Sites in Sri Lanka

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Abstract

Bioremediation is the most harmless and efficient method for degrading petroleum hydrocarbons. The objective of the present study was to isolate and identify potential crude oil degrading bacteria from chronically contaminated sites located at Colombo and Gampaha districts of Sri Lanka. Primary enrichments were prepared by inoculating sludge and water samples separately into the Bushnell Hass Minimal Salt medium amended with 2% crude oil as the source of carbon and energy, incubated at 28 °C for 5 days at 130 rpm. Eleven bacterial isolates were obtained from spread plates prepared after fifth enrichment. The Gram's staining showed that all the isolates were gram negative rod shape. Growth kinetics were assessed by culturing each isolate in BHMS amended with 2% crude oil under the constant culture conditions stated above with two replicates and a control. OD₆₂₀ was measured in every 24 hours interval and several isolates showed excellent growth (Isolate 1, 2, 3 and 8), high growth (isolate 5), moderate growth (isolate 11, 12 and 14) and low growth (isolate 10 and 13) in respect to OD value. Partial gene sequences of 16S rRNA was shown 99% and 100% identities for *Pseudomonas aeruginosa*. However, the morphological variations observed in the crude oil layers and in the culture media due to the production of pigments pertaining to different isolates revealed that among the isolates, different strains of *Pseudomonas aeruginosa* might be present.

Keywords - Crude oil, Bacterial strains, BHMS medium, Pseudomonas aeruginosa