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Screening and Molecular Identification of Biosurfactant-Producing Bacteria Isolated from Contaminated Soil and Vegetables in Ago-Iwoye, Nigeria

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Abstract

Biosurfactants play a crucial role in bioremediation processes, wherein bacteria producing these surfactants utilize contaminants or pollutants as energy and nutrient sources. This study aims to screen and molecularly identify bacteria recovered from soil contaminated by cassava effluent and various vegetables for biosurfactant production. Standard methods were used for bacterial counting, isolation, and identification. Blood hemolysis and oil spreading tests evaluated biosurfactant production. High producers were characterized by 16S rRNA sequencing. Mean values compared and significance tested using chi-square. The mean bacterial counts were 67.00±2.54 for Ago-Iwoye soil, 86.00±4.35 or Oru soil, 102.00±3.32 for Ago-Iwoye vegetables, and 109.00±4.83 for Oru vegetables. Isolates AS1, AS2, CSA34, CSA36, CSO48, CSO49 showed partial hemolysis (10.00 – 14.00 mm), while others exhibited complete hemolysis (Isolates FP11, LS15, CSO45). Isolate LS30 showed no hemolytic activity. Among the isolates tested, only LS15 and CSO45 exhibited positive oil spreading ability, with diameters of 5.00 ± 2.50 mm and 7.50 ± 3.54 mm, respectively. *Staphylococcus aureus* and *Bacillus* spp. proved to be the highest biosurfactant producer by showing better results. The molecular characterization of the identified isolate LS15 revealed a 100% match with *Staphylococcus aureus* 8 BWI (accession number KX456108.1), while isolate CSA45 was identified as *Bacillus marasmi* with 96.6% similarity (accession number NR_147397.1). Therefore, sustained attention to *Staphylococcus* spp. and *Bacillus* spp. is warranted, given their consistent success in screening tests as proficient biosurfactant producers.

Keywords: Biosurfactant, Bacteria, *Staphylococcus* spp., *Bacillus* spp., Cassava, Soil

Introduction

Biosurfactants are amphiphilic compounds produced and secreted extracellularly by microorganisms. These compounds contain both hydrophilic and hydrophobic regions, which enable them to reduce surface or interfacial tension (Onajobi et al., 2023; Banat et al., 2010). Also referred to as bio-emulsifiers, microbial surfactants exhibit surface activity and come in a variety of chemical structures, such as glycolipids, lipopeptides, lipoproteins, lipopolysaccharides, phospholipids, fatty acids, and polymeric lipids. Bio-emulsifiers serve various functions, including antibacterial, antifungal, and antiviral applications in the medical field, and play a crucial role in environmental protection, such as oil spill management, biodegradation, and the detoxification of oil-contaminated industrial effluents and soils (Adeyemi et al., 2019; Yalcin and Ergene, 2009).

The use of biosurfactants offers an eco-friendly alternative for bioremediation in hydrocarbon-contaminated environments. By increasing the surface area of hydrophobic, water-insoluble substrates, biosurfactants enhance the growth and hydrocarbon-degradation capacity of oil-degrading bacteria in bioremediation processes (Bodour et al., 2003). Unlike synthetic surfactants, microbial biosurfactants are biodegradable, effective, and particularly well-suited for environmental applications such as oil spill dispersion and bioremediation (Mohan et al., 2006).

In agriculture, biosurfactants improve the hydrophilicity of heavy soils, thereby preserving their nutrient capacity. The majority of surfactants currently used are petroleum-based, making them toxic and resistant to microbial degradation, which can cause significant pollution and environmental damage (Meenakshisundaram and Pramila, 2017). Microbial surfactants, or biosurfactants, are intracellular or extracellular metabolites produced by fungi and bacteria, classified into structural and functional categories such as lipopeptides, glycolipids, polysaccharide-protein complexes, phospholipids, neutral lipids, and fatty acids (Mnif and Ghribi, 2015; Thavasi et al., 2011).

To improve biosurfactant production efficiency, chemical surfactants derived from non-renewable resources should be replaced with alternatives made from inexpensive, renewable materials like food industry by-products. Effluents from agro-industrial wastes, agricultural processing, and oil-polluted sites have caused environmental harm, negatively affected agricultural practices and leading to social conflicts. Remediating these contaminated soils requires adopting environmentally sound practices, and biosurfactants, with their proven success in environmental remediation, present a viable solution (Onajobi et al., 2023).

Biosurfactants obtained from the environment will both reduce the cost of the clean-up process and ensure the utilization of ecologically friendly products for the remediation process. The aim of this research is to isolate and identify bacteria from cassava effluent-contaminated soils and vegetables, screening them for their biosurfactant production potential by blood hemolysis and oil spreading assays within the rural areas of Ago-Iwoye, Ogun State, Nigeria.

Materials and Methods

Study Area

This study between January and February 2023 in the regions of Ago-Iwoye, Oru-Ijebu, and Ijebu-Igbo within Ogun State. The research sites encompassed vegetable farms situated in the aforementioned locations, as well as various soil samples collected from areas affected by effluent contamination originating from cassava processing plants in Ago-Iwoye and Oru-Ijebu as shown in fig. 1.

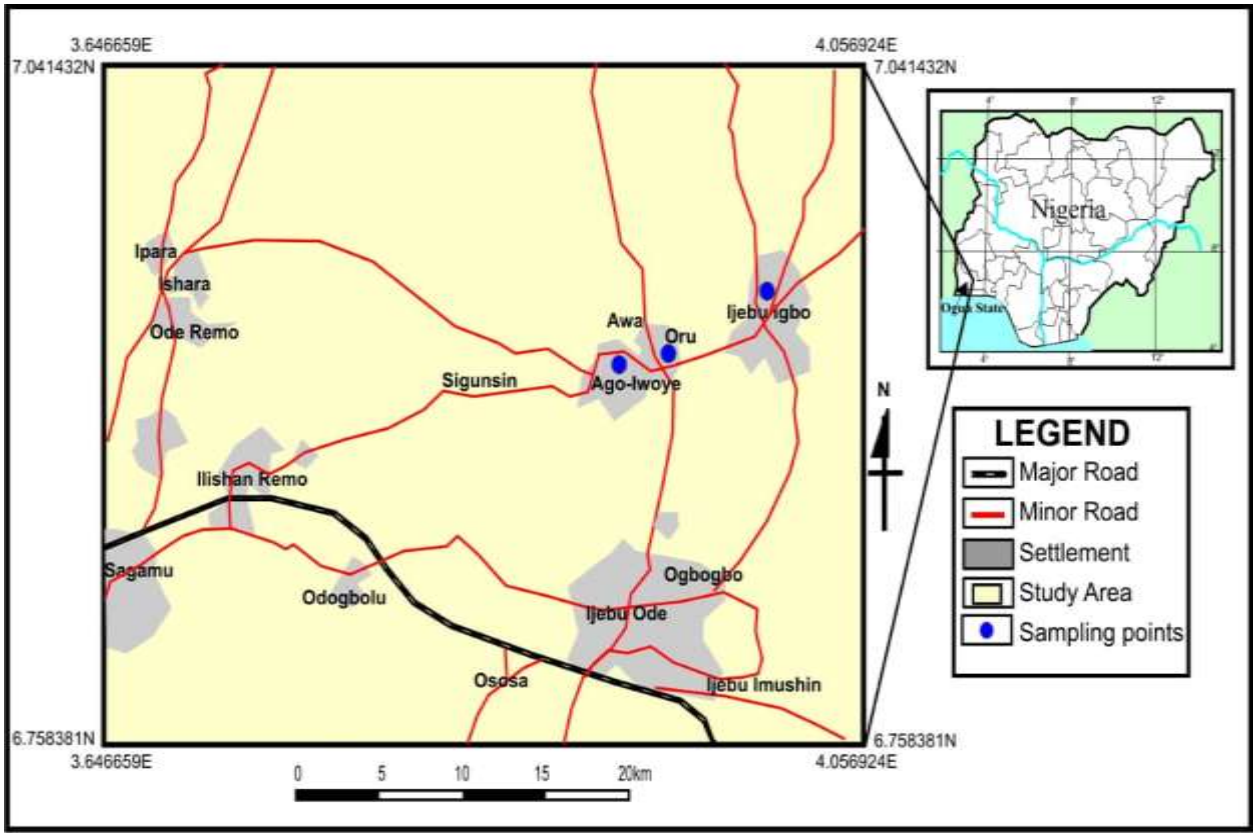

Figure 1: The study site with sampling points marked

Collection of Samples

Soil samples were collected according to the method described by John et al. (2020) with little modifications. Soil samples were randomly collected from cassava processing effluent-contaminated soil within Ago-Iwoye and Oru-Ijebu environment. Ten grams (10 g) of effluent-contaminated soil samples were collected from depths of 5 - 10 cm at ten (5) different locations within each of the sample collection sites. The samples were collected using a sterile spatula into appropriately labelled sterile polythene bags. The temperatures of the samples were taken immediately before the samples were transferred into an ice pack and transported to the laboratory for analysis.

Isolation of Biosurfactant-producing Bacteria

The serial dilution method described by Nwaguma et al. (2016) was adopted in this study. Each sterile test tube was filled with 9 mL of autoclaved normal saline solution (0.85% NaCl w/v), sterilized at 121°C for 15 minutes at 15 psi, and allowed to cool. A stock solution was made by dissolving 1g of dry soil in 9 mL of the sterile saline. Serial dilutions ranging from 10^1 to 10^5 were prepared. For bacterial isolation from water samples, each sample was streaked onto Nutrient Agar and Blood Agar plates, which were incubated at 37°C for 18-24 hours. Post-incubation, bacterial growth was examined, and morphologically distinct colonies were selected and repeatedly sub-cultured to ensure purity. The purified isolates were stored on nutrient agar slants at 4°C for subsequent analyses (Onajobi et al., 2020).

Biochemical Identification of Bacteria

The pure colonies of the isolates underwent further identification using Gram staining and standard biochemical tests. Additionally, the cultural morphology of the isolates was observed on selective media, including Eosin Methylene Blue Agar and Centrimide Agar, following the method described by Onajobi et al. (2023).

Gram Staining

Under sterile conditions, a 24-hour-old culture was smeared on a clean slide, air-dried, and heat-fixed. The smear was stained with crystal violet, Lugol's iodine, decolorized with alcohol, counterstained with safranin, and examined under oil immersion (x100) (Cheesbrough, 2006).

Biochemical Tests

The already Gram-stained isolates were further identified and characterized by the following biochemical tests:

Catalase Test

A colony of bacteria was picked with a sterile inoculating loop and emulsified in a 3% hydrogen peroxide solution on a clean free glass slide. The production of bubbles indicated a positive result and a negative result was identified by the absence of bubble production (Cheesbrough, 2006).

Coagulase Test

The coagulase test was performed by placing a drop of distilled water on a clean, labeled, grease-free slide. A colony of the test organism was emulsified in the water, followed by a drop of blood plasma. Visible agglutination within 1-2 minutes indicated coagulase presence (Malashree et al., 2024).

Triple Sugar Ion Test

Triple Sugar Iron Agar (TSI) is a differential medium containing lactose, sucrose, glucose, ferrous sulfate, and phenol red. It differentiates enteric bacteria based on sulfur reduction and carbohydrate fermentation. Fermentation of any sugar turns the medium yellow. If only glucose is fermented, the small glucose portion is consumed within 10 hours, after which the aerobic slant reverts to red (alkaline), while the anaerobic butt remains yellow due to continuous fermentation in oxygen-free conditions (Chauhan et al., 2020).

Screening Bacteria for Biosurfactant Production

Oil Spreading Technique

Twenty milliliters of distilled water were added to Petri dishes, followed by 1 mL of crude oil placed at the center. Then, 20 µL of the supernatant from the isolated microorganism cultures was dispensed onto the crude oil drop. The displacement of the crude oil, forming a ring, was measured with a meter rule. A 20 µL distilled water sample served as the control (Li et al., 2023; Adeyemi et al., 2019).

Haemolysis Test

Sterilized blood agar base was allowed to cool to about 45ºC and 20 mL of aseptically collected goat blood was added, mixed gently, and poured into sterile Petri dishes. About 24 hours freshly grown cultures were aseptically point-inoculated using wire loops at the centre of the blood agar media. The inoculated media were incubated at 37 ºC for 24 hours. The diameters of the clear zones around the colonies were measured using a meter rule (Onajobi et al., 2023).

Molecular Characterization of Isolated Bacteria (DNA Extraction Procedure using Zymo Kit)

The study conducted molecular characterization of isolated bacteria, focusing on DNA extraction, amplification, sequencing, and phylogenetic analysis.

DNA Extraction and DNA Amplification (PCR)

Bacterial template DNA was extracted at the International Institute of Tropical Agriculture (IITA) in Ibadan using the Zymo Kit. The extraction involved lysing bacterial cells, purifying DNA, and eluting it for downstream applications. The extracted DNA underwent Polymerase Chain Reaction (PCR) amplification at IITA. PCR cocktail mix containing buffer, primers, polymerase, and DNA was prepared (Ilesanmi et al., 2020). Cycling parameters involved denaturation, annealing, and elongation. Amplified fragments were visualized through gel electrophoresis as shown in table 1 below.

PCR Product Purification and DNA Sequencing

PCR products were purified using ethanol precipitation. Ethanol was added to the PCR product, followed by centrifugation, ethanol wash, and air drying. Purified amplicons were reconstituted in water and checked on agarose gel. Purified PCR products were sequenced at Inqaba Biotechnology Industrial

(Pty) Ltd, Pretoria, South Africa, using the dideoxy chain termination method. Sequencing was performed on an ABI PRISM® 350 XL DNA Sequencer (Ilesanmi et al., 2020).

Table 1: Primer sequence for amplification

Primer	Sequence	References
Forward (27F)	AGAGTTTGATCCTGGCTCAG	Bhutia et al. (2021)
Reverse (1492R)	GGTTACCTTGTTACGACTT	Ibrahim et al. (2019)

Sequence Analysis and BLAST search analysis of 16S rRNA gene sequence

Sequence data underwent modification and alignment using BioEdit. The 16S rRNA gene sequence was analyzed via a BLAST search using the Basic Local Alignment Search Tool (BLAST) program from the National Center for Biotechnology Information (NCBI), employing default parameters for homology identification. (Faniyan et al., 2023).

Molecular Phylogenetic Analysis

Molecular Evolutionary Genetics Analysis (MEGA) was used to generate a phylogenetic tree based on 16S rRNA gene sequences. The Maximum Likelihood method based on the Tamura-Nei model was employed to infer the evolutionary history of isolates. The final dataset comprised 511 locations. MEGA was utilized for evolutionary analyses and tree construction (Tamura et al. 2021).

Statistical Analysis

Comparison of the mean values with the results were presented as bar chat, mean±standard error of mean (SEM) and a probability value less than 0.05 which was considered to be statistically significant. Chi-square tested for significant association between the observed and expected percentage of distribution values of according to Oil spreading of Hydrocarbon Utilizing Bacterial isolates by Kock (2020).

Results

Table 2: Description of soil and vegetables samples collected from shops in Ago-Iwoye and Oru

S/N	Soil Location	Depth (in cm)	Sample codes
1	Ago-Iwoye	0-10cm	CSA3
2		10-15cm	CSA36
3		15-20cm	CSA38
4	Oru	0-10cm	CSO45
5		10-15 cm	CSO48
6		15-20cm	CSO49
	Vegetable Location		
7	**Ago-Iwoye**		AS1
8			AS2
9			FP11
10	Oru		LS15
11			LS22
12			LS30

Keys: AS- African Spinach, FP- Fluted Pumpkin, LS- Lagos Spinach, CSA Contaminated Soil Ago-Iwoye, CSO- Contaminated Soil Oru.

The total heterotrophic bacterial (THB) counts in soil and vegetables from Ago-Iwoye and Oru reveal significant differences (fig. 2). Oru's soil (86.00 ± 4.35^{b}) and vegetables (109.00 ± 4.83^{b}) have higher THB counts compared to Ago-Iwoye's soil (67.00 ± 2.54^{a}) and vegetables (102.00 ± 3.32^{a}). Both locations show higher bacterial counts in vegetables than in soil, likely indicating bacterial accumulation on vegetable surfaces. However, there's no significant difference between the THB counts of Ago-Iwoye soil and vegetables or Oru soil and vegetables, despite Oru samples having consistently higher bacterial loads.

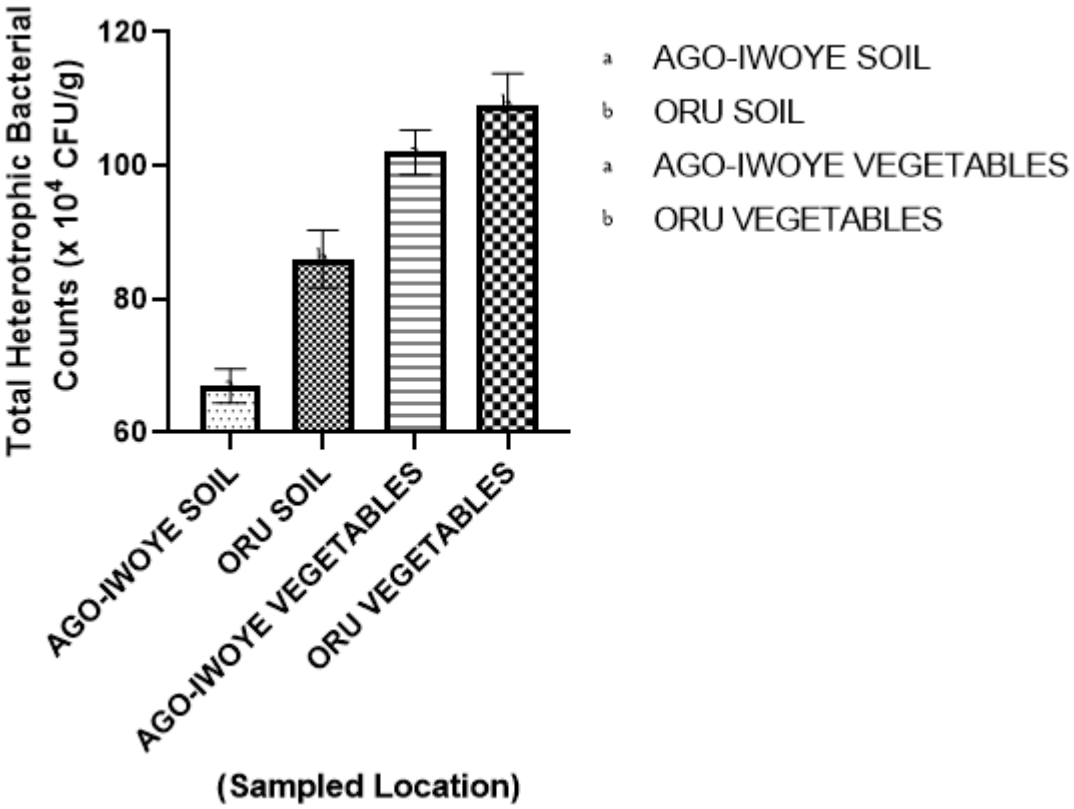

Figure 2: Total Heterotrophic Bacterial Bars Counts; column with similiar superscripts were not significantly different at $p<0.05$

The bacterial isolates exhibit various morphological forms, with most being Gram-positive, including cocci and rods in singles, clusters, and chains as shown in table 3. Two isolates, CSO48 and CSO49, are Gram-negative cocci and rods.

Table 3: Morphological Appearance and gram reaction of bacterial Isolates

S/N	Isolate code	Gram reaction and Microscopy
1	AS1	Gram positive cocci in singles
2	AS2	Gram positive rod in singles
3	FP11	Gram positive cocci in singles
4	LS15	Gram positive rods in clusters
5	LS22	Gram positive rods in chains
6	LS30	Gram positive rods in chains and clusters
7	CSA36	Gram positive rods in clusters
8	CSO45	Gram positive rods in singles
9	CSO48	Gram negative cocci in singles
10	CSO49	Gram negative rods in singles

The bacterial isolates show diverse biochemical characteristics (table 4). Most isolates are positive for sucrose and dextrose fermentation. *Bacillus spp.* is dominant, with *Escherichia coli*, *Staphylococcus* spp., and *Pseudomonas* spp. also present, based on coagulase, gas, and H_2S production results.

Table 4: Biochemical Identification of Bacterial isolates

S/N	Isolate code	Catalase	Sucrose	Coagulase	Dextrose	Lactose	Gas	H_2S	Probable Organism
1	AS1	-	+	+	+	+	+	-	*Bacillus* spp.
2	AS2	-	+	+	+	+	-	-	*Bacillus* spp.
3	FP11	+	+	-	+	+	+	-	*Escherichia coli*
4	LS15	+	+	+	+	+	+	-	*Staphylococcus* spp.
5	LS22	+	+	-	+	+	-	-	*Staphylococcus* spp.
6	LS30	-	+	-	+	+	+	-	*Bacillus* spp.
7	CSA34	+	+	-	+	+	+	-	*Bacillus* spp.
8	CSA36	+	+	+	+	+	-	-	*Bacillus* spp.
9	CSO45	+	+	-	+	+	+	-	*Bacillus* spp.
10	CSO48	+	+	-	+	+	+	-	*Pseudomonas* spp.
11	CSO49	+	+	-	+	+	-	-	*Pseudomonas* spp.

Keys: - Negative; + Positive

The hemolytic activity of hydrocarbon-utilizing bacterial isolates is shown in fig. 3. The data includes the zone of clearance (in mm) and hemolysis patterns: partial (μ), complete (b), and gamma (g). Partial hemolysis indicates partial breakdown of red blood cells, complete hemolysis signifies full destruction, and gamma hemolysis shows no activity. Isolates AS1, AS2, CSA34, CSA36, CSO48, and CSO49 exhibited partial hemolysis with clearance zones of 10.00 to 14.00 mm. FP11, LS15, and CSO45 showed complete hemolysis with clearance zones of 12.00, 13.00, and 14.00 mm, respectively. LS30 showed no hemolytic activity.

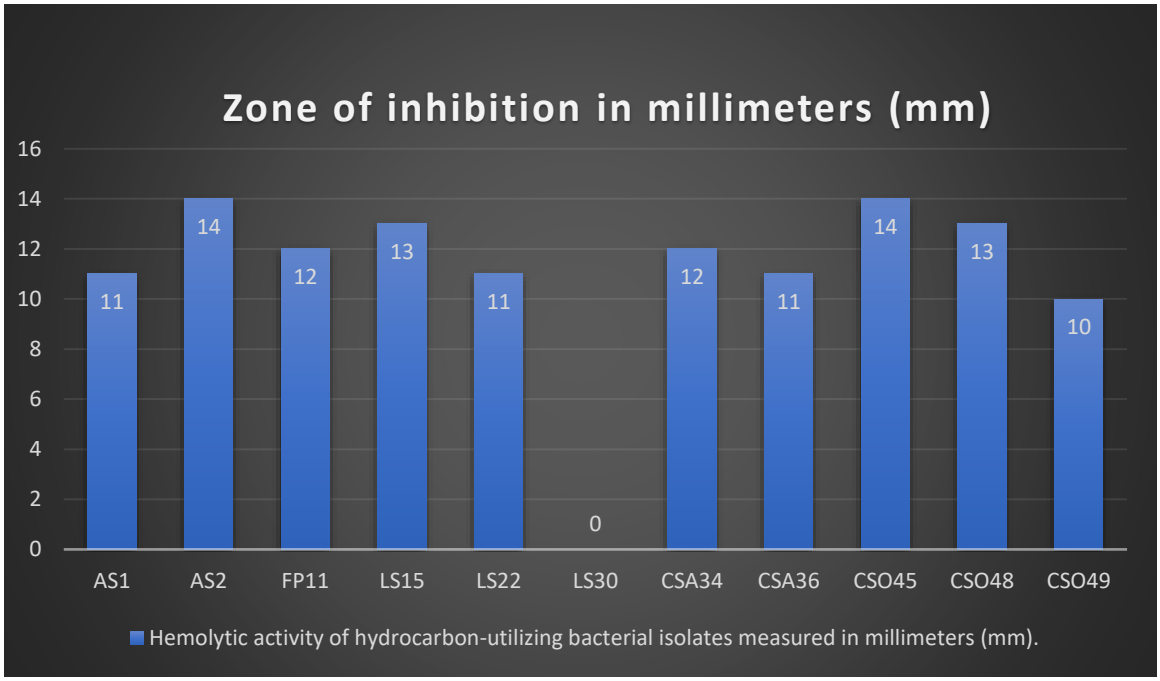

Figure 3: Hemolytic Activity of Hydrocarbon-Utilizing Bacterial Isolates

The oil spreading assay results for hydrocarbon-utilizing bacterial isolates (table 5). Only LS15 and CSO45 demonstrated positive spreading abilities (5.00 ± 2.50 mm and 7.50 ± 3.54 mm). Others showed no spreading. Statistical significance was set at $p < 0.05$.

Table 5: Oil spreading of Hydrocarbon Utilizing Bacterial Isolates

S/N	Isolate Code	Oil spreading	Diameter (mm)
1	AS1	-	0.00±0.00
2	AS2	-	0.00±0.00
3	FP11	-	0.00±0.00
4	LS15	+	5.00±2.50
5	LS22	+	0.00±0.00
6	CSA34	-	0.00±0.00
7	CSA36	-	0.00±0.00
8	CSO45	+	7.50±3.54
9	CSO48	-	0.00±0.00
10	CSO49	-	0.00±0.00

Keys: - Negative; + Positive; Mean±standard error of mean (SEM)

Chi-square test results assessing the link between bacterial isolate sources and oil spreading behavior (table 6). It includes observed and expected frequencies, the chi-square statistic (2.54), and contributions of each cell to the statistic.

Table 6: Chi square test association using contingency analysis and expected frequencies for Oil spreading of Hydrocarbon Utilizing Bacterial Isolates

S/N	Isolate Source	Observed Oil spreading (+)	Observed Oil spreading (-)	Total	Expected Oil Spreading (+)	Expected Oil Spreading (-)	(Observed - Expected)^2/ Expected
1	African Spinach	0	2	2	0.33	1.67	0.33
2	Fluted Pumpkin	0	1	1	0.16	0.83	0.16
3	Lagos Spinach	1	3	4	0.66	3.33	0.22
4	Contaminated Soil Ago-Iwoye	0	2	2	0.33	1.67	0.33
5	Contaminated Soil Oru	1	2	3	0.50	2.50	0.50
	Total	**2**	**10**	**12**			**2.54**

Chi-Square (X^2) Value = 2.54
Note: ***A probability value <0.05 was considered to be statistically significant***

The molecular identification and genetic characterization of bacterial isolates (table 7). It includes unique codes, sequence IDs, identified organisms, strains, percentage identities, and accession numbers. LS15 matched *Staphylococcus aureus* with 100% identity for strain 8 BWI, while CSA45 identified as *Bacillus marasmi* with 96.6% identity.

Table 7: Molecular Identification of Biosurfactant- producing isolates

Isolates code	Sequence ID	Identified organism	Strain	Percentage identity (%)	Accession number
LS15	37_907R	*Staphylococcus aureus*	8 BWI	100.00	KX456108.1
		Staphylococcus aureus	AT1	98.71	MH603394.1
CSA45	38_907R	*Bacillus marasmi*		96.6	NR_147397.1
		Bacillus sp. (in: firmicutes)		96.37	MT397046.1

Key: LS- Lagos Spinach, CSO- Contaminated Soil Oru

PCR gel pics

This gel image (Plate 1) shows successful PCR amplification of bacterial DNA. Sample 1 (LS15 - *Staphylococcus aureus*) and Sample 2 (CSA45 - *Bacillus marasmi*) both exhibit clear bands at approximately 515 bp, indicating positive amplification.

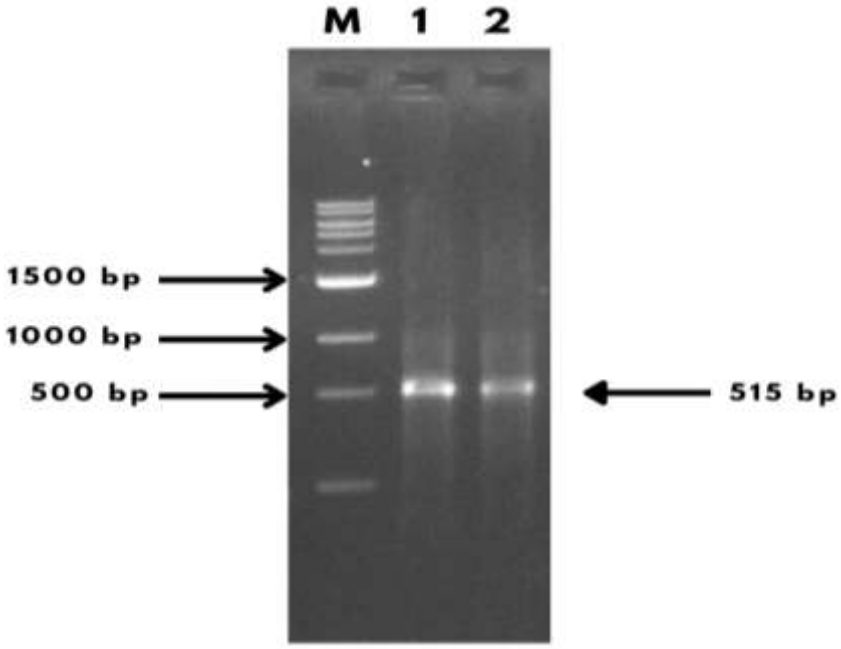

Plate 1: Agarose gel electrophoreses indicating a positive amplification of bacteria isolates using 16S rRNA specific Universal primers

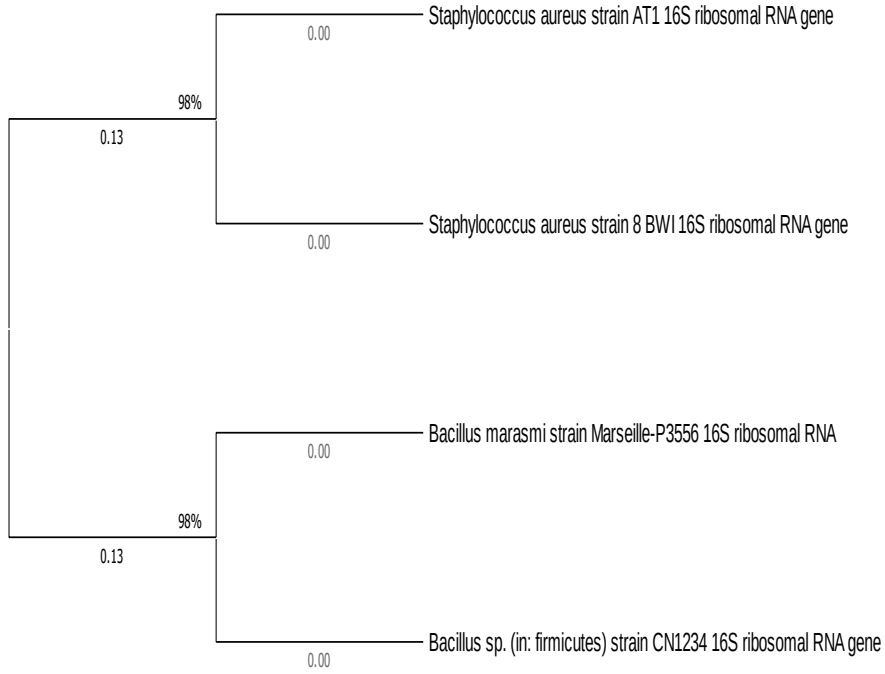

Figure 3: Phylogenetic tree of constructed using Neighbor-Joining method (MEGA 11)

Discussion

The use of biosurfactant is presented as a better alternate option because of its versatility, biodegradability and ecofriendly nature that maintains sustainability of the environment (Shah et al., 2016). Biosurfactants are compounds that are mostly used because they are easily degraded, environmentally friendly, low toxicity and produced from cheaper substrates (Meybodi et al., 2013). Although, biosurfactant producing bacteria are ubiquitous in nature. They are mostly found in hydrocarbon contaminated environment.

The study of total heterotrophic bacterial counts reveals high bacterial loads present in soil and vegetables from Ago-Iwoye and Oru. This is in line with the study of Meireles et al. (2017) that reported high heterotrophic bacteria from a minimally processed vegetables plant. The higher THBC in Oru samples could be due to various environmental or anthropogenic factors affecting that region. The statistical analysis indicates that while there are notable differences in bacterial counts between the locations, the bacterial loads within each location's soil and vegetables are not significantly different from each other (da Silva et al., 2020). Understanding these patterns is crucial for addressing microbial contamination in agricultural produce and ensuring food safety.

In this study, the morphological characteristics of bacterial isolates using gram staining and microscopic techniques. Gram staining classifies bacteria into two primary groups: gram-positive and gram-negative, based on cell wall composition and staining properties. This method aligns with the approach used by Onajobi et al. (2023), who focused on the identification and characterization of bacterial isolates from environmental and food sources. By combining morphological observations with biochemical and molecular tests, it can better classify and identify these isolates. Such comprehensive characterization is essential for understanding their roles in various ecosystems and their potential applications or implications, as emphasized by Al-Marri et al. (2023). This integrated approach aids in deciphering the functional significance of bacterial isolates and their interactions within different environments, thus contributing valuable insights into their practical uses and ecological impacts.

The results obtained in this study reveals that soil from effluents of cassava have the highest biosurfactant producer. Most of the isolates were gram positive rods. In this study, two biosurfactant producing strains were isolated namely; *Bacillus marasmi.* isolated from soil from cassava effluents while *Staphylococcus aureus* was isolated from Lagos Spinach vegetables. From this research work, two different screening methods were assessed for selecting biosurfactant producing bacteria from different locations which are blood hemolytic assay and oil spreading. These methods have been reported for the identification of biosurfactant producing bacteria. Hemolytic assay (Adeyemi et al., 2019) and oil spreading (Patel et al., 2019).

In this present study, selected strains showed hemolytic activity which is a screening technique for biosurfactant producing bacteria. Oil spreading technique was also carried out for further confirmation (Ndibe et al., 2018). Hemolytic activity seems to be a good selection measure in the exploration for biosurfactant producing bacteria (Batool et al., 2017). Biosurfactant producing strains hemolysed the red blood cells and showed alpha and beta hemolysis. The hemolytic capabilities of various hydrocarbon-utilizing bacterial isolates, which may have implications in fields such as biotechnology, environmental remediation, or medical research (Patel et al., 2019).

In this current study, isolates exhibiting positive oil spreading ability, such as LS15 and CSO45, may be promising candidates for further investigation and potential applications in the remediation of hydrocarbon contamination in various environments. This supports the findings of Al-Marri et al. (2023) stating that the oil spreading assay evaluates the ability of these isolates to spread over an oil surface, which is an important characteristic in bioremediation processes involving hydrocarbon contamination. The oil spreading assay provides valuable insights into the potential bioremediation capabilities of hydrocarbon-utilizing bacterial isolates.

The widest zone with a diameter of 7.50mm was observed upon the addition of cell-free supernatant (CFS) by oil spreading technique. Selected strains also showed oil displacement activity which was confirmed by oil spreading technique (Patel et al., 2019). These screening techniques are partly in alignment with the work of (Poonguzhali et al., 2022) whiich screened and characterized biosurfactant producing bacteria. The ability of the bacterial isolates from vegetables and the soil contaminated with

cassava effluents to produce biosurfactant is important, considering the level of pollution and contamination and the need to use ecologically friendly products in remediation process (Onajobi et al., 2023). The chi square test table in this study provides insights into the relationship between hydrocarbon-utilizing bacterial isolate sources and their oil spreading behavior, helping researchers understand the factors influencing bacterial activity in hydrocarbon-contaminated environments and this is in line with Onajobi et al. (2020).

It is noteworthy that many researchers have typically employed a maximum of two or three screening methods for the selection of biosurfactant producers. They have emphasized that a single method may not be sufficient to identify all types of biosurfactants. Molecular Characterization of the identified isolate shows a very comparable percentage of *Bacillus* spp. (100%) and *Staphylococcus aureus* (100%) which indicate the isolate is a good biosurfactant producers (Al-Marri et al., 2023).

The molecular characterization of the obtained sequences revealed 100% similarity with deposited 16S rRNA sequences in the GenBank. This is similar to the work of Plaza and Achal (2020) and Alyousif et al. (2020) where *Bacillus* and *Staphylococcus* species were characterized. In addition, biosurfactant-producing organisms of great significance in environmental cleanup operations can be found in other soil sources besides those previously contaminated with hydrocarbons.

Bacillus species must be continually optimized and it must be focused on as from most literature it is always producing biosurfactant, of which it is the same with this research (Shah et al., 2016). *Staphylococcus* must also pass through the optimization process because it can be useful in enhancing the production of surface-active agents of which it is the same with this research (Nwaguma et al., 2016).

Conclusion

This study demonstrates the potential of biosurfactant-producing bacteria isolated from soil and vegetables in Ago-Iwoye and Oru. The research highlights the ubiquity of these bacterial isolates in various environments, particularly in hydrocarbon-contaminated areas. Two key biosurfactant producers were identified: *Bacillus marasmi* from cassava effluent soil and *Staphylococcus aureus* from Lagos Spinach vegetables. Multiple screening methods, including hemolytic assay and oil spreading technique, effectively identified biosurfactant-producing bacteria. The isolates showed promising results in both tests, with some exhibiting significant oil displacement activity. Molecular characterization confirmed the identity of these isolates with 100% similarity to known sequences in GenBank. The study revealed the importance of using multiple screening methods to identify diverse biosurfactant producers. It also emphasizes the potential of non-hydrocarbon contaminated sources for isolating biosurfactant-producing organisms. The identified *Bacillus* and *Staphylococcus* species show great promise for environmental cleanup operations and other biotechnological applications. These findings contribute to the growing body of knowledge on biosurfactants and their potential applications in bioremediation and environmental sustainability. Further optimization of these isolates could lead to more efficient and eco-friendly solutions for addressing environmental contamination and industrial processes.

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Competing Interest

The authors declare no potential conflicts of interest regarding this research.

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