

Surfactant Production by Epiphytic Bacteria, *Lysinibacillus Sphaericus* Bibi1 and *Proteus Mirabilis* Strain Bibi8 and their Potential for Hydrocarbon Degradation

Oso, Simisola Olabisi, Ashade, Ahmeed Olalekan, Fanu, Blessing Seide

Lagos State University, Lagos, Nigeria

DOI: <https://doi.org/10.51244/IJRSI.2025.12040079>

Received: 06 April 2025; Accepted: 12 April 2025; Published: 13 May 2025

ABSTRACT

In this study, the ability of *Lysinibacillus sphaericus* BiBi1 and *Proteus mirabilis* BiBi8 isolated from and identified using biochemical and 16S rRNA molecular typing from leaves of *Celosia argentea*, were tested for their ability to utilize diesel as sole carbon source and also produce surfactants. The growth dynamics of isolates on diesel over 13-day incubation on diesel mineral salt medium revealed that Strain BiBi1 grew exponentially from day 0 to Day 8, with growth reaching its peak at day 10. A gradual decrease in the pH of the medium was observed from day 0 to day 13. *Proteus mirabilis* had a steady growth rate on diesel from day 0 to day 10 where growth was at its peak on day 12. Bacterial strain BiBi1 and BiBi8 showed alpha and beta hemolysis on blood agar and also recorded E_{24} % value of 51.28 and 76.32 respectively. The surfactants produced were characterized using high performance liquid chromatography, *Lysinibacillus sphaericus* produced rhamnolipids and subtilin as its surface biomolecule whereas *Lysinibacillus sphaericus*, in addition to rhamnolipid also produced lipopeptides. The results obtained from this study shows that *Lysinibacillus sphaericus* strain BiBi1 and *Proteus mirabilis* strain BiBi8 have potential for remediation of hydrocarbon contaminated environments.

Keyword: Biodegradation; diesel; hydrocarbon; Enrichment; Surfactants

INTRODUCTION

Petroleum hydrocarbons are known to be composed of complex mixtures of alkanes, aromatics, asphaltenes, and resins (Abbasian *et al.*, 2015). They pose a threat and great hazard to life in terrestrial and aquatic environments (Ezeonu *et al.*, 2102, Uddin *et al.*, 2021). This is due to their persistent nature of remaining in the environment for a long period leading to an alteration in the physical and chemical properties of the soil and also, the microorganisms known to be indigenous to the soil (Abdel-Shafy and Mansour, 2016). Over the years, various methods such as chemical, physical, and thermal treatments have been employed to treat hydrocarbons. These methods have been proven to be expensive and also require secondary treatment of these hydrocarbons (Das and Chandran 2011; Bilal and Iqbal, 2020). Due to these reasons, it is important to find methods that are environmentally friendly and cost-effective when treating these recalcitrant hydrocarbons.

An example is the use of microorganisms in biodegradation which converts petroleum hydrocarbons to CO₂, H₂O, and biomass which are less harmful to the environment (Das and Chandran, 2011; Bala *et al.*, 2022). Biodegradation is a natural process that utilizes microorganisms to clean up environmental contaminants (Varjani and Upasani, 2017). During biodegradation, different microorganisms such as bacteria, fungi, protozoa, and algae utilize contaminants such as petroleum hydrocarbons as sources of carbon and energy. Bacteria are the commonly used ones and examples are *Pseudomonas*, *Sphingomonas*, *Rhodococcus*, *Micrococcus*, *Alcaligenes*, *Aeromicrobium*, *Flavobacterium*, *Bacillus* and *Williamsia* (Oso *et al.*, 2019, Imron *et al.*, 2020). Enzymes produced by microorganisms play an important role in the biodegradation process by breaking down the waste material into safe environmental compounds (Karigar and Rao, 2011). However, for biodegradation to be effective factors such as temperature, nutrients, and pH should be optimal to allow for the growth of microorganisms (Karamalidis *et al.*, 2010). The first step in the degradation of hydrocarbons by

microorganisms involves bacterial membrane oxygenase which requires direct contact with the substrate (Das and Chandran 2011; Xu *et al.*, 2018).

The bioavailability and concentration of the hydrocarbon and environmental factors are determinants of the successful degradation of hydrocarbons. The degradation of hydrocarbons using a mixed culture of microorganisms has also proven to be effective because they possess a pool of catabolic genes which are required for an accelerated rate of degradation of hydrocarbons that are not found in a pure culture. Therefore, a mixture of mixed cultures of microorganisms can lead to higher degradation rates of hydrocarbons (Janbandhu and Fulekar 2011, Abbasi *et al.*, 2017; D'ugo *et al.*, 2021).

The degradation of petroleum hydrocarbons is carried out by an enzyme system, attachment of microbial cells to the hydrocarbon, or by the production of surfactants (Das and Chandran 2011; Xu *et al.*, 2018). The use of surfactants to enhance the degradation of hydrocarbons by increasing the solubility of hydrocarbons which in turn increases the bioavailability of the hydrocarbons to the microorganisms has proven to be an effective method of hydrocarbon degradation (Oso *et al.*, 2019; Oso *et al.*, 2021). Surfactants are amphiphilic compounds known to reduce surface tension by accumulating at the interface of immiscible fluids therefore increasing the surface area of insoluble compounds leading to increase bioavailability and enhance hydrocarbon degradation (Li and Chen, 2009; Burch *et al.*, 2014).

The utilization of hydrocarbons by surfactant-producing bacteria as a degradation method however depends on the type of hydrocarbons, type of surfactants produced by the microorganisms, and the characteristics the microorganisms possess (Hua and Wang 2014; Sah *et al.*, 2022). The phyllo sphere, known to be the above-ground organs of plants is a habitat for a wide range of microorganisms such as bacteria, fungi, yeast, and oomycetes (Vorholt, 2012). Bacteria are the most abundant ranging from 10^{-4} – 10^{-5} bacteria per mm^2 . Examples of known bacteria on leaves are *Pseudomonas*, *Rhodococcus*, *Acinetobacter*, *Bacillus*, *Methyl bacterium*, *Pantoea*, *Sphingomonas*, etc (Remus-Emsermann and Schlechter, 2018).

Microorganisms in the phyllo sphere can produce surfactants that aid their effective survival and exploration of the phyllosphere e.g. syringafactin produced by *Pseudomonas syringae* (Krimm *et al.*, 2005; Burch *et al.*, 2014). Surfactants produced by these bacteria inhabiting the phyllosphere are useful in the areas of agriculture, cosmetics, pharmaceuticals, and the environment. These surfactants produced by bacteria in the phyllosphere aid the removal of organic compounds such as petroleum hydrocarbons by decreasing surface and interfacial tensions (Abdoli *et al.*, 2025, Patel *et al.*, 2021). The surfactant-producing bacteria in the phyllosphere possess hydrocarbon-degrading genes and are capable of degrading hydrocarbons (Xu *et al.* 2018). Though, it is not unknown if these epiphytic bacteria gain fitness by utilising the hydrocarbons present on plant leaf surfaces (Vorholt, 2012).

These leaves in the phyllosphere are covered by a hydrophobic cuticle which protects against biotic and abiotic stresses such as water loss reduction and UV radiation (Riederer and Schreiber 2001, Krajšek *et al.*, 2011, Zeisler and Schreiber 2016, Sivakumar *et al.*, 2020). The cuticle present on leaves is known to be a composite structure of phenolics, mineral elements, and polysaccharides cutin and cuticular waxes. Cuticular waxes are a polymer containing crosslinked very long aliphatics and soluble aliphatics (Pollard *et al.* 2008, Yeats and Rose 2013). It also acts as the primary interface for microorganisms on leaves and insect interaction (Riederer & Schreiber, 2001; Yeats *et al.*, 2012). Cuticles differ in composition, structure, and thickness depending on the type of plant (Ridge 1997; Barthlott *et al.*, 1998). A large percentage of these bacteria listed above inhabiting the leaf surface is known to be hydrocarbon degraders and might be able to utilize the waxes on the leaf surface as a carbon source. However, there is no evidence that these bacteria can utilize the waxes present on the leaf surface (Remus-Emsermann *et al.*, 2014; Schlechter *et al.*, 2019). These bacteria also perform many ecological roles such as fighting against plant diseases and host-specific pathogens for trigonistic functions in the microbiota (Bonaterra *et al.*, 2022). In this study, the ability of two bacterial strains, *Lysinibacillus sphaericus* strain BiBi1 and *Proteus mirabilis* strain BiBi8 isolated from *Celosia argentea* leaves to utilize hydrocarbon and produce surfactants were looked into extensively.

MATERIALS AND METHOD

Study site and Sample collection

The sample *Celosia argentea*, used for the study were collected from Lagos State University (LASU) farmland behind the Faculty of Education. The site has witnessed continuous farming practice for over three decades. It has a coordinate (Latitude 6°28'29" N and Longitude 3°11'52" E). The sites were selected among different farmland on campus due to its biological richness, accessibility, and the prominence of *Celosia argentea* as a plant species.

Isolation of diesel degrading bacteria

Diesel degrading bacteria were isolated from the sample (*Celosia argentea*) via continuous enrichment technique by aseptically punching out one-hundred plugs from 20 leaves (*Celosia argentea*) with the aid of cork borer, the plugs were washed in a sterile 20 ml normal physiological saline. Carbon free mineral salt medium (MSM) was prepared as prepared as described by Habe *et al.*, 2003. The MSM constituent l⁻¹ were 0.22 g/l Na₂HPO₄, 0.8 g/l KH₂PO₄, 3.0 g/l NH₄NO₃, 0.2 g/l MgSO₄·7H₂O, 0.05 g/l FeCl₃·6H₂O, 0.01 g/l CaCl₂·2H₂O and 0.05 g/l yeast extract and pH of the medium adjusted to 7.2. Trace elements solution (1 ml/L) were filter sterilized using membrane filter 0.45 µm and added aseptically to the medium as described by Bauchop and Elsden (1960), fungal growth was suppressed using 50 µg/ml nystatin. Forty-five (45) ml MSM was transferred into replicate 250 ml Erlenmeyer flask supplemented with 1 ml sterile diesel serving as sole carbon source, and 4 ml aliquot containing the washed cell were added. Incubation were done in the dark and flask subjected to constant agitation in an orbital shaker at room temperature (27 ± 2.0°C) for 14 days until there was growth (turbidity). Following 3 consecutive transfers at shorter intervals (7 to 14 days), hydrocarbon degraders were isolated by plating out the final flask on Luria-Bertani (LB) agar. The colonies that appeared were further purified by subculturing repeatedly on LB agar.

Growth of Isolates on diesel

Sets of flasks containing 50 ml carbon free mineral salt medium supplemented with 0.5 ml diesel (1%) were prepared. The test organism was inoculated into the flasks and, incubated at ambient temperature (27 ± 2°C) for 13-d under constant agitation in the shaker. The control flask containing mineral salt medium and diesel with heat killed cells. At intervals of 2-day flask were randomly selected and their cell density determined via optical density measurement (Beckman Coulter DU 720, Fullerton, CA) while entire content of flask sacrificed. The growth patterns of isolates were plotted using Prism version 5.0 (GraphPad Software, San Diego, CA).

Identification of Isolates

Bacterial isolates were identified using colony morphology, cellular and biochemical characteristics using the taxonomic scheme of Cowan and Steel's (Barrow and Feltham, 1995). The biochemical test carried out include Gram staining, catalase, oxidase, sugar utilization, indole, motility and hydrogen sulphide production. Biochemical characterization was complemented with 16 rRNA molecular typing.

Molecular identification of Isolates

Genomic DNA was extracted from the cultures on sterile broth using the Quick-DNATM Fungal/Bacterial Miniprep Kit (Zymo Research, Catalogue No. D6005). The 16S target region was amplified using One Taq® Quick-Load® 2X Master Mix (NEB, Catalogue No. M0486 with the primers 16S-27F forward AGAGTTTGATCMTGGCTCAG and 16S-1492R Reverse CGGTTACCTTGTTACGACTT. The PCR products were run on a gel and cleaned up enzymatically using the EXOSAP method. The extracted fragments were sequenced in the forward and reverse direction (Nimagen, BrilliantDye™ Terminator Cycle Sequencing Kit V3.1, BRD3-100/1000) and purified (Zymo Research, ZR-96 DNA Sequencing Clean-up Kit™, Catalogue No. D4050). The purified fragments were analyzed on the ABI 3500xl Genetic Analyzer (Applied Biosystems, ThermoFisher Scientific) for each reaction for every sample. BioEdit Sequence Alignment Editor

version 7.2.5 was used to analyze the .ab1 files generated by the ABI 3500XL Genetic Analyzer and results were obtained by a BLAST search (NCBI) (Stephen *et al.*, 1997). The 16S rRNA gene sequences were then deposited in the GenBank Database (accession number CP026120; CP053718).

Screening for Surface Active Biomolecules

Hemolysis test

The method described by Satpute *et al.*, 2008 was used to assay for the hemolytic activity of the isolates. Bacterial isolates were streaked on Luria-Bertani (LB) agar supplemented with 5% fresh sheep blood and incubated at $(27 \pm 2^\circ\text{C})$ for 48-72 h. Plates were observed for alpha, beta and gamma-hemolysis. Greenish clearing zones around the colonies indicated alpha-hemolysis, while complete clearing zone around the colonies indicated beta hemolysis. gamma-hemolysis was characterized by lack of changes around the colonies. Hemolysis was presumptive for surfactant production.

Emulsification index

The emulsification indices (E_{24}) was measured using the method described by Ilori *et al.*, 2005. surfactant activity is measured by adding 2ml of diesel to 2 ml of cell-free extract and vortexing at high speed for 2 minutes. Measurement was taken 24 hours later. The emulsification index(E_{24}) was determined as the percentage of height of the liquid column. The E_{24} was measured in three replicates for each surfactant (Schulz *et al.*, 1991). The surfactant content is correlated with E_{24} . Evaluating emulsification capacity is a basic screening method that is appropriate for the initial screening of surfactant generating microorganisms (Plaza *et al.*, 2005).

$$E_{24} = \frac{h_{\text{emulsion}}}{h_{\text{total}}} \times 100\%$$

Characterization of Surfactants

Using High performance liquid chromatography, surfactant samples were analyzed using HPLC system 1260LC syringe, filter 0.2cm:0.4mm equipped with proshell 120EC, C_{18} 4 micrometer, column gravity= $150 \times 4.6\text{mm}$ particle size 3-micrometer, machinery-nongel was used with DAD (VWD) diode array detector 257 nanometer, data acquisition was performed using chemo station software (Agilent Cooperation). Flow rate was set up at 0.700ml/min and the column oven temperature was set to 28°C . The mobile phase solution; Acetonitrile A and 0.1% formic acid in ultra-pure water B were used and running buffer. The method started with a ratio A:B from 70-30% and a linear gradient was applied to reach a ratio of 80:20% in 8 mins. The acetonitrile fraction was increased linearly from 80-100% between 9 and 10 mins and decreased linearly to 70% in 11 and 12.5 mins. The injection volume of sample was set at 20 μ l detected through a DAD (VWD) detector at 257nm. Each analysis was completed within 15mins.

RESULTS

The isolation of diesel degrading bacteria from the leaves of *Celosia argentea*, resulted in the isolation of two competent diesel degrading organisms (Table 1). The isolates were identified using biochemical and 16S rRNA molecular typing as *Lysinibacillus sphaericus* (strain Bibi1) and *Proteus mirabilis* (strain Bibi8)

Table 1: Biochemical characterization of isolates

Isolate code	Gram staining	Catalase	Indole	Simmon citrate	motility	oxidase	Hydrogen sulphide	Putative identity
BiBi1	+ve	+ve	-ve	+ve	+ve	+ve	+ve	<i>Lysinibacillus</i>

								<i>sphaericus</i>
Bibi8	-ve	+ve	-ve	+ve	+ve	-ve	+ve	<i>Proteus mirabilis</i>

The growth profile of the isolates on diesel as monitored via observation of physical turbidity and the disappearance of the diesel contaminant in the enrichment flask (Figure 1A and 1B). Strain BiBi1 grew exponentially from day 0 to Day 8 (OD = 0.7nm), whereas growth where at peak at Day 10 (OD = 0.8 nm). It was observed that a steady gradual decrease in the pH of the medium from 7.2 day 0 to 6.3 day 13. Also, *Proteus mirabilis* had a steady growth rate on diesel from day 0 to day 10 whereas growth was at its peak on day 12 (OD = 0.85 nm), acidic pH was also observed in the growth media with a steady fall in pH from 7.2 (day 0) to 6.2 (day 13).

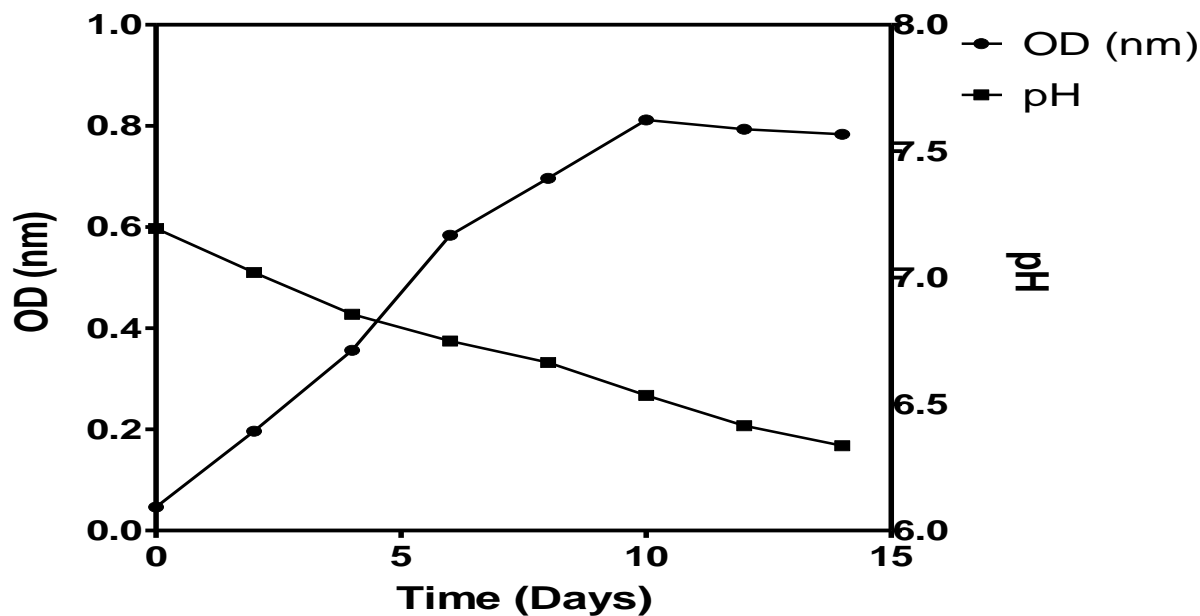


Figure 1A: Growth dynamics of *Lysinibacillus sphaericus* on diesel

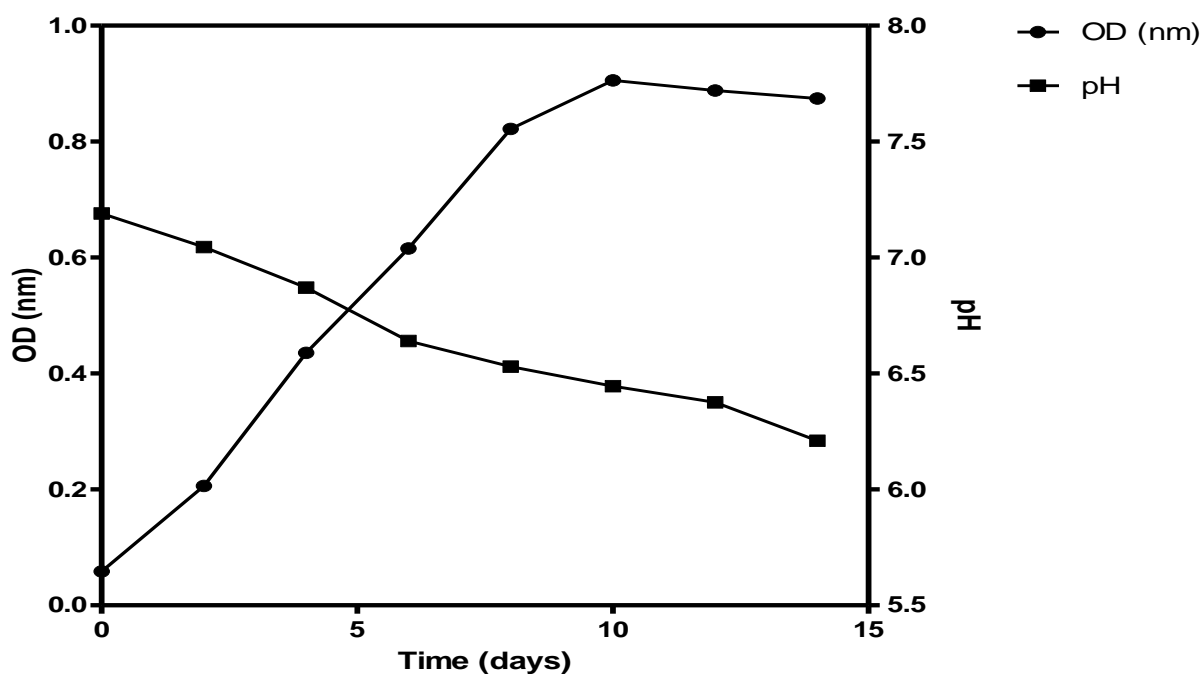


Figure 1B: growth dynamics of *Proteus mirabilis* on diesel

The potential of the isolates to produce surface active biomolecules were tested via hemolytic and E₂₄ test (Table 2). Strain BiBi1 showed incomplete zones of clearing on sheep blood agar which signify alpha hemolysis, whereas BiBi8 showed complete zone of clearing signifying beta hemolysis. Although hemolysis test is presumptive for biosurfactant production. Also, the emulsification index (E₂₄) test showed that strain BiBi1 had E₂₄ values of 51.28% whereas BiBi8 showed 76.32%. the values were remarkably high for a hydrocarbon utilizer since organism produced surface active molecule in response to degradation of hydrocarbon in the environment. It also implies that the organisms can be used to control the menace of hydrocarbon pollution in our local environment

Table 2: Biosurfactant producing capability of isolates

Isolate code	Hemolytic assay	Emulsification indices (E ₂₄)
BiBi1	Alpha hemolysis	51.28
BiBi8	Beta hemolysis	76.32

Following biosurfactant production, the nature of the surface-active biomolecule produced by diesel degrading isolates were characterized using the high-performance liquid chromatography (HPLC) (Figure 2A and 2B). *Lysinibacillus sphaericus* produced rhamnolipids and subtilisin as its surface biomolecule although rhamnolipid was produced more than the former. Also, the production of rhamnolipid by *Proteus mirabilis* was massive (Figure 2B) as compared to with *Lysinibacillus sphaericus*, in addition to rhamnolipid, lipopeptides was also produced by strain BiBi8.

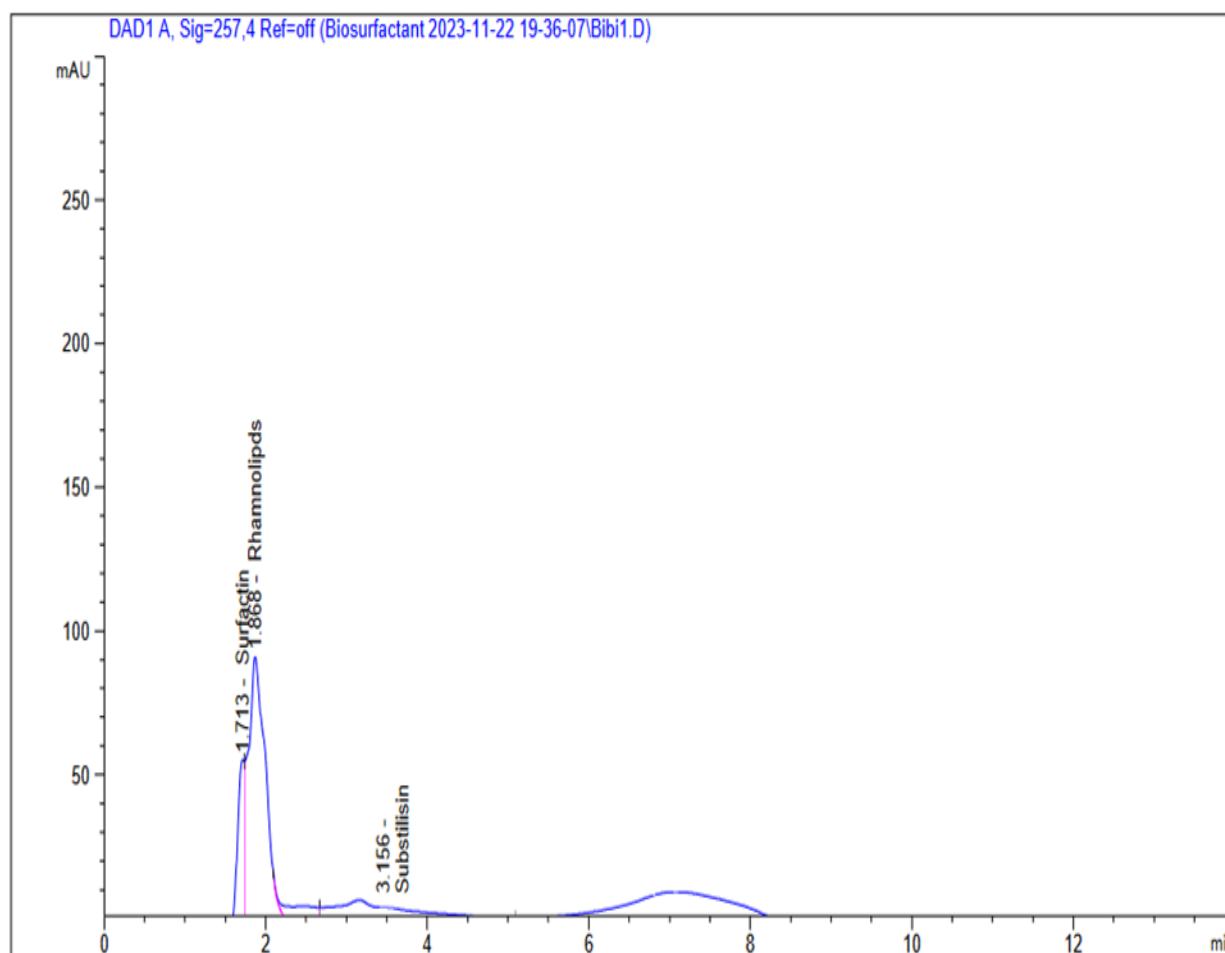


Figure 2A: HPLC profile of the Rhamnolipid and Subtilisin biomolecules produced by *Lysinibacillus sphaericus*

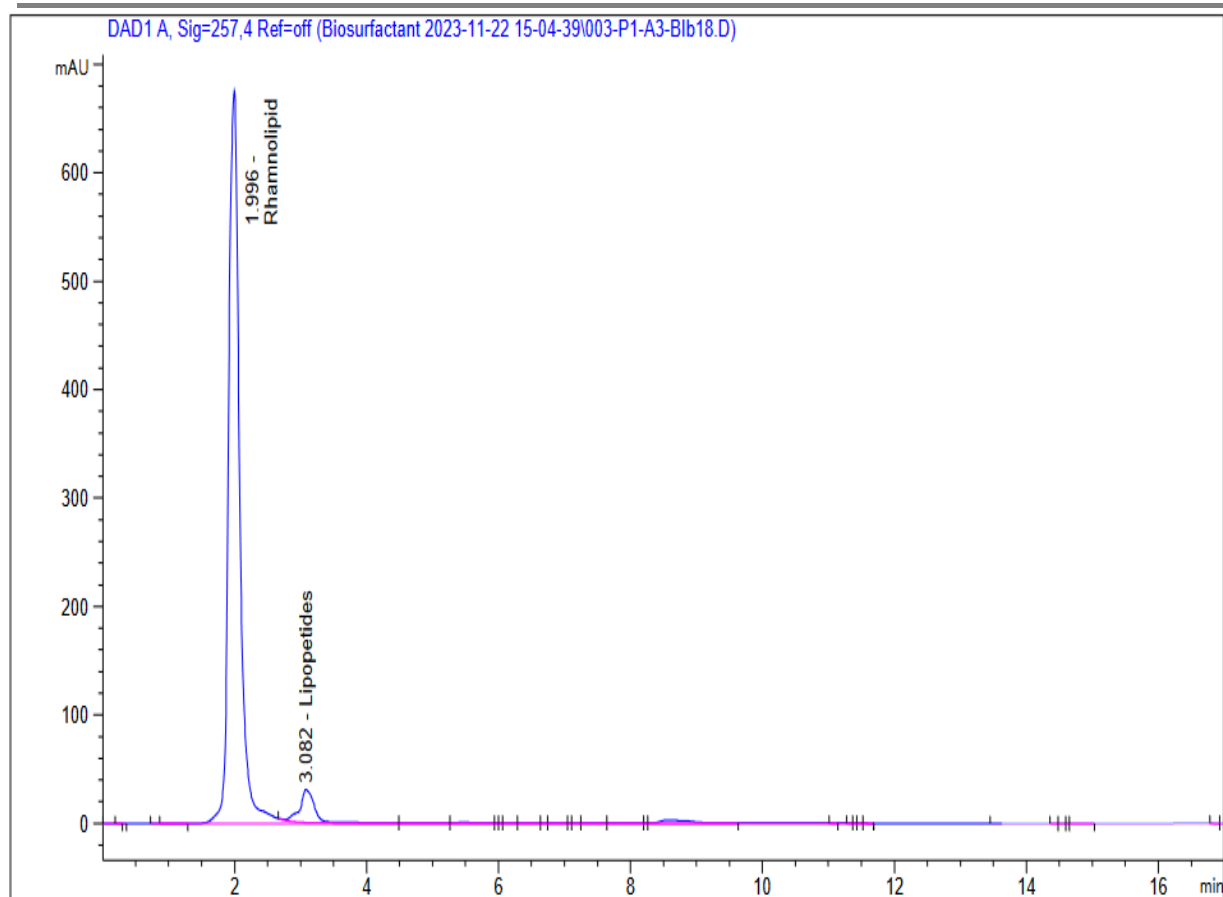


Figure 2B: HPLC profile of the rhamnolipid and lipopeptide biomolecules produced by *Proteus mirabilis*

RESULTS AND DISCUSSION

Studies over the years have confirmed that bacteria present in aquatic and terrestrial environments can be excellent hydrocarbon degraders. In recent times, there has been research looking into bacteria present on the leaf surface and their potentials to degrade hydrocarbons and produce surfactants. Biodegradation is known as a major mechanism for removing recalcitrant hydrocarbons in the environment. The organisms used in this study have been well studied with excellent results (Hernandez-Santana and Dussan, 2018; Ke *et al.*, 2021; Shettima *et al.*, 2023). This study further proves that phyllosphere bacteria are hydrocarbon utilizers and surfactant producers. In the study two diesel degrading bacterial identified using biochemical and molecular typing were *Lysinibacillus sphaericus* strain BiBi1 and *Proteus mirabilis* strain BiBi8 (Table 1). Isolating the bacterial strain showing good degradation capacity on diesel is not surprising as findings from this study corroborated previous studies when tested for their ability to utilize diesel as source of carbon and energy and also produce surfactants which will enhance their bioavailability to the hydrocarbon (Oso *et al.*, 2019; Oso *et al.*, 2021). The bacterial strains were first grown on LB agar which does not determine their hydrocarbon utilising ability. This study therefore establishes that bacteria present on *Celosia argentea* can be excellent hydrocarbon degraders which can aid the removal of this recalcitrant from the environment. Many bacteria have been studied extensively in degradation studies and surfactant production; Pseudomonads however remains one of the most studied bacteria. As observed in Burch *et al.*, 2014, the surfactant produced by *Pseudomonas syringae* B728a helped the organism thrive in the phyllosphere when the environment becomes unfavorable. This is achieved when the surfactant breaks the surface tension of water and lower the contact angle of water droplet on the leaf. This in turn increases the area where the water droplets cover on the leaf and allow for the movement of the bacteria and for nutrients to diffuse toward the bacteria. Surfactant production in the phyllosphere also increases wettability and permeability of leaf cuticle. This shows that surfactants on leaf surface are beneficial to epiphytic bacteria in the phyllosphere.

To test for surfactant production in *Lysinibacillus sphaericus* and *Proteus mirabilis*, two complementary assays were used i.e. drop collapse method and emulsification index (Ilori *et al.*, 2005; Oso *et al.*, 2019; Wu *et*

al., 2022). Both methods indicated the presence of surfactants in the bacterial strains. Although, the drop collapse assay proved to be a faster detection method in comparison to emulsification index which supports studies from Burch *et al.*, 2014 and Oso *et al.*, 2019 had previously studied surfactant production in phyllosphere bacteria isolated from spinach and romaine lettuce (Fig 2A and 2B, Table 2). In terms of hydrocarbon utilization, both strains showed to be hydrocarbon utilizers when grown in MSM supplemented with diesel while no growth was observed in the control flasks which contained no carbon source. This further confirms that these bacterial strains are hydrocarbon utilizers as carbon source and surfactant producers based on previous studies which also show the presence of alkane degrading genes *alkB* in trees found in urban areas (Gandofi *et al.*, 2017). The results obtained broadens the understanding of the presence of hydrocarbon utilizers in the phyllosphere and can contribute to the creation of an effective biodegradation programme, thereby contributing to a greener environment.

CONCLUSION

The phyllosphere has been proven to be an excellent habitat for hydrocarbon utilizers and surfactant producing bacterial strains. The strain used in this study were able to utilize bacteria and also produce surfactants. It is however unclear if these strains are able to utilize the long chain aliphatics present on the cuticular waxes. This will be looked into in the future. A thorough investigation into biodegradation studies will also be carried out to determine what components of diesel the bacterial strains able to degrade.

REFERENCES

1. Abbasi M. P., Nasrabadi T., Wu W., Al Dianty M. (2017). Evaluation of Oil Pollution Dispersion in an Unsaturated Sandy Soil Environment Pollution, 3 (4), 701-711.
2. Abbasian F., Lockington R., Mallavarapu M., Naidu R. (2015). A Comprehensive Review of Aliphatic Hydrocarbon Biodegradation by Bacteria. Applied Biochemistry and Biotechnology. 176 (3), 670-699.
3. Abdel-Shafy H.I., Mansour M.S.M. (2016). A Review on Polycyclic Aromatic Hydrocarbons: Source, Impact, Effect on Human Health and Remediation. Egyptian Journal of Petroleum. 25(1), 107-123.
4. Abdoli S., Asgari Lajayer B., Bagheri Novair S., Price, G. W. (2025). Unlocking the Potential of Biosurfactants in Agriculture: Novel Applications and Future Directions. Sustainability, 17(5), 2110.
5. Bala S., Garg D., Thirumalesh B. V., Sharma, M., Sridhar K., Inbaraj, B. S., Tripathi, M. (2022). Recent Strategies for Bioremediation of Emerging Pollutants: A Review for a Green and Sustainable Environment. Toxics, 10(8), 484.
6. Bauchop T., Elsdon S.R. (1960). The growth of microorganisms in relation to their energy. Journal of General Microbiology 23,457-459.
7. Bonaterra A., Badosa E., Daranas N., Francés J., Roselló G., Montesinos E. Bacteria as Biological Control. 2022. A, Agents of Plant Diseases. Microorganisms. 10(9),1759.
8. Barthlott W., Christoph N., David C., Friedrich D., Iris M., Inge T., Wilhelmi H. (1998). Classification and Terminology of Plant Epicuticular Waxes. 126 (3), 237-260.
9. Bilal M., Iqbal H. M. N. (2000). Microbial Bioremediation as a Robust Process to Mitigate Pollutants of Environmental Concern. Case Studies in Chemical and Environmental Engineering. 2,1-4.
10. Burch A.Y., Zeisler V., Yokota K., Schreiber L., Lindow S.E. (2014). The Hygroscopic Biosurfactant Syringafactin Produced by *Pseudomonas syringae* Enhances Fitness on Leaf Surfaces During Fluctuating Humidity. Environmental Microbiology. 16,2086-98.
11. Das N., Chandran P. (2011). Microbial Degradation of Petroleum Hydrocarbon Contaminants: An Overview. Biotechnology Research International. Article ID 941810.
12. D'ugo E, Bruno M., Mukherjee A., Chattopadhyay D., Giuseppetti, R., De Pace R., Magurano F. (2021). Characterization of Microbial Response to Petroleum Hydrocarbon Contamination in a Lacustrine Ecosystem. Environmental Science and Pollution Research. 28, 26187-26196.
13. Ezeonu C.S., Tagbo R., Anike E.N., Oje O.A., Onwurah I.N., (2012). Biotechnological Tools for Environmental Sustainability: Prospects and Challenges for Environments in Nigeria—a Standard Review. Biotechnology Research International. 1-26.

14. Gandolfi I., Canedoli C., Imperato V., Tagliaferri I., Gkorezis P., Vangronsveld J., Padoa Schioppa E., Papacchini M., Bestetti G., Franzetti A. Diversity and hydrocarbon-degrading potential of epiphytic microbial communities on *Platanus x acerifolia* leaves in an urban area. *Environmental Pollution*. 220, 650–8.
15. Hernández-Santana A., Dussán, J. (2018). *Lysinibacillus sphaericus* proved to have potential for the remediation of petroleum hydrocarbons. *Soil and Sediment Contamination: An International Journal*. 27(6), 538–549.
16. Hua F, Hong Q. W. (2014). Uptake and Trans-Membrane Transport of Petroleum Hydrocarbons by Microorganisms. *Biotechnology, Biotechnological Equipment*. 28(2), 165–75.
17. Ilori M.O., Amobi C.J., Odocha A.C. (2005) Factors affecting biosurfactant production by oil degrading *Aeromonas* sp., isolated from a tropical environment. *Chemosphere* 61,985–992.
18. Imron M.F, Kurniawan S.B, Ismail, N.I, Abdullah S.R.S. (2020) Future Challenges in Diesel Biodegradation by Bacteria Isolates: a Review. *Journal of Cleaner Production*. 251, 119716.
19. Janbandhu A., M. H. Fulekar. (2011). Biodegradation of Phenanthrene Using Adapted Microbial Consortium Isolated from Petrochemical Contaminated Environment. *Journal of Hazardous Materials*.187(1-3), 333-340.
20. Karamalidis A.K., Evangelou A.C., Karabika E.; Koukkou A.I., Drinas C., Voudrias, E.A. (2010). Laboratory Scale Bioremediation of Petroleum-contaminated Soil by Indigenous Microorganisms and Added *Pseudomonas aeruginosa* strain Spet. *Bioresources Technology*. 101, 6545–6552.
21. Karigar C.S, Rao S.S. (2011). Role of Microbial Enzymes in the Bioremediation of Pollutants: a Review. *Enzyme Research*. 2011, 1-11.
22. Ke C., Chen L., Qin F., Sun W., Wang S., Zhang Q., Zhang X. (2021). Biotreatment of oil sludge containing hydrocarbons by *Proteus mirabilis* SB. *Environmental Technology & Innovation*. 23: 101654.
23. Krajšek, S. S., Kreft S., Kladnik A., Drašlar K., Jogan N., Dermastia M. (2011). Morphology and Glandular Activity of Unicellular Trichomes of *Epilobium hirsutum*. *Biologia Plantarum*. 55, 149-152.
24. Krimm U., Abanda-Nkpwatt D., Schwab W, Schreiber L. (2005). Epiphytic Microorganisms on Strawberry Plants (*Fragaria ananassa* cv. Elsanta): Identification of Bacterial Isolates and Analysis of their Interaction with Leaf surfaces. *FEMS Microbiology Ecology*. 53,483–492
25. Li J.L., Chen B.H. 2009. Surfactant mediated biodegradation of contaminated soil and water. *Materials*. 2,76-94
26. Oso S., Walters M., Schlechter R.O., and Remus-Emsermann, M. N. P. (2019). Utilization of Hydrocarbons and Production of Surfactants by Bacteria Isolated from Plant leaf Surfaces. *FEMS Microbiology Letters*. 366(6), 1-10.
27. Oso S, Fuchs F, Übermuth C, Zander L, Daunaraviciute S, Remus DM,Stötzel I, Wüst M, Schreiber L, Remus-Emsermann MNP. (2021). Biosurfactants Produced by Phyllosphere-Colonizing *Pseudomonads* Impact Diesel Degradation but Not Colonization of Leaves of Gnotobiotic *Arabidopsis thaliana*. *Applied and Environmental Microbiology*. 87(9),1-13
28. Patel P., Modi A., Minipara D., Kumar A. (2021). Microbial Biosurfactants in Management of Organic Waste. In *Sustainable Environmental Clean-up*. Amsterdam: Elsevier. 211-230
29. Plaza G.A., Ulfig K., Brigmon R.L. (2005). Surface active properties of bacterial strains isolated from petroleum hydrocarbon-bioremediated soil. *Polish Journal of Microbiology* 54,161–167.
30. Pollard M., Beisson F., Li Y., Ohlrogge J. B. (2008). Building Lipid Barriers: Biosynthesis of Cutin and Suberin. *Trends in Plant Science*. 13 (5), 236-46.
31. Remus-Emsermann M. N. P., Lückner S., Müller D. B., Potthoff E., Daims H., Vorholt J. (2014). Spatial Distribution Analyses of Natural Phyllosphere-colonizing Bacteria on *Arabidopsis thaliana* Revealed by Fluorescence in situ Hybridization. *Environmental Microbiology*. 16(7), 2329-2340.
32. Remus-Emsermann M. N. P., and R. O. Schlechter. (2018). Phyllosphere Microbiology: At the Interface between Microbial Individuals and the Plant Host. *New Phytologist*. 218, 1327-1333
33. Ridge I. 1997. Plant Cuticles: An Integrated Functional Approach. *The Journal of Agricultural Science*. 47, 50-60.
34. Riederer M., L. Schreiber. 2001. Protecting against Water Loss: Analysis of the Barrier Properties of Plant Cuticles. *Journal of Experimental Botany* 52 (363): 2023-32.

35. Sah D, Rai J.P.N., Ghosh A., Chakraborty M. (2022) A Review on Biosurfactant Producing Bacteria for Remediation of Petroleum Contaminated Soils. *Biotech.* 12(9),218.
36. Satpute S.K., Bhawsar B.D., Dhakephalkar P.K., Chopade B.A. (2008). Assessment of different screening methods for selecting biosurfactant producing marine bacteria. *Indian Journal of Marine Science.* 37,243–250.
37. Schlechter R. O., Miebach M., Remus-Emsermann M. N. P. (2019). Driving Factors of Epiphytic Bacterial Communities: A review. *Journal of Advertising Research.* 19, 57-65.
38. Schulz D., Passeri A., Schmidt M., Lang S., Wagner F., Wray V., Gunkel W. (1991). Marine biosurfactants, I. Screening for biosurfactants among crude oil degrading marine microorganisms from the North Sea. *Z. Naturforsch.* 46c, 197-203.
39. Shettima H., Sanda M. B., Musti N. Salamon I. A., a Ismail H. Y. (2023). Isolation and Characterization of Biosurfactant-producing Bacteria from Ternary Effluent within Maiduguri Metropolitan Council. *Asian Journal of Plant Biology.* 5(1), 37-42.
40. Varjani S. J., Upasani V.N. (2017). A New Look on Factors Affecting Microbial Degradation of Petroleum Hydrocarbon Pollutants. *International Biodeterioration & Biodegradation.* 120, 71-83.
41. Vorholt J.A. Microbial Life in the Phyllosphere (2012). *Nature Review.* 10,828–840.
42. Wu B., Xi, J., Yu L., Huang L., Yi L., Ma Y. (2022). Biosurfactant production by *Bacillus subtilis* SL and its potential for enhanced oil recovery in low permeability reservoirs. *Scientific Reports.* 12, 7785.
43. Xu X., W. Liu, S. Tian, W. Wang, Q. Qi, P. Jiang, X. Gao, F. Li, H. Li, and H. Yu. (2018). Petroleum Hydrocarbon-Degrading Bacteria for the Remediation of Oil Pollution Under Aerobic Conditions: A Perspective Analysis. *Frontiers in Microbiology.* 9,1-11.
44. Yeats T. H., Rose J. K. C. (2013). The Formation and Function of Plant Cuticles. *Plant Physiology.* 163(1),5-20.
45. Zeisler V., Schreiber L. (2016) Epicuticular Wax on Cherry Laurel (*Prunus laurocerasus*) Leaves does not Constitute the Cuticular Transpiration Barrier. *Planta.* 243, 65-81.