

Isolation and Identification of Long Chain *n*-Alkane Degrading Bacteria from Crude Oil-contaminated Soil in Sapugaskanda, Sri Lanka

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The devastating impacts of anthropogenic petroleum pollution affect all organismal life in marine and terrestrial ecosystems in many ways. Alkanes being the major constituent of crude oil do not degrade efficiently and thus persist in the environment for long periods. In this study, we aimed to isolate novel microbial species from the crude oil-contaminated soil in Sapugaskanda, Sri Lanka, and to identify them at the molecular level for future bioengineering purposes. Soil samples were collected from a crude oil-contaminated site in the Sapugaskanda oil refinery. Soil samples (2.5 g) were inoculated with 50 ml of BSM supplemented with 1 g/L *n*-alkanes (*n*-hexadecane (C16), *n*-docosane (C22) and *n*-octacosane (C28)) separately and incubated at 25 °C for 7 days, continued up to six enrichments. Each enrichment culture was plated onto LB (Luria Bertani) agar plates and sub-cultured. Bacterial colonies with different morphologies were further purified by four-way streaking and incubated at 25 °C overnight. The pure cultures were examined morphologically, followed by Gram staining. Bacterial genomic DNA was extracted with Phenol-Chloroform extraction and the 16s rRNA gene was amplified and sequenced using 16s rRNA universal primers. The fragments were sequenced with Oxford nanopore sequencing and identified using Genius prime®, the sequences were annotated with the NCBI database. A total of seven different bacterial isolates (i.e., *Pseudomonas* sp., *Acinetobacter* sp., *Stenotrophomonas* sp., *Enterobacter* sp., *Serratia* sp., *Bacillus* sp. and *Brucella* sp.) were identified from the contaminated site. Future work will involve evaluating the growth of isolated strains in the presence of long chain *n*- alkanes of varying chain lengths as the sole carbon source. Additionally, the *n*-alkane degradation efficiencies of these strains will be assessed using gas chromatography-mass spectrometry (GC-MS).

Keywords: *Hydrocarbons, Long Chain n-Alkane Degrading Microorganisms, Pseudomonas sp., Stenotrophomonas sp., Serratia sp.*