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Research Article

Isolation and Screening of Biosurfactant-Producing Bacteria from Hydrocarbon-Contaminated Mechanic Workshop Soil in Samaru, Zaria, Nigeria

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ABSTRACT

Hydrocarbon contamination arising from automobile workshop activities poses significant environmental challenges and necessitates the development of sustainable remediation strategies. This study investigated the physicochemical characteristics of hydrocarbon-contaminated soil from a mechanic workshop in Samaru, Zaria, Nigeria, and evaluated the biosurfactant-producing potential of indigenous bacterial isolates. Physicochemical properties were determined using standard analytical methods. Bacteria were isolated and identified using morphological, microscopic, and biochemical tests. Screening for biosurfactant production was conducted using oil spread and drop collapse assays. Selected isolates were further evaluated for biosurfactant production in mineral salt medium supplemented with 2% (v/v) engine oil as the sole carbon source, and biosurfactant production was assessed through oil spreading and drop collapsed assay. The soil exhibited sandy-loam characteristics with mean values of 77.18 ± 0.06% sand, 12.32 ± 0.03% silt, and 10.57± 0.02% clay. The mean soil temperature, pH, moisture content, CEC, and EC were 28.45 ± 0.21°C, 6.07 ± 0.04, 8.25 ± 0.07%, 9.70 ± 0.28 meq/100 g, and 101.5 µS/cm, respectively. Seven bacterial isolates belonging to five genera were identified; *Bacillus* spp., *Klebsiella* spp., *Pseudomonas* spp., *Proteus* spp. and *Staphylococcus* spp. and three of them demonstrated positively for the biosurfactant production potential. *Bacillus* spp. produced 2.5±0.15 g/L of biosurfactant with an activity of 1.07 ± 0.21 cm oil displacement zone, *Pseudomonas* spp. produced 2.0±0.06 g/L of biosurfactant with an activity of 6.03 ± 0.15 cm oil displacement zone and *Klebsiella* spp. produced 1.1±0.12 g/L with 3.10 ± 0.26 cm displacement zone. The findings indicate that hydrocarbon-contaminated mechanic workshop soils harbor indigenous biosurfactant-producing bacteria with potential application in bioremediation.

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1.0 INTRODUCTION

Petroleum hydrocarbons are major environmental pollutants arising from industrial activities, transportation, petroleum exploration, and automobile maintenance. Mechanic workshops, particularly in developing countries, represent significant sources of hydrocarbon contamination due to the improper disposal of used engine oil, lubricants, and other petroleum products. The continuous accumulation of these contaminants in soils negatively affects physicochemical properties, reduces agricultural productivity, disrupts microbial communities, and poses risks to both human and environmental health (Ameh et al., 2011; Das & Chandran, 2011).

Bioremediation has gained recognition as an environmentally friendly and cost-effective strategy for restoring hydrocarbon-contaminated environments. A key component of this approach is the use of biosurfactants—amphiphilic, surface-active compounds produced by microorganisms. Biosurfactants enhance the bioavailability of hydrophobic pollutants by reducing surface and interfacial tension, emulsifying hydrocarbons, and facilitating their mobilization and biodegradation (Georgiou et al., 1992; Banerjee et al., 2022; Sarubbo et al., 2022). Unlike synthetic surfactants, biosurfactants are biodegradable, less toxic, biocompatible, and effective under a wide range of environmental conditions, including extreme pH, temperature, and salinity (Bhadra et al., 2022). Their applications extend beyond environmental remediation to agriculture, pharmaceuticals, cosmetics, food processing, and enhanced oil recovery (Barbosa et al., 2020). In addition, they can be produced from renewable and inexpensive substrates such as vegetable oils, agricultural wastes, dairy by-products, and industrial effluents, making them economically sustainable (Pinto et al., 2022).

Microorganisms produce biosurfactants as secondary metabolites that aid nutrient acquisition, motility, biofilm formation, and adaptation to hydrophobic environments. Biosurfactants are generally classified into glycolipids, lipopeptides, fatty acid derivatives, and polymeric compounds, with glycolipids and lipopeptides being the most extensively studied because of their strong surface activity and industrial relevance (Gayathiri et al., 2022; Ingsel et al., 2022). Biosurfactant-producing bacteria and yeasts enhance hydrocarbon degradation by increasing the accessibility of insoluble hydrocarbons for microbial metabolism (Gayathiri et al., 2022).

Several bacterial genera, including *Bacillus*, *Pseudomonas*, *Klebsiella*, and *Acinetobacter*, have demonstrated significant biosurfactant-producing and hydrocarbon-degrading capabilities (Das & Chandran, 2011; Sarubbo et al., 2022). *Bacillus* species are particularly important because of their metabolic versatility, endospore-forming ability, and production of potent lipopeptide biosurfactants such as surfactin, fengycin, and iturin.

Despite increasing interest in biosurfactant-mediated bioremediation, limited information exists on indigenous biosurfactant-producing microorganisms from hydrocarbon-contaminated soils in Northern Nigeria. Mechanic workshop soils around Samaru, Zaria, provide selective environments for hydrocarbon-tolerant microbes that may possess valuable bioremediation potential. Consequently, this study evaluated the biosurfactant-producing potential of indigenous bacterial isolates from contaminated mechanic workshop soil by determining soil physicochemical properties, isolating and identifying bacterial species, screening for biosurfactant production, and assessing biosurfactant production in engine oil-supplemented liquid media (Ameh et al., 2011; Sarubbo et al., 2022).

2. MATERIALS AND METHODS

2.1 Study Area and Sample Collection

Soil samples were collected from a hydrocarbon-contaminated automobile workshop located near the North Gate of Ahmadu Bello University (ABU), Samaru Campus, Zaria, Kaduna State, Nigeria (Latitude 11°09′41.07″N; Longitude 7°38′39.03″E). The area experiences intensive automobile repair activities with

frequent oil spillages and lies within the Guinea Savannah ecological zone.

Composite soil samples were obtained from three contaminated points at a depth of 0–10 cm using sterile sampling tools. Samples were homogenized, transferred into sterile polyethylene bags, labeled appropriately, and transported to the Microbiology Laboratory, Ahmadu Bello University, for analysis.

2.2 Physicochemical Analysis of Soil

Soil samples were air-dried, crushed gently with a mortar and pestle, and passed through a 2-mm sieve and analyzed for texture, moisture content, pH, cation exchange capacity, and electrical conductivity using standard procedures described by Adeleke et al. (2021) and Ogunbanwo and Adejumo (2023).

2.2.1 Soil Temperature: Temperature measurements were obtained in situ using a calibrated digital soil thermometer inserted to a depth of 10 cm during soil sampling at the field

2.2.2 Soil Texture: Soil texture was determined using the hydrometer method as described by Adeleke et al. (2021). Aggregates were dispersed in 5% sodium hexametaphosphate solution and sedimentation readings were obtained using an ASTM 152H hydrometer. Percentages of sand, silt, and clay were calculated and classified using the USDA textural triangle.

2.2.3 Moisture Content: Moisture content was determined gravimetrically by oven-drying soil samples at 105°C to constant weight.

2.2.4 Soil pH: A 1:2.5 soil-to-water suspension was prepared and analyzed using a calibrated pH meter.

2.2.5 Cation Exchange Capacity: CEC was determined using the ammonium acetate saturation method followed by ammonium displacement and quantification.

2.2.6 Electrical Conductivity: Electrical conductivity was measured in saturated soil paste extracts using a conductivity meter at 25°C.

2.3 Isolation and Identification of the Bacteria from Mechanic Workshop Soil.

2.3.1 Nutrient Agar (NA) Preparation

Commercially available Nutrient Agar (Oxoid, UK) was prepared following the manufacturer's instructions. The required amount of dehydrated medium was suspended in distilled water, mixed thoroughly, and sterilized by autoclaving at 121 °C for 15 minutes. The sterile medium was cooled to 45–50 °C, dispensed aseptically into sterile Petri dishes, and allowed to solidify before serial dilution and inoculation (Cheesbrough, 2006).

2.3.2 Serial Dilution

The sterile test tube was labeled with different dilution factor of 10^{-1} to 10^{-5}.Twenty-five gram (25g) of the soil sample was weighed and aseptically transferred into a sterile test tube containing 225mL of sterile distilled water and thoroughly mixed using a vortex mixer to obtain a homogeneous suspension. A series of 10-fold serial dilutions (up to 10^{-5}) was prepared by transferring 1 mL of the initial suspension into a fresh tube containing 9 mL of sterile distilled water. This process was repeated sequentially for subsequent dilutions, ensuring the reduction of microbial load and facilitating bacterial enumeration and isolation. (Ogbonna & Inana, 2018).

2.3.3 Inoculation and Incubation

A 0.1 mL from the 10^{-2}, 10^{-3}, and 10^{-4} dilutions was aseptically inoculated onto sterile Nutrients agar plates. The spread plate technique was employed using a sterile bent glass rod to evenly distribute the inoculum across the agar surface. The plates were incubated in an inverted position at 37°C for 3–5 days under aerobic conditions to allow for the growth of hydrocarbon degrading bacteria (Satpute *et al.*, 2010). Distinct colonies were purified and characterized based on colony morphology, Gram reaction, and biochemical properties including catalase, oxidase, citrate utilization, methyl red, Voges–Proskauer, indole, motility, urease, starch hydrolysis, sugar fermentation, and Triple Sugar Iron (TSI) tests.

2.4 Gram Staining

Gram staining was performed to differentiate bacterial isolates into Gram-positive and Gram-negative groups (Rivenson *et al*., 2023). A thin smear of bacterial was prepared on a glass slide, heat-fixed, and sequentially stained with crystal violet for 60 seconds, followed by Lugol's iodine for 60 seconds. The smear was then decolorized with 95% ethanol for 10–15 seconds and counterstained with safranin for 30 seconds. After air drying, the stained slide was observed under an oil immersion microscope at 100× magnification. Gram positive bacteria appeared purple, while Gram-negative bacteria appeared red (Bergey & Holt, 2000)

2.5 Biochemical Tests

It was performed according to (Ravi & Praveen, 2016).

2.5.1 Motility Test

The bacterial culture was inoculated into the motility medium by stabbing the needle into the agar, then incubate at 37°C for 1 to 2 days. Motility was identified by diffused growth radiating outward from the stab line while growth along the stab-line was indicated non-motility

2.5.2 Indole Test

The bacterial culture was inoculated into tryptone broth or peptone water and the broth was incubated at 37°C for 1 to 2 days, a positive result was indicated by a cherry-red ring after the addition of Kovac's reagent while no color change indicates indole-negative.

2.5.3 Methyl Red Test

The bacterial culture was inoculated into the MR-VP broth (Buffered Peptone Glucose Broth), then incubate at 37°C for 1 to 2 days. Methyl red indicator of 4 drops was added to the broth, a red color indicated stable acid production, while yellow indicated a negative result.

2.5.4 Voges-Proskauer Test

The bacterial culture was inoculated into the MR-VP broth (Buffered Peptone Glucose Broth), then incubate at 37°C for 1 to 2 days. Barritt's reagents A (α-naphthol) and B (KOH) each of 4 drops was added to the broth, a red or pink coloration after adding reagents was confirmed acetoin production, a precursor to 2,3-butanediol.

2.5.5 Citrate Utilization Test

The bacterial culture was inoculated onto the Simmons' Citrate Agar, then incubate at 37°C for 1 to 2 days. A blue color change indicated citrate utilization (alkaline reaction) and also growth on the agar.

2.5.6 Catalase Test

A drop of hydrogen peroxide was placed on a sterile glass slide, then a small amount of bacteria isolate will be added to the hydrogen peroxide (3%). Oxygen bubbles after adding bacteria isolate indicated catalase activity.

2.5.7 Oxidase Test

The oxidase test strip was moistened with distilled water, then a small amount of the isolate was added to the strip. A blue-purple coloration confirmed a positive result.

2.5.8 Sugar Fermentation Test

The bacterial culture was inoculated into the sugar fermentation broth, then the broth was incubated at 37°C for 1 to 2 days. A color change from red to yellow indicated acid production due to pH change, while gas bubbles was confirmed as gas production in the Durham tube.

2.5.9 Starch Hydrolysis Test

The bacterial culture was inoculated onto the starch broth, then the broth was incubated at 37°C for 1 to 2 days, then iodine solution was added to the broth. A clear brown zone around bacterial growth indicated starch hydrolysis while blue-black color indicates hydrolysis-negative.

2.5.10 Triple Sugar Iron Agar (TSIA) Test

The bacterial culture was inoculated onto the TSI agar slant by stabbing the butt and streaking the slant, then the TSI agar slant was incubated at 37°C for 1 to 2 days. Results were interpreted based on color changes, slant color: (Red-no fermentation, Yellow-fermentation,), butt color (Red- no fermentation, Yellow-fermentation), gas production due to cracks or bubbles, and hydrogen sulfide (H_2S) precipitation due to blackening of the agar.

2.5.11 Preservation of the Isolates

Pure cultures were preserved on nutrient agar slants and stored at 4°C for further biosurfactant production studies (Fracchia *et al.,* 2012).

2.6 Screening for Biosurfactant Production

Biosurfactant-producing bacteria were screened using oil spread and drop collapse assays as described by Satpute et al. (2010) and Yadav et al. (2021).

2.6.1 Drop Collapse Test: A drop of Engine oil (2µL) was placed on a grease free slide and 5µL drop of the cell free supernatant was also placed at the center of the drop using a micropipette. The shape of the drop on the oil surface was observed for flattening and the collapse of the oil was observed after 1 minute under a light source and recorded.

2.6.2 Oil spreading method: A 40mL of distilled water was added to a Petri dish followed by the addition of 100µL of Engine oil using a micropipette on the surface of the water. Then one drop of the cell-culture broth was put on the Engine oil surface using a sterile syringe. The diameter of the clear zone on the oil surface was measured using a meter rule. The time taken to achieve the spread was also noted.

2.6.3 Emulsification capacity test: Emulsification activity of the biosurfactant was carried out. Four ml (4mL) of Engine oil was added to an equal amount of cell-free supernatant in a centrifuging tube and was vortexed at 5000 r.p.m for 10 min. After 24hours, the height of the stable emulsion layer was measured using a meter rule. The emulsification layer was calculated as the ratio of the height of the emulsion layer and the total height of the liquid.

2.7 Biosurfactant production

The Engine oil used in the research was obtained from NNPC Filling Station Samaru (Zaria, Nigeria) and was used throughout the study. Out of the five isolated Bacteria, three were used for Biosurfactant production using Mineral salt medium (MSM) which was the medium for the experiment. The medium was prepared by dissolving 0.5g Potassium phosphate (KH_2PO_4), 0.2g Sodium phosphate (Na_2HPO_4), 0.2g $FeSO_4$, 1.0g of Sodium chloride (Nacl), 0.2g Magnesium sulphate ($MgSO_4$), 0.5g Calcium chloride ($CaCl_2$) in a 250ml conical flask containing 200ml of distilled water. The preparations were sterilized and supplemented with 4ml of petroleum Engine oil as the carbon source, 0.5 gram of yeast extract as nitrogen source and fused with cotton wool. The prepared media were inoculated with the test organisms each and incubated in an incubator shaker for 7 days. The culture broths were centrifuged using centrifuging tubes at 5000 rpm for 10 minutes to obtain cell-free supernatants. The activities of biosurfactants produced were assessed using the oil spreading assay, where significant clear zones recorded, indicating high surface activity while control show no zone formation. The results are reported in terms of biosurfactant produced in gram per millimeter (g/mL) and the activities of the biosurfactants produced using Oil Spread Assay techniques.

2.7.1 Extraction and quantification of biosurfactant

The biosurfactant produced was extracted from the cell-free culture using the acid precipitation followed by solvent extraction, as described by Thavasi *et al.* (2007) and modified by Sena et al. (2018).

2.7.1.1 Acid Precipitation: This is done by lowering the pH of the cell-free broth to 2.0 using 6M HCl under constant stirring, followed by cold incubation at 4°C for 12-24 hours to precipitate the biosurfactant (Dubey and Juwakar 2001).The acidified broth was transferred into the centrifuging tubes and spin at 10,000 rpm for 20minutes therefore forming a pallet at the bottom. The precipitate was extracted with chloroform methanol (2:1 v/v) and evaporated using a rotatory evaporator at 45℃ to obtain the biosurfactant extract. The Crude biosurfactant dried, weighed and stored at 4℃ for characterization.

2.7.1.2 Determination of Biosurfactant Activity: A 10 µL drop of cell-free supernatants was placed on a thin layer of Engine oil spread over 20ml of distilled water in a petri dish, and the diameter of the clear zone was measured after 30 seconds (Nnabugwu et al 2023).

3. RESULT

The physicochemical properties of the contaminated soil are presented in Table 1. The soil was predominantly sandy-loam with

mean sand, silt, and clay contents of 77.18 ± 0.06%, 12.32 ± 0.03%, and 10.59 ± 0.02%, respectively. Soil pH was slightly acidic (6.07 ± 0.04), while temperature averaged 28.45 ± 0.21°C. pH, Moisture content, CEC, and EC were 6.07 ± 0.04, 8.25 ± 0.07%, 9.70 ± 0.28 meq/100 g, and 101.75 ± 0.35 µS/cm, Seven bacterial isolates coded A1 to A7 belonging to five genera were identified; *Bacillus* spp., *Klebsiella* spp., *Pseudomonas* spp., *Proteus* spp. and *Staphylococcus* spp. table 2. The frequency of occurrence of each of the bacteria isolated from soil samples; *Bacillus* spp. and *Pseudomonas* spp. had the highest percentage of occurrence with 28.57% each as shown in table 3.

The results for Screening of biosurfactants production using Oil spreading assay and Drop collapse assays were presented in Table 4 which shows that 3 out of 5 demonstrated positive biosurfactant activity, showing oil displacement zones ranging from 0.5±0.10 to 1.4±0.10 cm, and were also positive for drop collapsed assays and the most active isolates are *Pseudomonas* spp.

The result of biosurfactant production by the potent isolates are shown in Table 5. *Bacillus* spp. produced the highest quantity (2.5±0.15 g/L), followed by *Pseudomonas* spp. (2.0±0.06 g/L) and the least was produced by *Klebsiella* spp. (1.1±0.12 g/L) and the Activity of biosurfactants produced by *Bacillus* spp. was 1.07 ± 0.21 cm oil displacement zone, 3.10 ± 0.26 cm and 6.03 ± 0.15 cm oil displacement zone for *Klebsiella* spp. and *Pseudomonas* spp. respectively.

The results of the emulsification index (E24) values of the biosurfactants produced by the bacterial isolates was shown in Table 6 which shows that *Pseudomonas* spp. have the highest E24 values (47.73 ± 0.15%) indicating effective emulsification of kerosene.

Table 1. Physicochemical properties of mechanic workshop soil from Samaru, Zaria. (n=2)

Parameter	Mean ± SD
Temperature (°C)	28.45 ± 0.21
pH	6.07 ± 0.04
Moisture Content (%)	8.25 ± 0.07
CEC (meq/100 g)	9.70 ± 0.28
EC (µS/cm)	101.75 ± 0.35
Sand (%)	77.18 ± 0.06
Silt (%)	12.32 ± 0.03
Clay (%)	10.59 ± 0.02

Table 1: Morphological and Biochemical Characteristics of Bacteria Isolated from Mechanic Workshop Soil.

I/C	Gram reaction	Spore staining	CT	OX	CU	MR	VP	TSI	IN	H_2S	UR	MOL	Suspected organism
A1	+ rod	+	+	+	+	+	–	K/A	–	–	–	+	*Bacillus* spp.
A2	– rod	–	+	–	+	–	+	K/A	–	–	+	–	*Klebsiella* spp.
A3	+ cocci	–	+	–	+	+	–	K/A	–	–	+	–	*Staphylococcus aureus*
A4	_ rod	–	+	+	+	+	–	K/K	–	–	–	+	*Pseudomonas* spp.
A5	– rod	–	+	–	+	+	–	K/A	–	+	–	+	*Proteus* spp.
A6	+ rod	+	+	+	+	+	–	K/A	_	–	–	+	*Bacillus* spp.
A7	– rod	–	+	+	+	–	–	K/K	–	–	–	+	*Pseudomonas* spp.

Key: CT=Catalase, OX=Oxidase, CU=Citrate Utilization, MR=Methyl Red, VP=Voges-Proskauer, TSI= Triple Sugar Iron, IN=Indole, H_2S=Hydrogen Sulphide, UR= Urease, MOL= Motility

Table 3: The Frequency of Occurrence of Bacteria Isolated from Soil Samples

Bacteria	Frequency (N)	Percentage (%)
Bacillus spp.	2	28.57
Klebsiella spp.	1	14.29
Proteus spp.	1	14.29
Pseudomonas spp.	2	28.57
Staphylococcus spp.	1	14.29
TOTAL	**7**	**100**

Table 4: Screening of Biosurfactants Production Using Oil Spreading and Drop Collapse Assays (n=3)

Bacteria	Oil spread assay in cm Mean ± SD	Drop collapsed assay
Bacillus spp	0.5±0.10	+
Klebsiella spp.	1.0±0.16	+
Staphylococcus. Spp	0±0.00	–
Proteus spp.	0±0.00	–
Pseudomonas spp.	1.4±0.10	+

Key: A= Isolates, cm = centimeter, + = Drop Collapsed, - = No collapsed

Table 5: Biosurfactant Produced from Bacteria Isolated From Mechanic Workshop Soil (n=3)

Parameters	***Bacillus* spp.** Mean ± SD	***Klebsiella* spp.** Mean ± SD	***Pseudomonas* spp.** Mean ± SD
Biosurfactant Produced (g/L)	2.5±0.15	1.1±0.12	2.0±0.06
Activity of Biosurfactants Produced Using Oil Spread Assay (cm)	1.07 ± 0.21	3.10 ± 0.26	6.03 ± 0.15
Appearance	Light Brown	Creamy White	Brownish

Key: g/L = gram per Litrer, cm = centimeter

Table 6: Emulsification index (E24) of Biosurfactants Produced by Bacteria using Kerosene. (n=3)

Test organism	Emulsification Height(cm) Mean ± SD	Total Height(cm) Mean ± SD	Emulsification index-E24 (%) Mean ± SD
Bacillus spp	1.80 ± 0.10	4.4 ± 0.00	40.6.0 ± 0.53
Klebsiella spp	1.9 ± 0.38	4.4 ± 0.00	43.30 ± 0.17
Pseudomonas spp.	2.1 ± 0.20	4.4 ± 0.00	47.73 ± 0.15

4. DISCUSSION

This study demonstrates both scientific and practical relevance by focusing on

indigenous bacterial isolates obtained from hydrocarbon-contaminated mechanic workshop soils. These microorganisms are naturally adapted to local environmental conditions and contaminants, making them suitable candidates for bioremediation. The research integrated physicochemical soil characterization with microbiological analyses and biosurfactant screening, providing a comprehensive understanding of factors influencing microbial activity and biosurfactant production. The use of both oil spread and drop collapse assays enhanced the reliability of biosurfactant detection, with consistent results supporting the biosurfactant-producing ability of selected isolates. The findings revealed that locally isolated *Bacillus* and *Klebsiella* species could utilize engine oil as a carbon source for biosurfactant production, highlighting their potential for petroleum-contaminated site remediation. The study also provides valuable baseline data from Northern Nigeria, where such investigations remain limited. However, limitations include restricted sampling, lack of molecular identification and advanced biosurfactant characterization techniques, and the absence of stability assessments under varying environmental conditions which are important factors for industrial and environmental applications.

The sandy-loam texture, slightly acidic pH, and moderate CEC observed in this study are characteristic of hydrocarbon-contaminated mechanic workshop soils and provide favorable conditions for microbial colonization and hydrocarbon degradation. Similar findings have been reported for contaminated soils where continuous petroleum input promotes the establishment of hydrocarbon-degrading microbial communities and is consistent with previous reports that sandy soils promote aeration and hydrocarbon infiltration, thereby enhancing microbial colonization and biodegradation processes (Adeleke et al., 2021). The slightly acidic pH (6.07 ± 0.04) recorded in the contaminated soil agrees with the findings of Ameh et al. (2011), who reported similar pH values in mechanic workshop soils exposed to petroleum residues. The predominance of *Bacillus* spp. among the isolates may be attributed to their ability to form resistant endospores and survive under stressful environmental conditions. This finding corroborates earlier reports by Bodour et al. (2003) and Das and Chandran (2011), who observed that *Bacillus* spp. frequently dominate hydrocarbon-polluted environments due to their endospore-forming ability and metabolic versatility. Members of this genus are known to degrade a wide range of petroleum hydrocarbons and produce potent biosurfactants such as surfactin (Morikawa et al., 2000; Ongena & Jacques, 2008).

Positive responses in both oil spread and drop collapse assays confirmed the biosurfactant-producing capacity of the isolates despite the different oil-spread values between the screening-stage and production-stage for the same three organisms; this variations may be due to different cells used. At the screening-stage, cell-culture broth was used, while at production-stage, cell-free supernatants containing biosurfactants were used. Biosurfactants facilitate hydrocarbon degradation by increasing the solubility and bioavailability of hydrophobic compounds, thereby enhancing microbial utilization. Similar observations have been reported by Georgiou et al. (1992), Satpute et al. (2010), and Banerjee et al. (2022), who described these assays as rapid and reliable screening methods for biosurfactant-producing microorganisms.

Among the isolates examined, *Bacillus* spp. produced the highest biosurfactant yield (2.5±0.15 g/L). This result is consistent with the findings of Jha et al. (2016), who reported significant biosurfactant production by Bacillus subtilis during crude oil degradation. The enhanced performance may be attributed to the production of lipopeptide biosurfactants such as surfactin, which possess excellent emulsifying and surface-active properties (Stein, 2005; Ongena & Jacques, 2008). The superior oil displacement activity exhibited by *Bacillus* spp., *Klebsiella* spp. and *Pseudomonas* spp. suggests their potential applications in

bioremediation programs aimed at restoring hydrocarbon-polluted soils. Further optimization of production conditions and molecular characterization of the biosurfactant produced are recommended.

5. CONCLUSION

Mechanic workshop soils in Samaru, Zaria harbor indigenous bacterial populations capable of producing biosurfactants. The physicochemical characteristics of the soil supported microbial growth and hydrocarbon degradation. Seven bacterial isolates belonging to five genera; *Bacillus* spp., *Klebsiella* spp., *Pseudomonas* spp., *Proteus* spp. and *Staphylococcus* spp. with *Bacillus* spp. and *Pseudomonas* spp. were isolated and identified from sampling site (Mechanic workshop soils in Samaru, Zaria). However, the three out of five identified bacteria demonstrated an ability to produced Biosurfactant and the highest yield was recorded by *Bacillus* spp. and these findings highlight the potential application of indigenous Bacillus species in the bioremediation of hydrocarbon-contaminated environments.

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