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Validation of diesel degradation potential of selected bacterial isolates from petroleum contaminated soil samples in Sri Lanka

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Petroleum, a natural fuel composed of hydrocarbon compounds, is commonly used as an energy source in industries and in daily life. Petroleum contaminants in soil may remain as persistent organic pollutants causing long-term damage to ecosystems. Bioremediation is an economical, eco-friendly treatment method for the recovery of soil quality following contamination. Indigenous bacteria from contaminated soil samples often possess metabolic processes for hydrocarbon utilization or degradation, which can be used to degrade and remove petroleum pollutants from soil during bioremediation. This study aimed to validate diesel degrading (DD) potential of selected bacterial isolates from a petroleum-contaminated soil sample in Sri Lanka, using redox assay techniques and spectrophotometry. Bacterial strains were isolated in Bushnell Haas mineral salt medium supplemented with crude oil, streaked on nutrient agar and sub-cultured. Fourteen fast-growing isolates were screened for DD potential using two redox indicator dyes 2,6-dichlorophenolindophenol (2,6-DCPIP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) to detect the degradation rate. Spectrophotometric readings for decolorization of dyes by each bacterial isolate were obtained where the amount of decolorization or oxidation coincided with the level of hydrocarbon utilization. Absorbance readings for each isolate were obtained in triplicates and expressed as mean±standard deviation. Statistical analyses were performed using R Studio 3.5 where absorbance readings from the 2,6-DCPIP assay were analysed using Two-way ANOVA and Tukey's test, and DPPH assay readings were analysed using Kruskal-Wallis test and Dunn's test. The isolation of DD bacteria here suggests the emergence of hydrocarbon degradation ability in indigenous soil bacteria following exposure to petroleum contaminants. The redox assays confirmed that all fourteen isolates could utilize diesel as the sole carbon source; isolates BN02, BN06 and BN44 from the soil sample showed the highest degradation rates in both redox assays (>88%). These results suggest that the DPPH redox assay is an effective method to validate the results of conventional hydrocarbon degradation analysis assays which use 2,6-DCPIP to assess degradation potential. This study can be expanded further to verify the efficiency, mechanisms of DD, molecular identification, and synergy of mixed bacterial consortiums in soil prior to exploring their potential in bioremediation of petroleum-contaminated soil.

Keywords: Diesel degradation, hydrocarbon degrading bacteria, bioremediation, redox assay techniques

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