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MORPHOLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF EFFICIENT DIESEL-DEGRADING BACTERIA ISOLATED FROM PETROLEUM-CONTAMINATED SUBSURFACES

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The widespread use of petroleum products like diesel has led to environmental contamination, necessitating studies aimed at minimizing its impact upon release into the environment. Among the potential petroleum-degrading microbes, previous studies have identified bacteria as the most effective. However, bioremediation success relies on identifying native bacteria from contaminated sites, as they are better suited to local conditions. Degradation of complex hydrocarbons like diesel requires multiple bacterial species, as no single strain can break down all its components. Thus, this study aimed to characterize and identify efficient diesel-degrading bacteria from petroleum-contaminated soil collected from garages in Kandy, Sri Lanka, and to design bacterial consortia that can efficiently degrade diesel. Diesel-utilizing bacteria in collected soil samples were enriched using Bushnell Haas broths supplemented with diesel. Potential diesel-degrading bacteria were isolated using spread plating and streak plating techniques. These isolates were subjected to turbidity assay and 2,6-Dichlorophenolindophenol (2,6-DCPIP) assay to quantitatively determine the most effective degrading bacteria. The recognised efficient bacteria were characterised using morphological and biochemical tests and were used to design bacterial consortia. Based on the findings of the quantitative tests, three of the isolated diesel-degrading bacteria were determined to be the most efficient. The morphological and biochemical test results led to the subsequent identification of these three isolates as belonging to the genera *Klebsiella*, *Enterobacter*, and *Staphylococcus*. Of these, *Klebsiella* was shown to be 5.87% and 10.87% more effective than the other two isolates, respectively. Of the four bacterial consortia that were designed using pure bacterial isolates, three consortia were more successful at degrading diesel than the individual bacterial isolates based on the 2,6-DCPIP assay. It showed that the consortium consisting of *Enterobacter* and *Klebsiella* was the most successful. The findings of this study can be employed for bioremediation of diesel-contaminated sites.

Keywords: 2,6-Dichlorophenolindophenol assay, Bacterial consortia, Bioremediation, Diesel-degrading bacteria, Turbidity assay