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Isolation and Screening of Biosurfactant-Producing Bacteria from Hydrocarbon-Contaminated Mechanic Workshop Soils in Sokoto Metropolis, Nigeria

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Abstract: **Hydrocarbon-contaminated soils are important reservoirs of biosurfactant-producing bacteria due to the selective pressure exerted by petroleum pollutants on indigenous microbial communities. Biosurfactants are surface-active compounds produced by microorganisms and have gained considerable attention because of their applications in environmental remediation and various industrial processes. This study aimed to isolate and screen biosurfactant-producing bacteria from hydrocarbon-contaminated soils collected from automobile mechanic workshops in Sokoto Metropolis, Nigeria. Soil samples were collected from three contaminated sites and analyzed using standard physicochemical and microbiological methods. Aerobic heterotrophic bacterial counts ranged from 2.5×10^7 to 1.38×10^8 CFU/g across the sampling locations. A total of sixteen bacterial isolates were recovered and identified as Pseudomonas aeruginosa, Bacillus subtilis, Bacillus pumilus and Staphylococcus epidermidis. Biosurfactant production was screened using haemolysis, drop collapse and oil displacement assays, while emulsification activity was used to evaluate selected isolates. Eight isolates showed positive haemolytic activity, five were positive for the drop collapse test and eight exhibited oil displacement activity. Among the identified isolates, Pseudomonas aeruginosa and Bacillus subtilis demonstrated the strongest biosurfactant-producing potential based on their performance in the screening assays and emulsification activity. The findings indicate that hydrocarbon-contaminated soils in Sokoto Metropolis harbour indigenous bacteria with promising potential for biosurfactant production and possible application in bioremediation of petroleum-contaminated environments. Further studies on molecular characterization and optimization of biosurfactant production are recommended.**

Keywords: **Biosurfactants; hydrocarbon-contaminated soil;** ***Pseudomonas aeruginosa; Bacillus subtilis*****; bioremediation.**

I. INTRODUCTION

Biosurfactants are surface-active biomolecules synthesized by microorganisms and are increasingly attracting attention because of their environmental compatibility and industrial applicability. These compounds possess both hydrophilic and hydrophobic moieties, enabling them to reduce surface and interfacial tension between different phases. Based on their chemical composition, biosurfactants are classified into glycolipids, lipopeptides, lipoproteins, phospholipids, lipopolysaccharides and polymeric surfactants (Ekprasert et al., 2020). Microorganisms such as bacteria, yeasts and fungi are capable of producing a diverse range of biosurfactants with significant biological and industrial importance.

Among the various microbial biosurfactants studied, bacterial biosurfactants such as rhamnolipids, surfactin and lichenysin produced mainly by Pseudomonas and Bacillus species have received considerable scientific attention due to their high efficiency and broad applications (Brumano et al., 2016). These compounds possess several advantages over synthetic surfactants, including biodegradability, low toxicity, environmental compatibility, stability under extreme environmental

conditions and high specificity. They have demonstrated remarkable effectiveness in the remediation of petroleum hydrocarbon-contaminated soils and industrial effluents as well as in enhanced oil recovery processes (Luna et al., 2015).

Biosurfactants also play significant roles in several industrial sectors, including cosmetics, pharmaceuticals, food processing, polymerization and environmental biotechnology. Unlike synthetic surfactants commonly used in detergents and personal care products, biosurfactants are environmentally sustainable and less harmful to living organisms (Banat et al., 2010). According to Mulligan et al. (2005), biosurfactants can be grouped into glycolipids, phospholipids, lipopeptides and lipoproteins, fatty acids, neutral lipids, polymeric surfactants and particulate biosurfactants based on their structural and functional properties. Their structural diversity and multifunctional characteristics make them highly attractive for numerous biotechnological applications (Pacwa-Plociniczak & Zhang, 2011).

The functional properties of biosurfactants include emulsification, dispersion, solubilization, mobilization, wetting, foam formation and reduction of surface tension. These properties arise from their ability to accumulate at interfaces such as liquid-liquid, liquid-solid and liquid-gas boundaries (Satpute et al., 2017). Several fungal species, including Candida bombicola, Candida lipolytica, Candida ishiwadae, Candida batistae and Aspergillus ustus, are recognized producers of sophorolipid biosurfactants (Bhardwaj et al., 2013). Owing to their biodegradability and reduced toxicity, biosurfactants are preferred alternatives to synthetic surfactants in many environmental and industrial processes (Kaur et al., 2017).

Hydrocarbon pollutants derived from petroleum products represent one of the major environmental challenges globally. Industrial discharges, transportation activities, leakages and accidental oil spills contribute significantly to hydrocarbon contamination of soil and water environments. These contaminants adversely affect soil fertility, microbial ecology and groundwater quality, thereby posing serious ecological and public health concerns. Hydrocarbons are generally classified into alkanes, aromatic compounds, asphaltenes and resins, each exhibiting different levels of persistence and toxicity (Elgazali et al., 2023).

Previous studies have shown that hydrocarbon pollution is particularly severe in regions associated with intense industrial and petroleum-related activities. Conventional remediation methods such as incineration, chemical oxidation and landfilling are often expensive and may generate secondary environmental pollution. Consequently, there is increasing interest in environmentally sustainable remediation strategies such as bioremediation using biosurfactant-producing microorganisms (Liu et al., 2018). Hydrocarbon-contaminated soils provide favorable ecological niches for microorganisms capable of utilizing hydrocarbons as carbon sources while simultaneously producing biosurfactants that enhance hydrocarbon degradation.

Despite the increasing interest in microbial biosurfactants, there remains limited information on the diversity and biosurfactant-producing potential of indigenous bacterial species present in hydrocarbon-contaminated environments within Sokoto Metropolis, Nigeria. Therefore, this study was conducted to determine the physicochemical characteristics of hydrocarbon-contaminated soils and to isolate and screen biosurfactant-producing bacteria from contaminated environments. The study is expected to contribute to the development of environmentally sustainable biotechnological approaches for pollution control and environmental remediation.

II. MATERIALS AND METHODS

Study Area and Sample Collection

Hydrocarbon-contaminated soil samples were collected from selected automobile mechanic workshops within Sokoto Metropolis, Sokoto State, Nigeria, following standard environmental sampling procedures (American Public Health Association [APHA], 2017). Approximately 200 g of soil was collected in triplicate from each of three contaminated locations designated A, B and C. Samples were placed in sterile zip-lock bags, labelled appropriately and transported to the Microbiology Research Laboratory, Usmanu Danfodiyo University Sokoto, for analysis.

Physicochemical Analysis of Soil Samples

Soil pH

Soil pH was determined following the International Institute of Tropical Agriculture (IITA) protocol. Twenty grams of air-dried soil were mixed with 20 mL of distilled water and allowed to stand for 30 min with intermittent stirring. The pH meter was calibrated using a standard buffer solution (pH 7.0). The electrode was immersed in the suspension and readings were taken in triplicate.

Particle Size Distribution

Particle size distribution was determined using the Bouyoucos hydrometer method (Gee & Or, 2002). Soil samples were dispersed using sodium hexametaphosphate and hydrometer readings were taken at specified sedimentation intervals to determine sand, silt and clay fractions.

Organic Carbon

Organic carbon was determined using the Walkley–Black wet oxidation method as described in Nelson and Sommers (1996).

Total Nitrogen

Total nitrogen was determined using the Macro-Kjeldahl method (Juo, 1979). Soil samples were digested with concentrated sulfuric acid in the presence of a catalyst. Distillation and titration were performed and nitrogen content was calculated using standard procedures:

$$\text{Nitrogen (\%)} = \frac{\text{N} \times 0.014 \times \text{V}_\text{d} \times 10 \times 100}{\text{A} \times \text{W}} \quad (1)$$

Where N is normality of acid, V_d is volume of digest A is aliquot of digest used for titration W is weight of soil sample and 0.014 is milliequivalent weight of nitrogen

Cation Exchange Capacity (CEC)

CEC was determined using the barium chloride saturation method (Abdallah and Ibrahim, 2023). Soil samples were equilibrated with barium chloride and magnesium sulfate solutions. Magnesium concentration in the filtrate was measured using Atomic Absorption Spectrophotometry and CEC was calculated accordingly.

$$\text{CEC (meq/100 g)} = \frac{(\text{C}_0 - \text{C}_1)\text{V}_2 \times 100}{\text{W}} \quad (2)$$

where C_0 is initial concentration or blank value, C_1 concentration of magnesium in extract, V_2 is volume of extract (L or appropriate unit) and W is weight of oven-dried soil

Microbiological Analysis

Bacterial Enumeration

Soil samples were serially diluted in sterile distilled water up to 10^{-7}. Aliquots (0.1 mL) from appropriate dilutions (10^{-5}, 10^{-6} and 10^{-7}) were inoculated onto Nutrient Agar (NA) plates using the spread plate technique and incubated at 30 °C for 24 h. Following incubation, bacterial colonies were counted and expressed as colony-forming units per gram (CFU/g) of soil (APHA, 2017).

Distinct colonies were selected and repeatedly subcultured on fresh Nutrient Agar plates to obtain pure cultures. Pure isolates were subsequently preserved on nutrient agar slants at 4 °C for further characterization and screening studies.

Isolation and Identification of Bacteria

Isolates were identified based on colonial morphology, Gram staining and biochemical tests. Morphological features such as colony shape, size, colour and elevation were recorded. Gram staining followed Harley and Prescott (2002). Biochemical tests included catalase, oxidase, citrate utilization, indole, methyl red, Voges–Proskauer, urease, starch hydrolysis and motility (Cheesbrough, 2006).

Biosurfactant Screening

Hemolytic activity was assessed using blood agar plates incubated at 30 °C for 24 h, based on zone formation around colonies

The drop collapse test was performed using cell-free supernatant on a hydrophobic surface. Spreading or collapse indicated positive activity. Oil displacement activity was assessed by adding crude oil to water followed by cell-free supernatant. Clear zone diameter was measured after 30 s.

The emulsification index (E24) was determined by mixing equal volumes of supernatant and hydrocarbon, vortexing for 2 min and standing for 24 h following Cooper and Goldenberg (1987):

$$E_{24}(\%) = \frac{h_{emulsion} \times 100}{h_{total}} \quad (3)$$

Where $h_{emulsion}$ is height of emulsified layer h_{total} is total height of liquid column

Biosurfactant Production

Selected isolates were grown in mineral salt medium supplemented with trace elements. The medium pH was adjusted to 7.2 and sterilized at 121 °C for 15 min. Flasks containing 100 mL medium were incubated at 30 °C for 7 days under aerobic conditions (Abouseoud et al., 2008).

Data Analysis

All experiments were conducted in triplicate and results were expressed as mean ± standard deviation. Descriptive statistical analysis was used to summarize the data obtained. Results were presented in tables and figures.

III. RESULT AND DISCUSSION

Table 1: Physicochemical Properties of Hydrocarbon-Contaminated Soil Samples

Parameter	Sample A	Sample B	Sample C
pH	6.95	6.96	6.99
EC (µS/cm)	141.8	187.1	67.9
Ca (cmol/kg)	0.75	0.65	0.55
Mg (cmol/kg)	1.05	0.55	1.30
Na (cmol/kg)	0.48	0.57	0.65
K (cmol/kg)	0.58	0.65	0.88
CEC (cmol/kg)	2.20	2.00	2.40
Nitrogen (%)	0.07	0.07	0.16
Organic Carbon (%)	1.20	1.40	1.50
Sand (%)	91.7	93.7	95.6
Silt (%)	5.9	3.9	4.0
Clay (%)	2.4	2.4	0.4
Phosphate (mg/kg)	0.14	0.15	0.15

Key: EC = Electrical Conductivity; CEC = Cation Exchange Capacity.

The physicochemical characteristics of hydrocarbon-contaminated soil samples collected from mechanic workshops along Abdullahi Fodio Road, Sokoto, are presented in Table 1. The soil pH ranged from 6.95 to 6.99, indicating slightly acidic to near-neutral conditions suitable for bacterial growth and metabolic activities. Electrical conductivity varied among the sampling locations, with Sample B exhibiting the highest value (187.1 µS/cm), while Sample C recorded the lowest (67.9 µS/cm). The concentrations of exchangeable cations varied across samples. Magnesium ranged from 0.55 to 1.30 cmol/kg, while potassium ranged from 0.58 to 0.88 cmol/kg. The cation exchange capacity (CEC) ranged between 2.00 and 2.40 cmol/kg. Organic carbon content varied from 1.20% to 1.50%, while nitrogen content ranged from 0.07% to 0.16%. Particle size analysis revealed that sand constituted the dominant soil fraction (91.7-95.6%), indicating a sandy soil texture. Silt and clay fractions were relatively low. The predominance of sandy particles may enhance aeration and permeability, creating favourable conditions for microbial survival and hydrocarbon biodegradation.

Table 2. Aerobic Heterotrophic Bacterial Counts in Hydrocarbon-Contaminated Soil Samples

Sample	Mean Bacterial Count (CFU/g)
A	1.38×10^8
B	3.50×10^7
C	2.50×10^7

The aerobic heterotrophic bacterial counts obtained from the contaminated soils are presented in Table 2. Sample A recorded the highest bacterial population (1.38×10^8 CFU/g), followed by Sample B (3.5×10^7 CFU/g) and Sample C (2.5×10^7 CFU/g). The relatively high bacterial counts indicate the presence of active microbial communities adapted to hydrocarbon-contaminated environments.

Table 3. Frequency of Occurrence of Identified Bacterial Isolates from Hydrocarbon-Contaminated Soil

S/n	Identified isolates	Frequency of occurrence	% Frequency of occurrence
1	*Pseudomonas aeruginosa*	6	37.5
2	*Bacillus subtilis*	5	31.3
3	*Bacillus pumilus*	4	25.0
4	*Staphylococcus epidermidis*	1	6.2
	Total	16	100

A total of sixteen bacterial isolates were recovered and identified. Four bacterial species were identified, namely *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Bacillus pumilus* and *Staphylococcus epidermidis*. *Pseudomonas aeruginosa* was the most frequently occurring species (37.5%), followed by *Bacillus subtilis* (31.3%), *Bacillus pumilus* (25.0%) and *Staphylococcus epidermidis* (6.2%).

Table 4. Identified Bacterial Species and Biosurfactant Screening Results

Isolate Code	Identified Species	Haemolysis	Drop Collapse	Oil Displacement
A1	*Bacillus pumilus*	+	–	+
A2	*Bacillus pumilus*	+	–	+
A3	*Bacillus pumilus*	+	–	+
B1	*Bacillus subtilis*	+	+	+
B2	*Pseudomonas aeruginosa*	+	+	+
B3	*Pseudomonas aeruginosa*	+	+	+
C1	*Pseudomonas aeruginosa*	+	+	+
C3	*Bacillus subtilis*	+	+	+

Key: (+) Positive; (–) Negative.

Morphological and biochemical characterization resulted in the identification of four bacterial species (Table 3). Gram-positive spore-forming rods were identified as *Bacillus subtilis* and *Bacillus pumilus*, whereas Gram-negative rods were identified as *Pseudomonas aeruginosa*. The only coccoid isolate recovered was identified as *Staphylococcus epidermidis*. Biosurfactant screening showed that eight isolates were positive for haemolytic activity and oil displacement assays, while five isolates demonstrated positive drop collapse activity. Isolates C1 and C3 exhibited the strongest biosurfactant-producing potential, showing positive results in all screening assays and the highest emulsification activity.

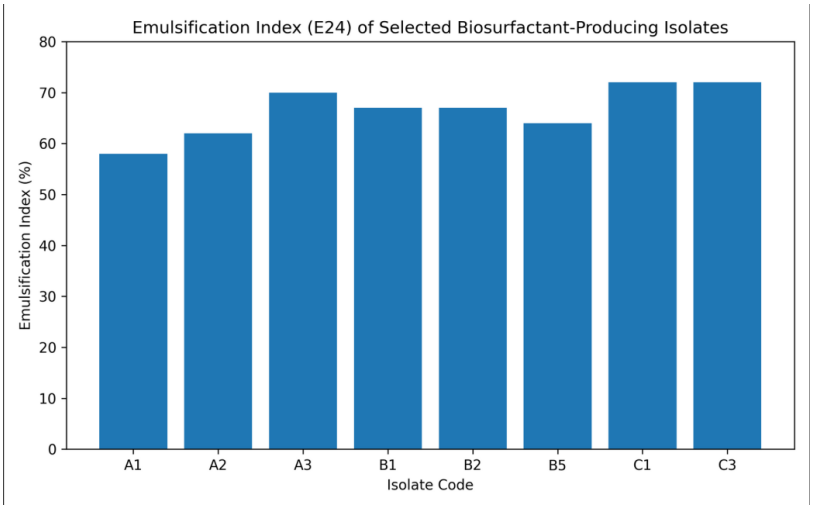

Figure 1. Emulsification Index (E_{24}) of Selected Biosurfactant-Producing Isolates

The emulsification index (E_{24}) of selected bacterial isolates is presented in Figure 1. Isolates C1 and C3 exhibited the highest emulsification activity (60%), indicating strong biosurfactant production capacity. In contrast, isolate A1 recorded the lowest emulsification activity (42%). The high emulsification indices observed among the selected isolates further confirm their potential for biosurfactant production and possible application in hydrocarbon bioremediation.

IV. CONCLUSION

This study investigated the physicochemical characteristics of hydrocarbon-contaminated soils from selected automobile mechanic workshops in Sokoto Metropolis and evaluated the biosurfactant-producing potential of indigenous bacterial isolates. The soils were characterized by slightly acidic to near-neutral pH, low cation exchange capacity, moderate organic carbon content and predominantly sandy texture, conditions that support the growth and activity of hydrocarbon-degrading microorganisms. A total of sixteen bacterial isolates were recovered and identified as Pseudomonas aeruginosa, Bacillus subtilis, Bacillus pumilus and Staphylococcus epidermidis. Pseudomonas aeruginosa was the most frequently occurring species among the isolates. Biosurfactant screening using haemolysis, drop collapse, oil displacement and emulsification assays revealed that several isolates possessed biosurfactant-producing capabilities. In particular, isolates identified as Pseudomonas aeruginosa and Bacillus subtilis exhibited the highest biosurfactant activity, demonstrating positive responses in all screening tests and high emulsification indices. The findings indicate that hydrocarbon-contaminated soils in mechanic workshop environments constitute important reservoirs of biosurfactant-producing bacteria with potential applications in environmental biotechnology. These indigenous microorganisms may be exploited for the development of cost-effective and environmentally friendly strategies for the remediation of petroleum-contaminated soils. Further studies involving molecular characterization, quantitative biosurfactant production and optimization of fermentation conditions, quantitative biosurfactant production and optimization of culture conditions are recommended to enhance their potential for large-scale bioremediation applications.

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