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RESEARCH ARTICLE

On-Site Study of Lipophilic Bacteria in the Bioremediation of Polycyclic Aromatic Hydrocarbons (PAH) from Automotive Grease / Engine Oil Spills

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ABSTRACT

In this study, the isolated strains that are able to grow on grease / oil contaminated spills and used as a sole carbon source were isolated from Coimbatore regions. The physicochemical analysis of oil-contaminated soil revealed a pH range of 6.8–8.4, indicating a slightly acidic to neutral environment suitable for microbial growth. Electrical conductivity remained below 2 dS/m, supporting microbial viability. Enrichment techniques yielded eight distinct bacterial isolates, initially characterized by morphology, Gram staining, and biochemical tests. Molecular identification via 16S rRNA confirmed and MALDI-TOF analysis identified two isolates with high confidence, including species like *Pseudomonas stutzeri* and *Exiguobacterium aurantiacum*. FTIR spectra confirmed the presence of polycyclic aromatic hydrocarbons (PAHs), validating the soil for bioremediation studies. It showed reduction in hydrocarbon peaks and appearance of new functional groups, suggesting PAH degradation. Spray plate assays revealed clear degradation zones with *Pseudomonas stutzeri* and also showing the most effective PAH breakdown than the *Exiguobacterium aurantiacum*. The Dioxygenase enzyme assays further supported these findings, showing highest activity in *Pseudomonas stutzeri* and *Exiguobacterium aurantiacum*. SDS-PAGE analysis displayed distinct protein bands between 53–78 kDa, with a prominent 37 kDa band in strong degraders, possibly linked to degradative enzymes.

Keywords: Polycyclic Aromatic Hydrocarbons, Biodegradation, Spray Plate technique, Dioxygenase enzyme, Phenanthrene

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INTRODUCTION

Environmental pollution caused by the improper disposal of used engine oil and grease spills in automotive sheds is a growing concern. Engine oil and grease are complex mixtures of hydrocarbons, heavy metals, and other toxic compounds that pose significant environmental and health risks. When released into the environment, these pollutants can contaminate soil and water, leading to long-term ecological damage. Polycyclic Aromatic Hydrocarbons (PAHs) are a class of organic compounds composed of two or more fused aromatic rings made up of carbon and hydrogen atoms. They are produced primarily through the incomplete combustion of organic materials such as coal, oil, gas, wood, and waste. PAHs are widely distributed in the environment and are considered significant environmental pollutants due to their persistence, toxicity, and potential carcinogenic and mutagenic properties. These compounds are hydrophobic, meaning they have low water solubility, which allows them to bind to soil, sediments, and particulate matter, leading to long-term environmental contamination. PAHs are categorized into low-molecular-weight (LMW) and high-molecular-weight (HMW) compounds based on the number of aromatic benzene rings; for example, low molecular weight (LMW) PAHs have two or three benzene rings while high molecular weight (HMW) PAHs have more than three benzene rings. Classical examples of LMW PAHs include acenaphthene, anthracene, naphthalene, fluorene and phenanthrene, whereas HMW PAHs include benzo (a) pyrene (B(a)P), dibenz (a, h) anthracene, pyrene, and chrysene (Bright *et al.,* 2023). LMW PAHs, such as naphthalene, anthracene, and phenanthrene, are more water-soluble and volatile, whereas HMW PAHs, such as benzo[a] pyrene, chrysene, and dibenz [a, h] anthracene, are more stable and toxic due to their complex ring structures. Petroleum in its natural state is called crude oil. Crude oil, because of its characteristics, is one of the most significant pollutants in the environment as it is capable of causing serious damages to human and the ecosystem (okpokwasili GC, 1996).

Mechanism of Lipophilic bacteria

Lipophilic bacteria play a crucial role in degrading polycyclic aromatic hydrocarbons (PAHs) in used engine oil and grease spills through specialized biochemical mechanisms. These bacteria produce bio surfactants, such as rhamnolipids and surfactant, which reduce surface tension and emulsify oil droplets, increasing the bioavailability of PAHs. Their hydrophobic cell surfaces allow them to adhere directly to oil droplets, facilitating the uptake of hydrocarbons. The degradation process begins with the oxidation of PAHs by monooxygenases and dioxygenases, which introduce oxygen into the aromatic rings, forming hydroxylases intermediates like dihydrodiols and catechol. These intermediates are then subjected to ring cleavage by catechol 1,2-dioxygenase (ortho-cleavage) or catechol 2,3-dioxygenase (meta-cleavage), producing compounds like cis, cis-muconate and 2-hydroxymuconate semi aldehyde. These products enter central metabolic pathways, such as the tricarboxylic acid (TCA) cycle, generating energy and biomass. High-molecular-weight PAHs, which are more resistant to degradation, are often broken down through co-metabolism, where the presence of simpler hydrocarbons stimulates enzyme production. (Van Hamme *et al.,* 2003).

Sources and Impact of PAHs in the Atmosphere

Polycyclic aromatic hydrocarbons (PAHs) in the atmosphere primarily arise from the incomplete combustion of organic materials such as fossil fuels, biomass, and tobacco. They are released through vehicle exhaust, industrial activities, residential heating, and wildfires. Once emitted, PAHs bind to airborne particulate matter, enabling them to travel long distances and stay suspended in the air. Because of their low volatility, PAHs can linger in the atmosphere for extended periods, contributing to air pollution. PAH concentrations tend to be higher in urban areas due to dense traffic and industrial operations. These pollutants pose significant health risks, including respiratory issues, cardiovascular diseases, and a higher likelihood of cancer. PAHs in the air can also settle onto land and water, leading to further contamination. Additionally, atmospheric PAHs can undergo photochemical reactions, creating secondary pollutants. When PAHs are discharged into the atmosphere, they exist in two distinct states: a vapour phase and a solid phase, where they adhere to particulate debris (Wang *et al.,* 2013).

Toxicity and Health Risks of PAHs

Polycyclic aromatic hydrocarbons (PAHs) are recognized for their toxicity, with several compounds in this group being carcinogenic, mutagenic, and teratogenic. Prolonged exposure to PAHs can lead to the development of cancer, particularly lung, skin, and bladder cancers, due to their ability to cause genetic mutations and alter cellular functions. These substances can also harm fetal development, leading to birth defects when exposure occurs during pregnancy. Humans can be exposed to PAHs through various routes, including inhalation of contaminated air, ingestion of contaminated food or water, and skin contact with contaminated surfaces or substances. Such exposure poses significant health risks, especially in areas with high industrial or vehicular emissions, or where oil and fuel spills occur. Bacterial biodegradation offers a low-energy, cost-effective alternative to traditional industrial remediation methods, which often require significant energy and resources. By utilizing naturally occurring microorganisms, this bioremediation process is both economically and environmentally sustainable. It promotes sustainability in automotive workshops and industrial facilities, supporting global environmental conservation efforts. This research encourages industries to adopt eco-friendly waste management practices, reducing their carbon footprint. Additionally, it helps reduce harmful PAH levels in the environment, contributing to the mitigation of long-term soil and water contamination. By promoting bacterial bioremediation, this study supports cleaner, greener industries while effectively reducing environmental pollution (Varjani, S. J. 2017). In this study, we have analyzed the samples which have PAH compounds and it would give a solution to protect the environment from hazardous compounds pollutants and assure the safe environment.

MATERIALS AND METHODS

Sample collection: Samples were aseptically collected from the automotive shed in and around Coimbatore city. Processing on sample began immediately upon the arrival of laboratory. Sample were sieved using 2mm mesh and thoroughly mixed. About 100g of sample were collected from each location in a sterile airtight cover and stored at 4ºC.

Sample characteristics: The collected grease/ oil spilled samples were prepared for pH and Conductivity analysis by mixing 10g of soil with 20ml of distilled water and the results were recorded (Winter and Behan-Pelletier, 2007). The presence of PAH compounds in the sample have been measured with FT-IR analysis to confirm the presence of PAH compounds. To enumerate microbial load, 10g of sample was added to the 90 ml of sterile distilled water, and the mixture was vortexed for 10-15minutes. Serial dilutions of 10^{-1} to 10^{-10} were performed, and 0.1-1ml of diluted soil suspension was plated onto sterile Nutrient agar plates. The plates were incubated at 37º C for 24 - 48 hours, and the colonies were counted using a colony counter. The colony forming units (CFU) per gram of soil were recorded.

Isolation of Lipophilic bacteria: After incubation, 15 out of the 40 samples showed growth of lipophilic bacteria, inwhich 25 samples did not show any lipophilic growth. The selected colonies were sub cultured on fresh Minimal agar and olive oil plates to obtain pure cultures. The sub cultured plates were incubated at 30-37º C for 24 hours.

Following this, Gram's staining was performed to determine the morphology and the arrangements of the organisms. Biochemical tests, such as Catalase, Oxidase and Urease were also conducted to further characterize the isolates. Besides, IMVIC test were also performed as per the requirement. Microbial identification was performed using the keys provided by Bergey's Manual of Determinative Bacteriology (1994).

Characterization by MALDI-TOF Analysis

Molecular tools were used to characterize the organisms by MALDI-TOF analysis

PROTOCOL: Matrix-Assisted Laser Desorption / Ionization Time-of-Flight Mass Spectroscopy (MALDI-TOF MS) is a high throughput technology based on the comparison of the protein fingerprint obtained by microbial cells with a database of reference spectra by means of the use of various algorithms integrated in systems recently made. (Przybilla *et al.,* 2004) For MALDI-TOF MS analysis, 2-5mg of bacterial cells were suspended in 0.1% trifluoroacetic acid, vortexed, and centrifuged. The supernatant was removed, and the process was repeated thrice. The extracted cells were mixed with acetonitrile, formic acid and α-cyano-4-hydroxycinnamic acid matrix. The mixture was spotted onto MALDI target plates and analyzed using polyethylene glycol sodium adducts (Singhal, N., *et al.*, 2015). In this study, the MALDI-TOF study was out sourced at Royal care hospitals, Coimbatore.

Spray plate assay: The PAH spray plate assay was adopted to test the PAH degradability of isolates. The PAHs (anthracene, pyrene, flourene and acenapthalene.) were dissolved in solvents and applied in spray plate methods. Bacterial colonies were pre-grown on YEPG agar plates and then sprayed with 1% (w/v) PAH dissolved in acetone. After evaporating the acetone by placing the plates in a fume hood, the plates were placed in plastic bags and incubated at 28ºC for up to 3 weeks. The plates were observed daily upto observable zone appearance. (Kiyohara *et al.*, 1982).

Dioxygenase test: Dioxygenase activity was examined using indole because the formation of indigo from indole is presumptive for aromatic ring dioxygenases. Bacterial colonies were pre-grown on YEPG agar plates, and then indole crystals were placed in the lids of the Petri dishes. Then, the plates were incubated for 1 day at 20ºC. (Ensley *et al.*, 1983).

Drop collapsed method: The drop collapse assay was conducted using crude oil as the hydrocarbon substrate, following a method. With slight modifications, since the primary objective of this study was to assess the degradation of crude oil, the same crude oil was used as the substrate for the assay. A single drop of crude oil was placed on a glass slide, and then a drop of 48-hour-grown culture broth was added onto the crude oil drop. (Bodour and Miller-Maier 1998)

Fourier transform infrared (FTIR) analysis: This technique is based on the interaction of electromagnetic radiation in the thermal infrared region (Hammes, 2005). Also known as vibrational spectroscopy, it measures different types of vibrations between the atoms according to their atomic bonds. The soil sample was mixed with a suitable broth and a PAH compound, and then incubated for a specified period to allow for potential degradation of PAHs by microorganisms present in the soil. Following incubation, the mixture was centrifuged at a suitable speed for a specified time to separate the solid and liquid phases. The centrifuged sample was then processed for FTIR analysis, where it was analyzed using FTIR spectroscopy to detect changes in the molecular structure of the PAH compound, indicating potential degradation. The samples were placed directly onto the FT/IR- 4600 type A spectrometer. The pressure exerted on the samples was ranging from 30 to 50 psi. The absorbance spectrum was recorded in the range of 399-7800$cm^{-1.}$ The FTIR analysis was carried out using a Perkin Elmer 4600 type A spectrum instrument. All absorbance spectra were obtained in the 4000–400 cm1 range by 100 scans at 4 cm^{-1} resolutions, and the signal-to-noise ratio was 45000:1. The FTIR spectra displays information regarding functional groups that are present 1 lm near the surface of an internal reflection element. Moreover, it possesses the ability to obtain precise spectra of the samples.

SDS- PAGE for enzyme production: To analyze PAH degradation, microorganisms are first inoculated into a suitable liquid growth medium containing PAH compounds (such as acenaphthalene or phenanthrene or pyrene or flourene) and incubated for 48-72 hours to allow PAH degradation (Bodour and Miller-Maier, 1998). After incubation, the cells are harvested by centrifugation (10,000-15,000 rpm for 10-15 minutes), and the supernatant is discarded. The cell pellet is then suspended in a lysis buffer, and cell lysis is performed using methods like sonication or freeze-thaw cycles (Wei *et al.*, 2010). Following lysis, the sample is centrifuged again to remove debris, and the protein-containing supernatant is collected. The protein concentration is quantified using a protein assay, such as the Bradford method (Bradford, 1976). The protein samples are mixed with an SDS-PAGE sample buffer containing Tris-HCl, SDS, glycerol, bromophenol blue, and β-mercaptoethanol (Laemmli, 1970), and the mixture is heated to 95°C for 5 minutes to denature the proteins. The denatured samples are loaded onto a polyacrylamide gel, and electrophoresis is performed at a constant voltage (100-150 V) until the dye front reaches the bottom (Laemmli, 1970). After electrophoresis, the proteins are stained with Coomassie Brilliant Blue or silver stain (Sambrook and Russell, 2001), and the gel is analyzed for changes in the protein profile. Proteins involved in PAH degradation, such as naphthalene dioxygenase or phenanthrene dioxygenase, may be upregulated and these changes are indicative of microbial activity in PAH degradation (Fuchs *et al.*, 2011). The presence of new or enhanced protein bands can be quantified using densitometry, offering insight into the enzymatic processes involved in PAH degradation.

RESULTS AND DISCUSSION

Soil chemical and properties

The pH and conductivity plays an important role influencing the growth of the organisms and enzyme production. The influence of the initial pH on enzyme and organisms was determined by varying the pH ranging from pH 6.8-8.4. Based on the chemical analyses, the pH and conductivity (Fig. 1) of the collected soil sample were measured, in which the pH has been found to be slightly acidic in environment. The conductivity of the soil samples were measured using the conductivity meter where the EC level was reported to be less than 2Ds/m (Win, P. P., *et al.* (2024).

Isolation and identification of lipophilic bacteria

The organisms were isolated from the oil- contaminated sample by inoculating 1g of sample into the minimal media with olive oil. The bacterial strains were found to be survived in the oil containing media. Out of 40 isolates there were 8 strains found to be capable of degrading PAH respectively (Plate 1, 2 and 3). The bacterial strains were identified as *Exiguobacterium aurantiacum, Brachybacterium conglomeratum, Sphingomonas trueperi Micrococcus luteus, Sphingomonas paucimobili, Staphylococcus pasteuri, Pseudomonas stutzeri* and *Trueperella pyogenes*. These bacterial isolates were enriches as pure culture in nutrient agar shown in plate 4 and 5. The biochemical test and Gram's staining of the organisms (Table 1.) was done to identify characterization of the isolated lipophilic bacteria, and to understand their metabolic capabilities.

MALDI-TOF Analysis

Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry (MALDI-TOF MS) was used to identify bacterial strains isolated from oil-contaminated soil based on their peptide mass fingerprinting profiles. The isolated organism was identified as *Exiguobacterium aurantiacum, Brachybacterium conglomeratum, Micrococcus luteus, Staphylococcus pasteuri, Pseudomonas stutzeri* and *Trueperella pyogenes*. All isolates were matched with high-confidence scores 99.9% (Table 2.), indicating reliable species-level identification. These bacteria have documented supporting their role in PAH biodegradation. These peaks used to differentiate the *Entero bacteriaceae* and allowing to identify genus and species (Clark, A. E**.** *et al.*, 2013). The sample were analyzed in VITEK MS PRIME, which is a mass spectrometry system using matrix- assisted laser desorption/ ionization time of flight mass spectrometry (MALDI-TOF MS) shows (Table 2.) that confirm the diverse taxonomic composition of the microbial community involved in PAH breakdown and provide molecular evidence of species with potential for bioremediation applications in oil-polluted environments (Przybilla *et al.*, 2004).

Spray plate assay: The spray plate assay demonstrated the PAH-degrading capabilities of bacterial strains isolated from oil-contaminated soil. Each isolate produced visible halo zones (Plate 6.) around colonies, indicating PAH breakdown. The following Table 3 summarizes the degradation efficiency based on zone diameter**:**

Dioxygenase test

All eight bacterial strains exhibited aromatic ring dioxygenase activity, evidenced by the conversion of indole to indigo and the formation of clearance zones on PAH-supplemented media. Meynet, P. *et al*., (2013) confirms their enzymatic ability to oxidative cleave aromatic rings—an essential mechanism in PAH biodegradation. Among the isolates, *Pseudomonas stutzeri* showed the highest activity with a 25 mm zone, degrading phenanthrene, fluorene, and anthracene. *Exiguobacterium aurantiacum* followed closely with a 20 mm zone, targeting naphthalene and phenanthrene. Moderate-to-high activity was observed in *Micrococcus luteus* (17 mm, pyrene) and *Staphylococcus pasteuri* (16 mm, naphthalene and fluoranthene). Lower activity was recorded in *Brachybacterium conglomeratum* (14 mm, fluorene) and *Trueperella pyogenes* (12 mm, naphthalene). These findings confirm the presence of functional dioxygenase enzymes in all isolates and highlight the strong bioremediation potential of *Pseudomonas stutzeri* and *Exiguobacterium aurantiacum* in oil-contaminated, PAH-rich environments.

Drop collapsed method

The drop collapse assay was used to assess biosurfactant production by bacterial isolates from oil-contaminated soil. This method detects surface-active compounds that reduce surface tension, causing droplet collapse on a hydrophobic surface. All six strains tested positive. *Pseudomonas stutzeri* and *Exiguobacterium aurantiacum* showed rapid and complete collapse, indicating high biosurfactant activity. *Micrococcus luteus* and *Staphylococcus pasteuri* exhibited moderate activity, while *Trueperella pyogenes* and *Brachybacterium conglomeratum* showed slower but visible collapse. These findings suggest that bio surfactant production, alongside enzymatic degradation, enhances PAH breakdown and hydrocarbon bioavailability, supporting the bioremediation potential of these isolates (Jain, D.K *et al*., 1991)

Fourier transform infrared analysis

Sample 1 shows strong aromatic C–H (3028, 2986 cm^{-1}) and C=C (1637 cm^{-1}) and Sample 5 shows reduced intensity of similar peaks, especially around 1633 and ~1400 cm^{-1}. This shows the Breakdown of aromatic rings (PAHs) observed in Sample 7 compared to 3 (Fig 2 and 6). Sample 2 has peaks at 3025, 2923, 2802 cm^{-1}, and 1637 cm^{-1} (C=C). Sample 6 shows 3019, 2920, 2815 cm^{-1}, but lower intensity at 1633 cm^{-1}.Sample 8 indicates further degradation of PAHs and aliphatic groups compared to 4. (Fig 3 and 7). Sample 3 has clear aromatic and aliphatic C–H (3022, 2933 cm^{-1}), and aromatic C=C (1640 cm^{-1}). Sample 7 maintains similar peaks but shows new intense bands around 1202 and 985 cm^{-1}. Biotransformation/oxidation indicated by new polar functional groups in Sample 9. (Fig 4 and 8). Sample 4 shows PAH features at 3019, 2927 cm^{-1} and 1633 cm^{-1}. Sample 8 shows new peaks at 2752 cm^{-1} and more intense polar bands at 1244–1179 cm^{-1}. Clear evidence of PAH degradation and emergence of breakdown products (e.g., alcohols, phenols, or carboxylic acids) in Sample 10. ((Fig 5 and 9). The FTIR spectra of Samples 3 to 10 demonstrate the presence and progressive breakdown of PAH compounds**.** Characteristic PAH peaks at ~3050 cm^{-1} (aromatic C–H), ~1630 cm^{-1} (C=C), and ~1000 cm^{-1} (C–H out-of-plane bending) diminish or shift in intensity across the sample pairs, while new polar functional group peaks emerge, suggesting oxidation and transformation processes during treatment or degradation. For interpretation of various functional groups present in the biosurfactant, the FTIR spectrum was compared (Pornsunthorntawee *et al*., 2008)

FTIR Analysis of Samples spectra(Fig 2 - 9)

SDS- PAGE evident the Enzyme production for biodegradation

The isolates were loaded onto SDS-PAGE (Plate 7.) and the molecular weight of the proteins present in the bio-surfactants was checked and found that they ranged between 53 kD and 78 kDa. This result confirms the presence of protein in the bio-surfactant (Ramani *et al.,* 2012) also reported their biosurfactant as lipoprotein biosurfactant in the presence of oil/ grease spilled contaminated soil. One prominent band is visible around the ~37 kDa region, suggesting the sample predominantly contains a single protein of this molecular weight. The clarity and sharpness of the band suggest that the protein is relatively pure. The protein sample analyzed by SDS-PAGE showed a single band at approximately 37 kDa, indicating a successful purification step and suggesting that the sample consists of a single major protein species.

SUMMARY AND CONCLUSION

The ability of various microorganisms to metabolize hydrocarbon is well known. In this study, the isolated strains that are able to grow on grease / oil contaminated spills and used as a sole carbon source were isolated from Coimbatore regions. The physicochemical analysis of oil-contaminated soil revealed a pH range of 6.8–8.4, indicating a slightly acidic to neutral environment suitable for microbial growth. Electrical conductivity remained below 2 dS/m, supporting microbial viability. FTIR spectra confirmed the presence of polycyclic aromatic hydrocarbons (PAHs), validating the soil for bioremediation studies. Enrichment techniques yielded eight distinct bacterial isolates, initially characterized by morphology, Gram staining, and biochemical tests. MALDI-TOF analysis identified all eight isolates with high confidence, including species like *Pseudomonas stutzeri* and *Exiguobacterium aurantiacum*. Spray plate assays revealed clear degradation zones, with *Pseudomonas stutzeri* and *Sphingomonas trueperi* showing the most effective PAH breakdown. Dioxygenase enzyme assays further supported these findings, showing highest activity in *Pseudomonas stutzeri* and *Exiguobacterium aurantiacum*. All isolates tested positive for biosurfactant production, with complete droplet collapse observed in the two most potent strains, indicating significant surface activity. FTIR analysis of treated samples showed reduction in hydrocarbon peaks and appearance of new functional groups, suggesting PAH degradation. SDS-PAGE analysis displayed distinct protein bands between 53–78 kDa, with a prominent 37 kDa band in strong degraders, possibly linked to degradative enzymes. These results highlight the potential of native bacterial strains, especially *Pseudomonas stutzeri* and *Sphingomonas trueperi*, for effective PAH degradation and supporting their future application in sustainable bioremediation strategies.

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Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this article.

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Table 1. Morphological and biochemical characteristics of isolates

ORGANISMS	GRAM'S REACTION	INDOLE	MR	VP	CITRATE	OXIDASE	CATALASE
Exiguobacterium aurantiacum	Positive	Negative	Positive	Positive	Negative	Negative	Positive
Brachybacterium conglomeratum	Positive	Negative	Negative	Negative	Negative	Negative	Positive
Sphingomonas trueperi	Negative	Negative	Negative	Negative	Positive	Positive	Positive
Micrococcus luteus	Positive	Negative	Positive	Positive	Positive	Positive	Positive
Staphylococcus pasteuri	Positive	Negative	Negative	Negative	Negative	Negative	Positive
Sphingomonas paucimobilis	Negative	Negative	Negative	Positive	Positive	Positive	Positive
Pseudomonas stutzeri	Negative	Negative	Negative	Negative	Positive	Positive	Positive
Trueperella pyogenes	Positive	Positive	Negative	Negative	Negative	Negative	Negative

Table 2. Molecular characteristics of isolates by MALDI-TOF Analysis

SAMPLES	UHIH/IP No.	LAB No.	IDENTIFIED ORGANISMS	PROBABILITY (%)
1	315158	2349644	*Exiguobacterium aurantiacum*	99.9%
2	315158	2349646	*Brachybacterium conglomeratum*	99.9%
3	315158	2349615	*Sphingomonas trueperi*	99.9%
4	315158	2349633	*Micrococcus luteus*	99.9%
5	315158	2349630	*Sphingomonas paucimobilis**	99.9%
6	315158	2349615	*Staphylococcus pasteuri*	99.9%
7	315158	2349631	*Pseudomonas stutzeri*	99.9%
8	315158	2349630	*Trueperella pyogenes*	99.9%

Table 3. Spray Plate Assay

Bacterial isolate	Degraded PAH Compounds	Zone Diameter (mm)	Degradation Efficiency
Pseudomonas stutzeri	Anthracene, Phenanthrene, Fluorene	22mm	Very High
Sphingomonas trueperi	Fluoranthene	22mm	Very High
Sphingomonas paucimobilis	**Phenanthrene**, **Pyrene**	21 mm	High
Exiguobacterium aurantiacum	Phenanthrene, Naphthalene	18mm	High
Micrococcus luteus	Pyrene	15mm	Moderate-High
Staphylococcus pasteuri	Naphthalene, Fluoranthene	14mm	Moderate
Brachybacterium conglomeratum	Fluorene	13mm	Moderate
Trueperella pyogenes	Naphthalene (low MW PAH)	10mm	Low

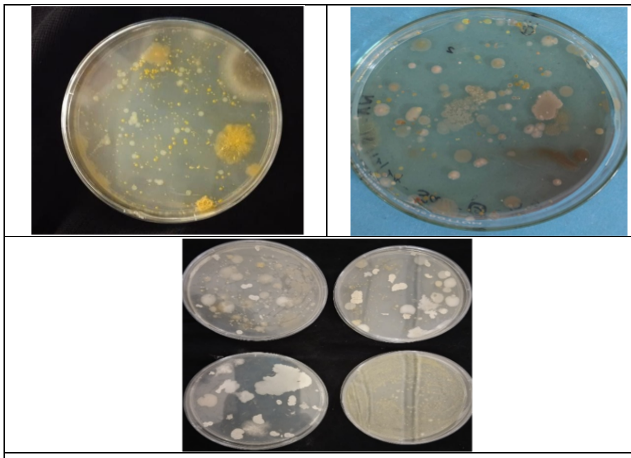

Plate **1, 2 and 3: Serial dilution of sample produce CFU on plates**

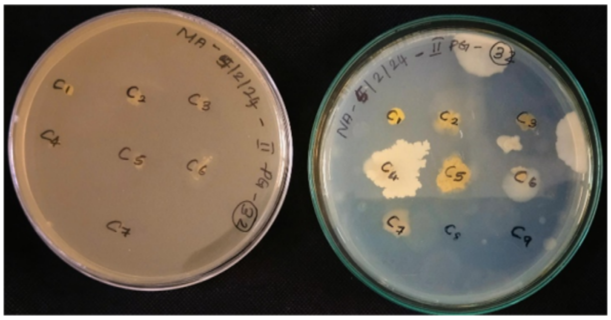

Plate 4. Enrichment culture in Nutrient agar (right) and Minimal agar (left)

Plate 5: Pure culture of lipophilic bacterial isolates

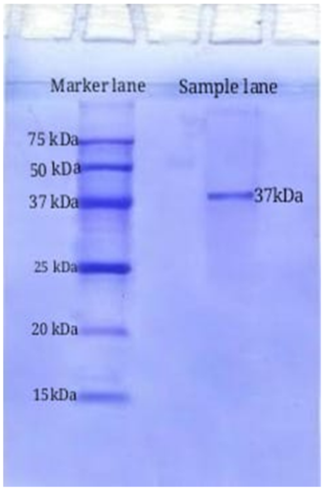

Plate 7: SDS- PAGE reveals the protein 37kDa in the sample lane

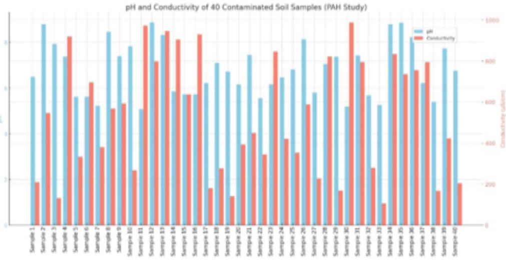

Fig 1:- Physiochemical analysis of sample - pH and Conductivity

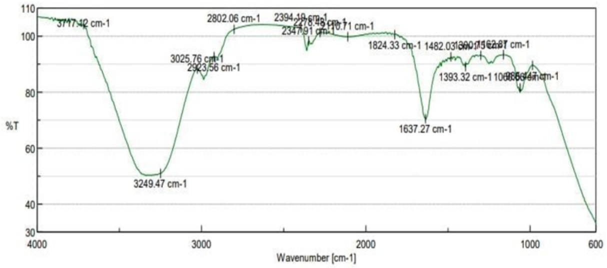

Fig 2: Sample 1

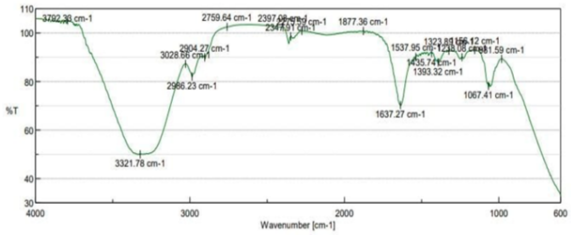

Fig 3: Sample 2

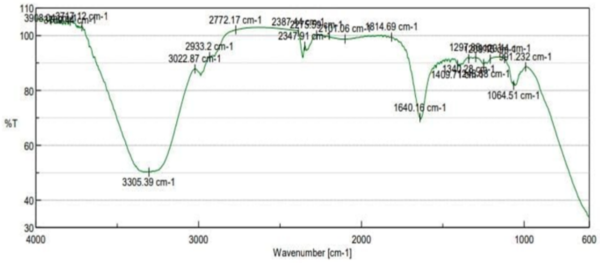

Fig 4: Sample 3

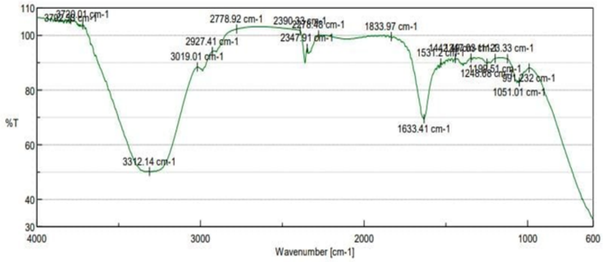

Fig 5: Sample 4

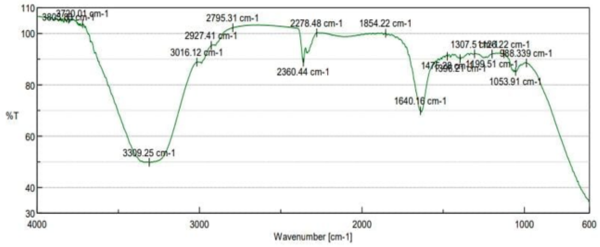

Fig 6: Sample 5

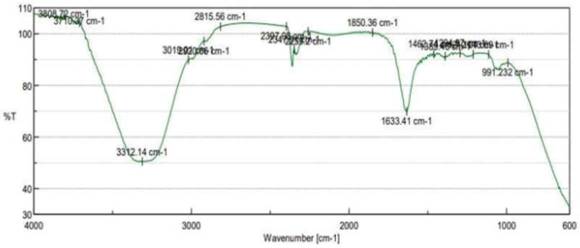

Fig 7: Sample 6

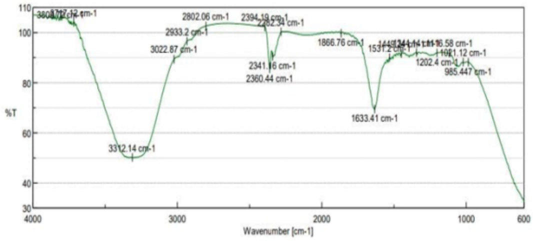

Fig 8: Sample 7

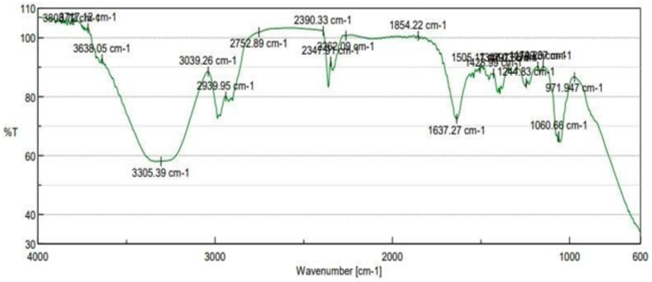

Fig 9: Sample 8