Isolation and Characterization of Bacteria from Soil Samples of Fuel Station

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Abstract-The study aimed to identify and isolate bacteria capable of breaking down hydrocarbons found in petrol-contaminated soil. To do this, an enrichment culture method and serial dilution techniques were used to isolate the bacteria. Selective media containing hydrocarbons was used to culture the bacteria, which were then placed on Petri plates and incubated for 24 hours. After this, the bacteria were sub-cultured on agar slants to maintain pure cultures. Gram staining was used to identify gram-positive bacteria, which were observed as purple and rod-shaped.

During the study, different bacteria colonies were observed, with varying colours, shapes, and sizes. This indicated the presence of various bacterial species. These bacterial isolates underwent different biochemical tests such as the catalase test to determine the presence of a catalyst enzyme and the methyl red test to assess the capacity of hydrocarbon-degrading bacteria to metabolize. A positive result from the methyl red test indicated the presence of glucose fermentation pathways. On the other hand, a negative result suggested the presence of alternative metabolic pathways or the inability of the bacteria to ferment glucose. The study's primary objective was to provide valuable insights into the metabolic processes of hydrocarbon-degrading bacteria.

The study investigates the potential of hydrocarbon-degrading bacteria to restore the environment through bioremediation. The indole test determines if bacteria can produce indole, a byproduct of tryptophan metabolism. The Voges-Proskauer test detects the production of acetoin during glucose fermentation. The study also evaluates the bacterial viability at different temperatures, revealing an optimum growth temperature range of hydrocarbon-degrading bacteria within the mesophilic range (20-40°C). Antibiotic susceptibility testing was performed on hydrocarbon-degrading bacteria using three antibiotics: erythromycin, azithromycin, and rifampicin. The results showed that the sensitivity of the tested bacterial isolates varied.

Keywords: Hydrocarbon – Degrading bacteria, Physiological characterization, Biochemical characterization, M.S Media, IMVC test, Anti-microbial studies, Bio-degradation Studies.

INTRODUCTION

Our planet's natural resources, particularly crude oil, have been carelessly depleted, leading to significant environmental pollution. The increasing demand for petroleum products has resulted in pollution risks that pose a significant environmental threat, especially with the growing population and urbanization. Industrialization has also led to the production of large amounts of waste that pollute the soil and water bodies. Exploiting natural resources to meet the population's energy demands has mainly relied on petroleum hydrocarbon, a global pollutant. Accidental spills and improper waste disposal have increased soil and marine ecosystem contamination, posing a risk of groundwater contamination and reduction in air quality. To mitigate the environmental impact of petroleum and its by-products, the use of hydrocarbon-degrading bacteria in environmental bioremediation has been highlighted. These bacteria break down hydrocarbons into simpler compounds and play a crucial role in reducing the harmful effects of hydrocarbon pollution on the environment and human health. Bioremediation strategies utilizing these bacteria have advantages over conventional methods, as they are cost-effective, eco-friendly, and minimize the generation of pollutants. Moreover, recent advancements in molecular biology, genomics, and metagenomics have led to the discovery of novel hydrocarbon-degrading bacteria that offer promising solutions for addressing environmental pollution challenges. These microorganisms contribute to the natural attenuation of hydrocarbon contaminants and are vital for restoring contaminated environments. Further research and development efforts will

facilitate the development of effective strategies for addressing environmental pollution challenges.

The primary objective of this research is to isolate and characterize hydrocarbon-degrading bacteria from contaminated environments, focusing on understanding their potential for environmental bioremediation.

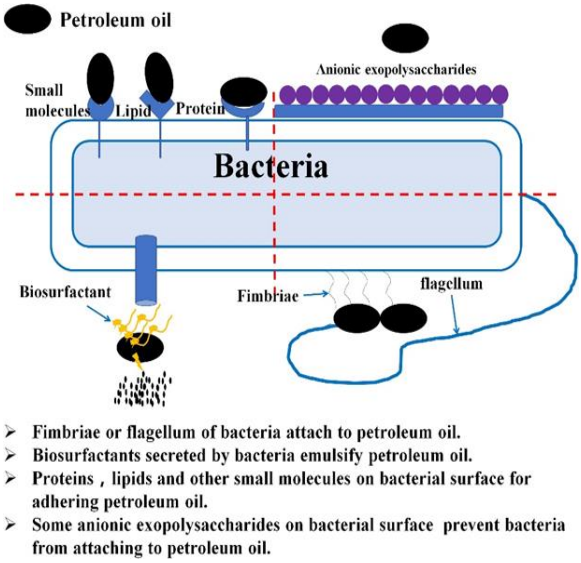

Fig: Hydrocarbon – degrading bacteria

MATERIALS AND METHODS

Collection of sample and isolation of bacteria: Petrol-contaminated soil was collected from the petrol pump, and the sample was taken and weighed 1gm. Then, it was mixed with distilled water to make up to 500ml volume in a conical flask and kept aside for some time to get sediment.

An enrichment culture method was used to isolate bacteria from it, and serial dilution techniques were employed to obtain pure cultures.

Fig : Preparation of supernatant solution

Media preparation and optimization: Selective media containing hydrocarbons was used to promote the growth of hydrocarbon-degrading bacteria. The contents below were mixed with 100ml of distilled water in a conical flask.

Chemicals	Quantity
K2HPO4	2.0gm/l
[NH4]2so4	0.5gm/l
KH2PO4	0.02gm/l
FeSO4.4H2O	300mg/l
MgSO4	0.05g/l
MnSO4.4H2O	400mg/l
ZnSO4.4H2O	200mg/l
CuSO4.7H2O	40mg/l
KI	300mg/l

Incubation Conditions and Duration: The supernatant solution, obtained before, was poured onto the M.S. media, which had previously been poured onto the Petri plates. After the media solidified, these petri plates were incubated at 30℃ for 24 hours. After 24 hours, the growth of bacteria was observed.

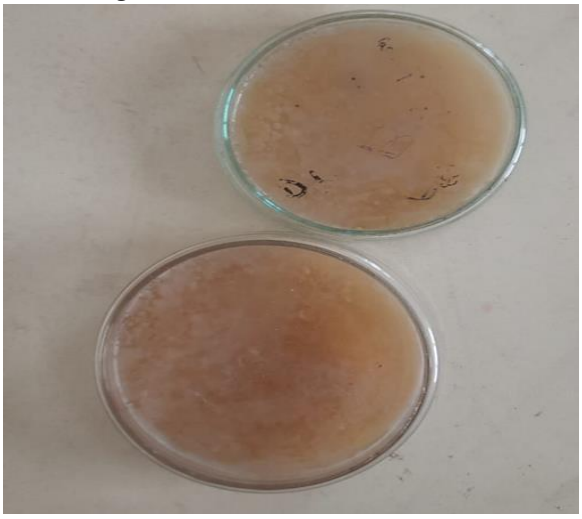

Fig : Growth of bacteria after 24 hours

Sub-Culturing Techniques:

The bacteria developed previously in Petri plates were sub-cultured by streaking on agar slants to maintain pure bacterial cultures and preserve isolate viability. These slants were again incubated at 30℃ for 24 hours.

Fig: Sub culturing on agar slants

STAINING TECHNIQUES AND INTERPRETATION

Smears of 24 hours were heat-fixed on the slide crystal Violet. First, it was applied and allowed to remain for 1 minute before being washed off with water. Gram's iodine was added; this stayed for another minute and was later washed off. The smear was decolorized with alcohol, washed with water, and counter-stained with one percent saffron. It was then blood-dried with clean filter paper and absorbed under the oil immersion lens of the microscope.

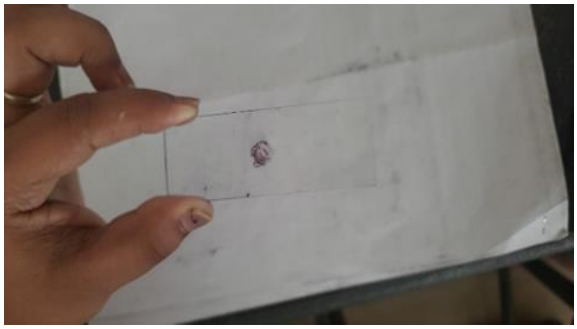

Fig: Gram's staining slide outside the microscope

BIOCHEMICAL CHARACTERIZATION OF ISOLATES:

Catalase activity: Inoculum from the pure cultures was streaked on nutrient agar slant and incubated at 37^0C for 24hours.Two drops of 3% hydrogen peroxide solution is added on to clean glass slide then with the help of nichrome wire loop cells from the center of well isolated colony were transferred onto drop of hydrogen peroxide.

Methyl Red Test:

The Methyl Red test evaluates bacteria's ability to produce stable acids during glucose fermentation. This helps determine the metabolic capacity of hydrocarbon-degrading bacteria. A positive result indicates the presence of glucose fermentation pathways, while a negative result suggests alternative metabolic pathways or a lack of glucose fermentation ability.

Indole Test:

The indole test detects bacteria's ability to produce indole, a byproduct of tryptophan metabolism. The enzyme tryptophanase catalyses the conversion of tryptophan into indole, pyruvic acid, and ammonia. Indole is detected by adding Kovac's reagent to a bacterial culture, which forms a red colour upon reaction with indole.

In this test, we inoculated isolated bacterial cultures into Tryptone broth tubes using standard sterile techniques, then incubated for 48 hours at 37ºC. After incubation, 1 ml of Kovac's reagent was added to the culture tubes, which are observed for a colour change.

Vogues Proskauer Test:

The Voges-Proskauer test detects the production of acetoin, a precursor of 2,3-butanediol, during glucose fermentation. Bacteria that produce acetoin in the presence of glucose and peptone react with alpha-naphthol and potassium hydroxide (KOH), resulting in a red colour due to the formation of a complex.

EVALUATION OF ANTIMICROBIAL ACTIVITY OF HYDROCARBON - DEGRADING BACTERIA

WELL DIFFUSION AGAR METHOD:

Agar well diffusion method is widely used to evaluate the antimicrobial activity of plants or microbial extracts . Similarly to the procedure used in disk-diffusion method, the agar plate surface is inoculated by spreading a volume of the microbial inoculum over the entire agar surface. Then, a hole with a diameter of 6 to 8 mm is punched aseptically with a sterile cork borer or a tip, and a volume (20–100 µL) of the antimicrobial agent or extract solution at desired concentration is introduced into the well. Then, agar plates are incubated under suitable conditions depending upon the test microorganism. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain tested.

RESULTS AND DISCUSSION:

STAINING TECHNIQUES AND INTERPRETATION:

Upon observation under the microscope, the gram staining was found to be ***gram-positive*** bacteria, which appeared purple with round-shaped bacteria.

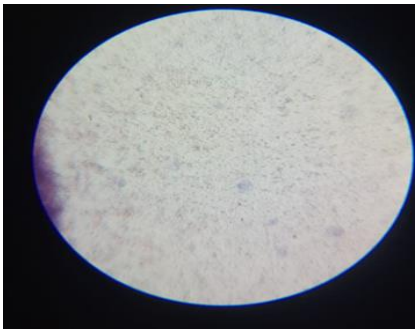

Fig: Gram's staining result

BIOCHEMICAL CHARACTERIZATION OF ISOLATES

Table: Biochemical charcterization of bacterial isolates

Chemical test	Observation	Reference	Sample image
Catalase activity	Efferenvense was observed (+)	The isolates utilizes the catalase in it	
Methyl Red Test	Stable red colour was formed.(+)	Indicating the production of mixed acids during glucose fermentation.	
Indole Test	Cherry red supernatant layer was observed. (+)	Bacterial isolate has ability to produce indole.	
Vogues Proskauer Test	Pink colour change was observed. (+)	Bacterial isolate has the ability for the of production of acetoin.	

ANTIMICROBIAL ACTIVITY OF HYDROCARBON – DEGRADING BACTERIA:

Antibacterial activity of Hydrocarbon degrading isolates was performed using two methods (i.e., Agar well diffusion method and Disc diffusion method.

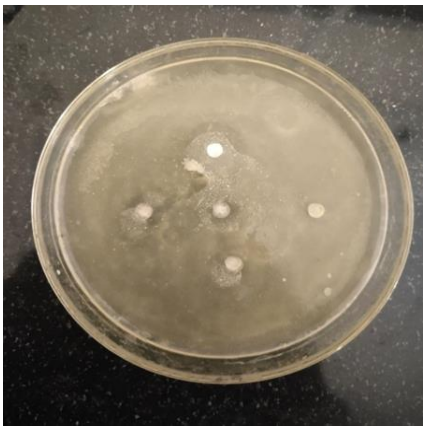

Fig : Azithromycin

In agar well diffusion method, the antibacterial activity of Hydrocarbon degrading isolates was performed against bacterial cultures using Azithromycin, and Erythromycin as standard. Hydrocarbon degrading bacteria showed antimicrobial activity against both and exhibited clear zone of inhibition.

Fig: Erithromycin

CONCLUSION

In conclusion, the study successfully isolated and characterized bacteria from petrol-contaminated soil samples obtained from a fuel station. The research aimed to identify bacteria capable of breaking down hydrocarbons found in the contaminated soil, and it utilized various enrichment culture methods and biochemical tests for this purpose. The findings revealed the presence of diverse bacterial species with varying characteristics, demonstrating the potential for environmental bioremediation. The study also assessed the metabolic processes, physiological characteristics, and antibiotic susceptibility of the isolated bacteria, providing valuable insights into their potential for biodegradation. Furthermore, the research highlighted the importance of hydrocarbon-degrading bacteria in mitigating environmental pollution caused by petroleum products, emphasizing the significance of bioremediation strategies. The results contribute to the understanding of microbial processes involved in environmental restoration and offer opportunities for further research and development in addressing pollution challenges. Overall, the study underscores the critical role of hydrocarbon-degrading bacteria in environmental conservation and the need for continued efforts to harness their potential for sustainable solutions.

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