Research & Reviews in Biotechnology & Biosciences **ISSN No: 2321-8681**
Website: www.biotechjournal.in **Research article**
Volume: 10, Issue: 1, Year: 2023 **PP: 7-13**
DOI: https://doi.org/10.5281/zenodo.8195112 **Peer Reviewed Journal**

Exploring hydrocarbon degrading ability of bacteria isolated from engine oil polluted soil.

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Article History

Received: 25/02/2023
Accepted: 15/03/2023

Article ID: RRBB/2

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Abstract:

This study was conducted to assess the capabilities of bacterial isolates obtained from oil-polluted sites with waste engine oil. Engine oil is made of crude petroleum, it mainly consists of hydrocarbons which includes aliphatic, aromatic hydrocarbons, alkyl compounds, resins etc. and trace amounts of Sulphur or nitrogen. To treat these toxic pollutants bioremediation is an environment friendly approach toreduce their intensity of toxicity.

Waste engine oil contaminated soil samples were collected from specific garage areas in Mangalore, and assessed for high hydrocarbon and oil degradation capacity. The screening process involved conducting both biochemical and molecular tests to assess the potential of the isolated organism.

Furthermore, the study employed emulsification index and gravimetric analysis to evaluate the isolates' efficacy in emulsifying and degrading potential of oil. Molecular tests confirmed the presence of three significant bacterial species—*Klebsiellaquasipneumoniae*, *Stutzerimonasstutzeri*, and *Klebsiella pneumonia*showing their potential for waste engine oil bioremediation. Moreover, the research sheds light on the microbial diversity and functionality within oil-contaminated environments.

In conclusion, this study uncovers the potential of bacterial isolates for effective waste engine oil bioremediation. It emphasizes the significance of employing these isolates to promote sustainable waste management practices, highlighting the need for further research to optimize

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bioremediation techniques and facilitate practical implementation in the field.

Keywords: Engine oil, Biodegradation, Emulsification index, Gravimetric analysis, bioremediation

Introduction:

Waste engine oil, a significant byproduct of machinery and vehicle operation, contaminates surrounding environments like soil and water. (1)Treating used motor oil is a challenge because it doesn't mix with water, has a thick texture, and may contain harmful substances. It breaks down slowly and sticks to surfaces, resulting in pollution of water and soil. It imposes great risk to the environment and various forms of life. Bioremediation provides a cost-effective and efficient solution for cleaning up contaminated environments. However, careful microbial handling and continuous monitoring are necessary to address concerns in this approach. (2) By implementing proper waste disposal methods and preventing soil contamination, we can mitigate the risks it poses to ecosystems and human health.

Physical remediation incineration and landfilling have minimal to no negative impact on marine biota, making them a sustainable choice. However, their effectiveness is influenced by various environmental and oceanic factors, including wave action, temperature, oil type, and the presence of natural dispersants.(3)

As a result, there is a growing need for sustainable and cost-effective approaches to tackle these environmental issues. (4)Bioremediation, using indigenous bacteria and transferred catabolic genes, offers a targeted and effective solution for waste engine oil treatment. Isolating native petroleum-degrading strains and screening enzymes for over-expression through proteomic identification are crucial steps. This approach holds promise for addressing petroleum contamination. (5)

Factors such as oil composition, dispersion, and absorption, as well as temperature, oxygen, and nutrients, influence biodegradation rates. Bacteria and fungi are key degraders, with adaptation and genetic amplification enhancing degradation. Seeding can accelerate biodegradation under controlled conditions. (6)

This research explores the potential of bacterial isolates in efficiently and sustainably remediating waste engine oil. Bacteria are isolated from oil-polluted sites, characterized, and tested for their oil degradation capabilities. Molecular tests confirm the presence and identity of oil degrading bacterial species. The findings enhance our understanding of microbial diversity and functionality in oil-contaminated environments, paving the way for scalable bioremediation strategies. Effective waste engine oil treatment is crucial to mitigate environmental pollution and health risks associated with oil spills and contamination.

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Sample collection: soil samples from the subsurface layer of soil from a depth of 5-30cm deep were collected from few engines.

Materials and Methods:

Fig1. Samples collected from oil contaminated sites of Mangalore.

1gm of the soil sample was added to 9ml of saline. It was vortexedand inoculated to Bushnell Hass (BH) broth supplemented with 2% engine oil as carbon source. After a brief growth period of 7 days the inoculums from this broth was spread plated on Bushnell Hassgrowth medium supplemented with engine oil as carbon source.(9, 10, 11).

The plates were incubated at 37°C for 48 hrs. (12)The bacterial isolates obtained from the BH agar medium showed consistent growth as BH medium serves as a selective medium,

A.Biochemical characterization: Biochemical tests were performed to further identify and characterize the bacterial isolates. The tests are given in the table 1. Biochemical test results helped in classifying the isolates into relevant taxonomic groups.(13)

B. Oil degradation screeninganalysis. (14): Oil degrading capabilities of the specifically designed to promote the growth of oil-degrading bacteria.(9)

Furthermore, after a thorough examination of the bacterial colonies on BH medium, distinct morphological characteristics such as variations in color, size, and shape were observed. Colonies representing unique characteristics were selected for further analysis and investigation.The pure cultures of the selected bacteria, individual colonies on BH media are shown in.Figure.2

isolatedbacteria were assessed using screening assays for their oil degrading ability. These assays involved the use of engine oil or specific hydrocarbon compounds as the sole carbon source. The degradation activity included changes in its Optical density, emulsification index and weight loss using analytical techniques such as gravimetric analysis.

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Figure2: Generation of pure bacterial cultures by sub culturing technique

Table: 1 Details of identified bacterial isolates and quantified results of gravimetric analysis.

Bacterial Isolate	Amount of oil degraded (gms)	Bacteria identified by 16s RNA Sequencing	Morphological features
CN1 CN8 CN9	0.25 0.32 0.2	***Klebsiella pneumoniae***	Gram-negative, non-motile, and rod-shaped bacteria, non-motile
CN7 CN14 CN16 CN17 CN18 CN19	0.15 0.23 0.36 0.48 0.48 0.49	***Klebsiella quasipneumoniae***	Gram-negative, non-motile, and rod-shaped bacteria, non-motile
CN13 CN15	0.31 0.46	***Stutzerimonas stutzeri***	Gram-negative motile, bacillus

Note- Results of gravimetric analysis showed that CN15, CN17, CN18, and CN19 possess high capacity for oil degradation with degradation rates above 0.4gms, while CN1, CN7, CN14, CN8, CN9, CN13, and CN16 exhibit moderate to lower abilities for oil degradation.

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Table 2. Biochemical tests of the bacterial isolates

Biochemical Tests	*Klebsiellapneumoniae*	*Klebsiellaquasipneumoniae*	*Stutzerimonas Stutzeri*
Carbohydrate fermentation test	+	+	+
Indole Test	-	-	-
Methyl red Test	-	+	+
VogesProskauer (VP) test	+	+	+
Citrate Test	+	+	+
Urease Test	+	+	+
Catalase Test	+	+	+
Oxidase Test	-	-	+

C. Molecular identification: For a more precise identification of the isolated bacteria, DNA sequencing was carried out. 16S rRNA sequencing was carried out to determine the bacterial genus and species.(15) Identified isolates were preserved for future use.(16)

Results

Based on DNA sequencing 3 bacterial species identified from oil-contaminated samples were *Klebsiellaquasipneumoniae, Stutzerimonasstutzeri, and Klebsiellapneumoniae.* The sequences were submitted to Gene bank. The accession numbers of gene bank submission in NCBI portal are

OP597535, OP597647, OP597648, OP597654, OP597675. These bacterial species provide valuable insights about the microbial composition of the oil-polluted sites.

The bacterial isolates CN15, CN17, CN18, and CN19 have demonstrated remarkable ability in breaking down more than 0.40 grams of oil, outperforming other bacterial isolates. The oil degrading capacity and biochemical test results of the isolates is given in the table 1 and 2

Conclusion:

In conclusion, oil degrading potential of these microorganisms, can be leveraged to standardize the environment friendly bioremediation protocols that will address oil pollution of water and soil due to waste engine oil.

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Declaration of competing interest: There is no conflict of interest

Acknowledgements: The authors express their gratitude to the University College, Mangalore University,Mangalore, Karnataka, India, for supporting this research work.

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