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Characterization of biosurfactant-producing bacterial strains isolated from agro-industrial wastes in southwestern, Nigeria

Authors: Ismail B. Onajobi[1], Jamiu O. Adeyemi[1], Frank A. Orji[3], Oyindamola J. Samson[1,*], Haneefat O. Egberongbe[1], Suliamon A. Aina[2], Oluwabukola T. Afolabi[1], Lawrence O. Adebajo[1], Obasola E. Fagade[4]

Abstract

Introduction. The difficulty of managing trash and cleaning up the environment prompted interest in biosurfactants and surface-active proteins made by microbes. The study aims to augment bacterial isolates from agro-industrial wastes targeted for possible mass production of biosurfactants. **Methods**. Six agro-industrial wastes from Cassava, Palm kernel, and Sawdust from six agro-industrial sites within Ijebu area in Ogun State were collected for standard laboratory analyses in the Biotechnology Unit of the Federal Industrial Institute for Research, Oshodi (FIIRO). Five screening methods; blood hemolysis, lipase activity, blue agar hydrolysis, oil spreading, and emulsification index (EI24) were carried out to confirm biosurfactant production. Isolates with the highest hyper-production were subjected to 16rRNA molecular identification. **Results**. The study justified efficient biosurfactant production from 4 bacterial isolates out of 26 screened bacterial isolates from hydrocarbon degraders and 29 heterotrophic screened bacterial isolates, making a total of 55 screened bacterial isolates. Screening results reveal the emulsification capacities of identified *Pseudomonas putida* strain SG1, *Acinetobacter baumanii* strain MS14413, *Bacillus zhangzhouensis* strain cdsV18, and *Burkholderia cepacia* strain 717. **Conclusion**. Biosurfactant bacteria produced in all agricultural and industrial wastes considered in this study are capable of mass production.

Key word: agro-industrial wastes, biosurfactants, bacteria, optimization, screening.

[1]Department of Microbiology, Faculty of Science, Olabisi Onabanjo University, Ago-Iwoye, Nigeria.

[2]Departamento de Zoología y Biología Ambiental, Facultad de Ciencias, Universidad Olabisi Onabanjo, Ago-Iwoye, Nigeria.

[3]Departamento de Biotecnología, Instituto Federal de Investigación Industrial, Oshodi, Nigeria.

[4]Departamento de Microbiología, Facultad de Ciencias, Universidad de Ibadan, Ibadan, Nigeria.

[*]https://orcid.org/0000-0003-0404-7634

Corresponding author:
Oyindamola John Samson
Address: *Departamento de Microbiología, Facultad de Ciencias, Universidad Olabisi Onabanjo, Ago-Iwoye, Nigeria, +2347065311985.
E-mail: oyindamolasamson1997@gmail.com

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Introduction

Wastes management has continued to be a serious concern as a substantial amount of wastes, pollutants, and contaminants are released into our environment daily from domestic and industrial sources(1-3). Thus, exploring safer, cheaper, less toxic, and eco-friendly means to reduce, reuse and recycle unwanted materials becomes imperative(4,5). Biosurfactants (BS) are amphipathic biomolecules with hydrophobic and hydrophilic activities obtained from microorganisms (bacteria, yeasts, and filamentous fungi) that have the ability to form micelles (active surface molecules) and break the surface tension of liquid surfaces extra-cellularly(5,6).

Their hydrophobic and hydrophilic nature enables these characteristics and has been vastly reported for several applications in many fields, including food processing, pharmaceutics, bioremediation, biodegradation, and enhanced oil recovery(7). Considering the characteristics above and the fact that they can be recovered from cheaper substrates, which include agro-industrial wastes, which are readily available, they are better than chemical surfactants(8). Due to their high production costs and ongoing struggles with optimization, these biosurfactant-producing microorganisms face a significant economic hurdle compared to their chemical counterparts(2,9).

On these bases, the present study focused on the identification of biosurfactant production potential in bacterial strains from agricultural and industrial wastes from southwestern Nigeria, with the following specific objectives(10,11) isolation and characterization of bacterial strains isolated from agro-industrial wastes, screening of isolated bacterial strains for biosurfactant production by blood hemolysis, hydrolysis on blue agar, oil spreading, lipolytic capacity and emulsification index and molecular

characterization of successfully isolated bacterial strains to the strain level by adopting molecular characterization techniques(12). The study aims to augment bacterial isolates from agro-industrial wastes targeted for possible mass production of biosurfactants and biosorption of heavy metals.

Materials and methods

Study area

The microbiological analysis of the study was carried out at the Federal Institute of Industrial Research Oshodi (FIIRO) in the biotechnology department. Agro-industrial waste samples were collected from the following sites, Agricultural Farm Settlement of the Olabisi Onabanjo University, Ago-Iwoye, Apoje Farms Ijebu-Igbo, Mini campus Sawmill Ago-Iwoye, Oke-Oru Sawmill Oru, Abusi Edumare Sawmill Ijebu-Igbo and Oke-Eri Sawmill, Odogbolu all within South-Western Nigeria.

Figure 1
Map of the study area

Source: www.viamichellin.com

Isolation of Bacteria

Each sample collected was serially diluted, total bacterial count present in the samples was determined by the pour plate method on nutrient agar. Sample suspensions were prepared by 10-fold serial dilutions with 1 gram of sample, using 10ml peptone water as diluents. Aliquots of the fourth and sixth dilutions were spread with an Eppendorf pipette on triplicates of sterile 12 ml of nutrient agar. The plates were incubated in the incubator for 24 hours in an inverted orientation at 37 °C(13). Colonies that formed during this incubation period were counted using this formula:

$$No\ of\ Colonies \times \frac{dilution\ factor}{volume\ of\ inoculum}$$

The enumeration of bacteria was carried out using the method reported by Chikere et al.(14) and Nwachukwu et al.(15). After incubation, morphologically and different colonial colonies were observed and sub-cultured on a nutrient agar by streak method to obtain pure cultures. They were subsequently seeded into 6 ml nutrient agar slants. The slants were kept in the bio-freezer at 4 °C as stock cultures.

Total Heterotrophic Bacterial Count

The total heterotrophic bacterial counts (THBC) were carried out by measuring one gram of each of the samples and serial diluting them nine-fold in sterile distilled water. One(1) ml of the diluents was aseptically poured into sterile Petri dishes. The dilutions were dispensed aseptically on sterile Petri dishes. The Petri dishes were incubated at 37°C for 24 h, after which the colonies were counted. These were done in triplicates. The colonies were further sub-cultured to obtain pure colonies(13,15).

Total Hydrocarbon Degrading Bacterial Count

Hydrocarbon using bacterial count was finished utilizing Mineral Salt Medium (MSM) agar on which 1% Double Reason Lamp oil (DPK) was added as the significant carbon source; before this, the DPK was sifted utilizing a Whatman channel paper No 1. Two percent (2%) agar was added to empower the hardening of the medium, as indicated by Venty *et al.*(16).

Colonial and morphological characterization of Bacterial isolates

Colonial and morphological characterization of the isolates was done using a standard microbiological method. Shape, pigmentation, elevation, size, appearance, and motility were used for morphological characteristics according to Onajobi et al.(5) and Venty *et al.*(16).

Screening of Bacterial isolates for Biosurfactant production

Blood hemolysis

Blood hemolytic activity was considered for the screening as a complimentary and qualitative test to confirm biosurfactant-producing bacteria. Bacteria cultures were streaked on nutrient agar supplemented with 5% fresh human blood and incubated at 370C for 48-72 hours. Visual inspections for hemolysis were by indication of red blood lysis by Gizele *et al.*(17).

Blue agar hydrolysis

Mineral Salts Agar (MSA) was supplemented with cetyltrimethylammonium bromide (CTAB: 0.5 mg/ml-methylene blue (MB: 0.2 mg/ml) and was prepared as reported by Nordiyana *et al.*(17,28). Carbon sources that were tested are glucose, sodium acetate, mannitol, glycerol, sodium acetate, and peptone. The dark blue halo coloration on the plate was considered positive for biosurfactant production.

Emulsification index (EI_{24})

The emulsification record for biosurfactant-delivering microscopic organisms was completed utilizing the technique of Raza *et al.*(18). Mannitol Salt Media was enhanced with 1% Lamp fuel for seven days in an orbital hatchery at 180 cycles each moment (rpm) at 28°C. Without cells, the supernatant was acquired by centrifuging the stock culture at 15,000 rpm for 15 min. Two milliliters of the supernatant of the organic entity inside the response cylinder and 2 ml of lamp fuel were added as hydrocarbon substrate test. The combination was vortexed at high velocity for 2 minutes and noticed for rate emulsification at stretches four h through 24 h. The emulsification record (EI24) was determined by estimation of the level of the emulsion layer (a) partitioned by the complete level (b), duplicated by 100 (〖EI〗 _24=a/b×100). This measure was acted in the same size glass test tubes as per Meenakshisundaram *et al.*(19).

Oil spreading test

The oil impregnation test was completed in polystyrene Petri dishes (100 mm × 15 mm) containing 20 µL of unrefined oil that was thoroughly stratified over 20 mL of refined water. A drop (~10 µL) of separated supernatant was cautiously pipette onto the focal point of the oil layer. The distance across the unmistakable zone on the outer layer of the oil layer was estimated and contrasted with the negative controls, as indicated by Ibrahim *et al.*(20).

Lipolytic test

The assay medium was composed of (g/l); Tributyrin, 2 ml; Gum Arabic, 4; Agar, 15; Phosphate buffer at pH 4.6, up to 1L. The lipase assay medium was prepared, and the cell-free filtrate of the biosurfactant producer was used as a source of lipase enzyme, as elucidated by Sidkey *et al.*(21). Lipolytic activity was detected by clearing zones around the hole in comparison to the turbid background of the assay plates.

Molecular characterization of Bacterial isolates

Bacterial isolates with very high potentials as hyper-producers of biosurfactant were identified further using Molecular Biology tools such as polymerase chain reactions, sequencing, and blast programs through the extraction of the genomic DNA of bacterial isolates, amplification by Polymerase Chain Reaction (PCR) using 16s-rRNA primer, sequencing of the isolate DNA and blast programs were used to reveal the name of the isolate according to the method of Joshi and Deshpande(22).

Statistical analyses

Data obtained were subjected to statistical analyses using the Statistical Package for Social Sciences (SPSS) version 20.0. Mean values were compared using Analysis of Variance (ANOVA) according to Zhang and Liang(23). Screening results were presented as Mean±Standard deviation. Post hoc test was adopted using the Student-Newman-Keuls (SNK). A probability value (p – value) less than 0.05 was considered statistically significant.

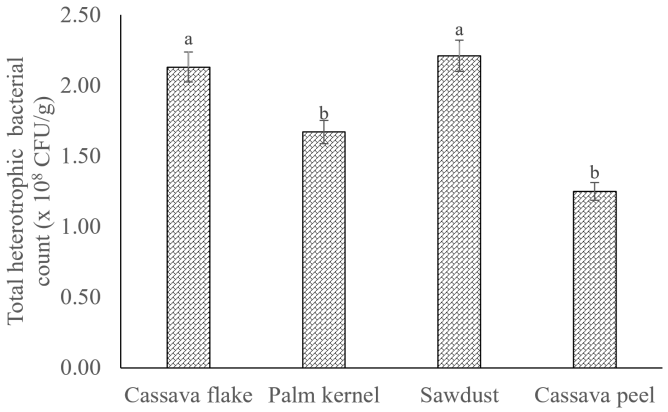
Results

Total Heterotrophic Bacterial counts

The results of the Total Heterotrophic bacterial counts on Cassava flake, Palm kernel, Sawdust, and Cassava peel showed no significant difference in the heterotrophic bacterial counts recorded with Cassava flakes and Sawdust. However, the highest heterotrophic bacterial count was recorded with Sawdust. On the other hand, the lowest heterotrophic bacterial count was recorded with Cassava peel, as presented in Figure 2.

Figure 2

Total Heterotrophic Bacterial counts; Bars with similar superscripts are not significantly different at p < 0.05

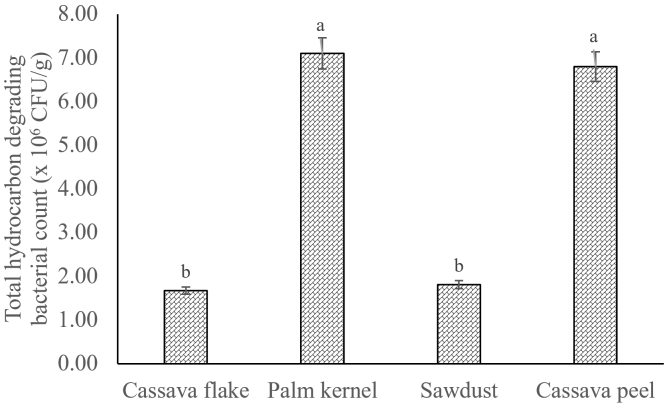


Total Hydrocarbon Degrading Bacteria count

The results of the hydrocarbon-degrading bacteria count are shown in Figure 3. Palm kernel had the highest hydrocarbon-degrading bacteria count (7.10 x 10^6 CFU/g). This was not significantly different from the Cassava peel (6.80 x 10^6 CFU/g). However, the hydrocarbon-degrading bacteria count recorded with the Palm kernel and Cassava peel were significantly higher than those of Sawdust and Cassava flake, respectively.

Figure 3

Total Hydrocarbon Degrading Bacteria count; Bars with similar superscripts are not significantly different at p < 0.05



Colonial and morphological characterization

The gram reaction and morphological appearance of the heterotrophic samples are presented in Table 1. Most of the samples are gram-positive rods though some have short rods, some appear to have single colonies, some are clustered, and some occur in chains. Likewise, most are mucoidal in colonial appearance, while some are milkfish in color.

Table 1

Morphological appearances and Gram reactions of Heterotrophic bacteria

S/N	Isolate code	Gram reactions and Microscopy
1	CF 124	Gram-positive rods in singles.
2	CF 125	Gram-positive rods in singles.
3	CF 126	Gram-positive rods in clusters.
4	CF 121	Gram-positive rods in chains and clusters.
5	CF 122	Gram-positive rods in clusters.
6	CF 123	Gram-positive rods in chains.
7	PKB 104	Gram-positive rods in clusters.
8	PKB 105	Gram-positive ellipsoidal cells in clusters.
9	PKB106	Gram-positive rods in singles.
10	PKB 107	Gram-positive slender rods in clusters.
11	PKB108	Gram-positive rods in chains and clusters.
12	SD 101	Gram-positive rods in long chains.
13	SD 102	Gram-positive short rods in chains.
14	SD 103	Gram-positive short rods in clusters
15	$S_2$33	Gram-positive fat rods in singles.
16	S_3Q	Gram-positive long rods in singles.
17	CS 113	Gram-positive rods in chains and clusters.
18	PKB 114	Gram-positive rods in chains.
19	CS 111	Gram-positive rods in chains and clusters.
20	CS 112	Gram-negative rods in singles and clusters.
21	CP 101	Gram-positive cocci in singles.
22	CP102	Gram-positive rods in clusters.
23	CP 103	Gram-positive rods in singles.
24	CP 104	Gram-negative rods in singles
25	CF 131	Gram-negative rods in singles.
26	CF 132	Gram-positive rods in clusters.
27	CF 133	Gram-negative rods in clusters.
28	CSP3MFR	Gram-positive rods in singles.
29	S33	Gram-positive rods in singles.

PK, Palm kernel bargasse; CF, Cassava flake; SD, Sawdust; CP, Cassava peel; CS, Cassava Shaft

The gram reaction and morphological appearance of the hydrocarbon samples are presented in Table 2. Most of the samples are gram-positive rods though some have short rods, some appear to have single colonies, while some are clustered, and some occur in chains. Also, most have a mucoidal colonial appearance, while some are milky.

Table 2

Colonial appearances and microscopy of hydrocarbon utilizing pure bacterial isolates

S/N	Isolate code	Colonial appearance	Microscopy and Morphology
1	CSP_1	Mucoidal and convex shape and entire with 10mm size in diameter	Gram-positive rods in single
2	$S_2$3	Milkish and translucent column shape is oval and about 4mm in size	Gram-positive rods in single
3	PKBJ	Mucoidal and convex with an entire shape of about 10mm size in diameter	Gram-positive rods in clusters
4	S_3(2)	Mucoidal and convex, with a wavy edge and about 12mm size	Gram-positive long rods in clusters
5	S_2Q	Milkish and raised colonies, with an entire shape 5mm in size	Gram-negative rods in clusters
6	$S_2$31	Milkish and raised colonies, with an entire shape 5mm in size	Gram-positive long rod in singles
7	S3	Mucoidal and convex with an entire shape size equal to 12mm	Gram-positive long rods in singles
8	S_3Q1	Convex shape with light yellow pigment shape equal to 2-4mm	Gram-negative long rods in clusters and single
9	PKBMY2	Translucent colonies	Gram-negative rods in singles
10	PKBMYQ	Pinkish colony with a convex structure size is 4mm	Gram-positive rods in clusters
11	PKBMR(2)	Mucoidal with wavy edge and size is 12mm	Gram-positive rods in clusters
12	PKBJ2	Mucoidal with wavy edge and size is 12mm	Gram-negative rods in single
13	CSP3MT(1)	Mucoidal with entire edge and size is 6mm	Gram-negative rods in singles
14	CJP2	Mucoidal and convex shape 6-8mm	Gram-positive rods in clusters
15	CSPMAR3	Translucent and convex structure size equal to 8-10mm	Gram-negative rods in singles
16	PKBMY(2)	Milky and translucent colonies in size 10mm	Gram-positive cocci in singles
17	PKBJ22	Mucoidal and oval in shape, translucent size equal to 10mm	Gram-positive rods in singles and cluster
18	S2	Mucoidal and with wavy edge size 12mm	Gram-positive long and slender rods in singles
19	S_1Q	Mucoidal and milky in color	Gram-negative rods in singles
20	CSF2	Mucoidal and milky in color	Gram-negative rods in singles
21	CSP_3	Mucoidal and milky in color	Gram-positive rods in single
22	$S_2$4	Mucoidal and milky in color	Gram-negative short and fat rods in single
23	PKBJ3	Mucoidal, translucent, 10mm in size	Gram-positive rod in singles and long chain
24	S_3(2)1	Mucoidal, translucent, 10mm in size	Gram-positive rods in singles
25	S_2Q1	Mucoidal, light yellow pigment size equal to 6m	Gram-positive rod in singles
26	$S_2$32	Wavy edge, Milkish and 8-10mm size in diameter	Gram-positive rod in short chain

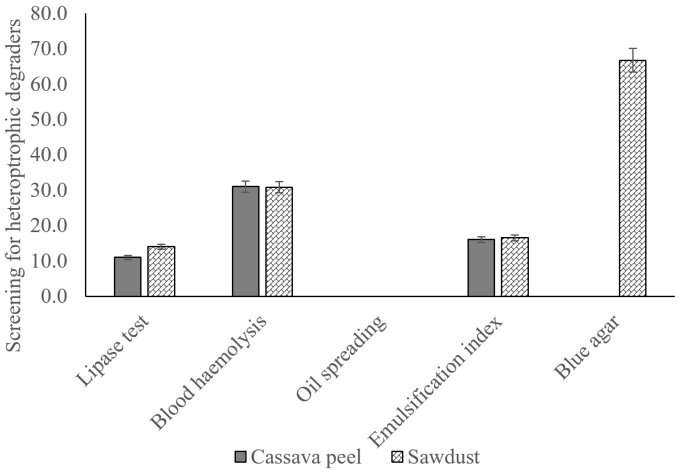
PK, Palm kernel bargasse; CF, Cassava flake; S, Sawdust; CP, Cassava peel; CS, Cassava Shaft

Substrate Screening results for Heterotrophic degraders

The sample screening results for heterotrophic degraders. Results showed no significant difference ($p > 0.05$) in the reactions of both the Cassava peel and Sawdust to Lipase test and Blood hemolysis. Also, Cassava peel and Sawdust showed no reaction to the oil spreading test. The emulsification index was highest with the Sawdust. This was, however, not significantly different from that of the Cassava peel. Similarly, Sawdust showed a positive response (66.7%) to the Blue agar test, as presented in Figure 4.

Figure 4

Substrates screening results for heterotrophic degraders



The substrate screening results for hydrocarbon degraders are presented in Table 3. Cassava flake showed no reaction to the Lipase test. However, this reaction to the Lipase test was significantly higher with the Palm kernel than with the Sawdust and Cassava peel, respectively. Similarly, Cassava peel showed no reaction to the Blood hemolysis test. Reaction to Blood hemolysis test was also highest with the Palm kernel and lowest with Sawdust. On the other hand, the Palm kernel showed no reaction to the oil spreading test.

Reactions to the oil spreading test were significantly higher with Cassava peel. This was followed by Sawdust and Cassava flake, respectively. Results, however, showed a significantly higher Emulsification index with the Palm kernel. However, the Emulsification index recorded with Sawdust, Cassava flake, and Cassava peel were not significantly different. This was lowest with cassava peel. Meanwhile, Cassava peel and Cassava flake showed a negative response (100%) to the Blue agar test. However, Sawdust (50.0%) and Palm kernel (14.3%) showed some positive responses to the Blue agar test.

Bacterial isolates screening results for heterotrophic degraders

The results for screening for the heterotrophic degraders are presented in Table 4.4, with isolate S233 with 18.3 having the highest average.

Table 3

Substrate screening results for Hydrocarbon degraders

Screening test Agro-industrial wastes	Lipase test	Blood hemolysis	Oil spreading	Emulsification index	Blue agar
Cassava peel	15.13 ± 3.12^{b}	0.00 ± 0.00^{c}	44.50 ± 12.02^{a}	9.67 ± 5.51^{b}	Negative (100%)
Cassava flake	0.00 ± 0.00^{c}	32.00 ± 2.00^{a}	11.00 ± 1.00^{c}	13.00 ± 3.00^{b}	Negative (100%)
Palm kernel	25.50 ± 5.66^{a}	33.75 ± 14.05^{a}	0.00 ± 0.00^{d}	31.43 ± 8.12^{a}	Positive (14.3%)
Sawdust	18.48 ± 8.19^{b}	22.13 ± 6.74^{b}	20.57 ± 8.08^{b}	18.43 ± 2.62^{b}	Positive (50.0%)

abcdMeans (±Standard deviation) in the same column having similar superscript are not significantly different at $p < 0.05$.

The results for screening for the hydrocarbon degraders are presented in Table 5, with isolate S2Q from Sawdust, with 35.7 having the highest average, and isolate CSP3MT(1) from cassava shaft having the lowest average of 3.7.

Table 4

Screening result for heterotrophic degraders

S/N	Isolate code	Lipase Test	BH	OS	Blue Agar	Average
01	S33	13	31	0	+	14.9
02	CSP3MFR	11	31	0	-	14
03	$S_2$33	20	35	0	-	18.3
04	S_3Q	9	26.5	0	+	11.8

Molecular identification of the four most successful biosurfactants hyper-producing strains of Bacteria

The molecular identities of the four most successfully screened isolates are represented in Table 6 with their different ascension numbers. The isolates are completely from Sawdust and Palm kernel.

The gel electrophoresis result is represented in Plate 1, revealing positions of DNA molecular markers, DNA bands of four isolates *Pseudomonas putida* strain SG1, *Acinetobacter baumanii* strain MS14413, *Bacillus zhangzhouensis* strain cdsV18 and *Burkholderia cepacia* strain 717 respectively.

Table 5

Screening results for hydrocarbon degraders

S/N	Isolate code	Lipase test	Blood hemolysis	Oil spreading	Blue agar	Average
1	CSP_1	16	-	36	-	17.3
2	S_23	31.5	17.5	14	+`	21
3	PKBJ	12.5	25.5	0	+	12.7
4	$S_3(2)$	26	27	14	+	22.3
5	S_2Q	25	21	61	-	35.7
6	S_23	18.3	35	17	-	23.5
7	S3	12.5	19.5	13	-	15
8	S_3Q	8	17.5	17	+	14.2
9	PKBMY2	0	13.5	0	-	4.5
10	PKBMYQ	17.5	26	0	-	14.5
11	PKBMR(2)	30	44.5	0	-	24.2
12	PKBJ21	31	-	0	-	10.3
13	CSP3MT(1)	11	-	0	-	3.7
14	CJP2	18.5	-	53	-	23.8
15	CSPMAR3	15	-	0	-	5
16	PKBMY(2)	10	44.5	0	-	18.2
17	PKBJ2	52	48.5	0	-	33.5
18	S2	14	25.5	0	-	13.2
19	S_1Q	12.5	14	8	+	11.5
20	CSF1	-	32	11	-	14.3

PK, Palm kernel bargasse; CF, Cassava flake; S, Sawdust; CP, Cassava peel; CS, Cassava Shaft

Table 6

Molecular identities of the four most successful biosurfactants hyper-producing strains of Bacteria

S/N	Isolate codes	Identity	% Similarity	Accession Number
1	S_2Q	*Pseudomonas putida* SG 1	100	MN318320.1
2	S_23	*Acinetobacter baumanii* MS14413	100	CP054302.1
3	PKBMR (2)	*Bacillus zhangzhouensis* cdsV18	100	MN826587.1
4	PKBJ2	*Burkholderia cepacia* 717	100	NR_029209.1

Plate 1

Gel electrophoresis showing DNA molecular markers, DNA bands of Pseudomonas putida strain SG1, Acinetobacter baumanii strain MS14413, Bacillus zhangzhouensis strain cdsV18 and Burkholderia cepacia strain 717 on lanes 2, 3, 4 and 5 respectively

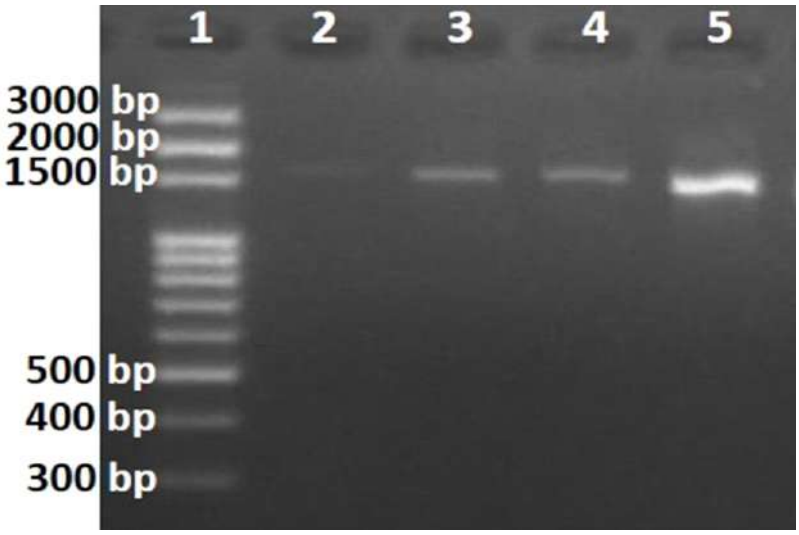
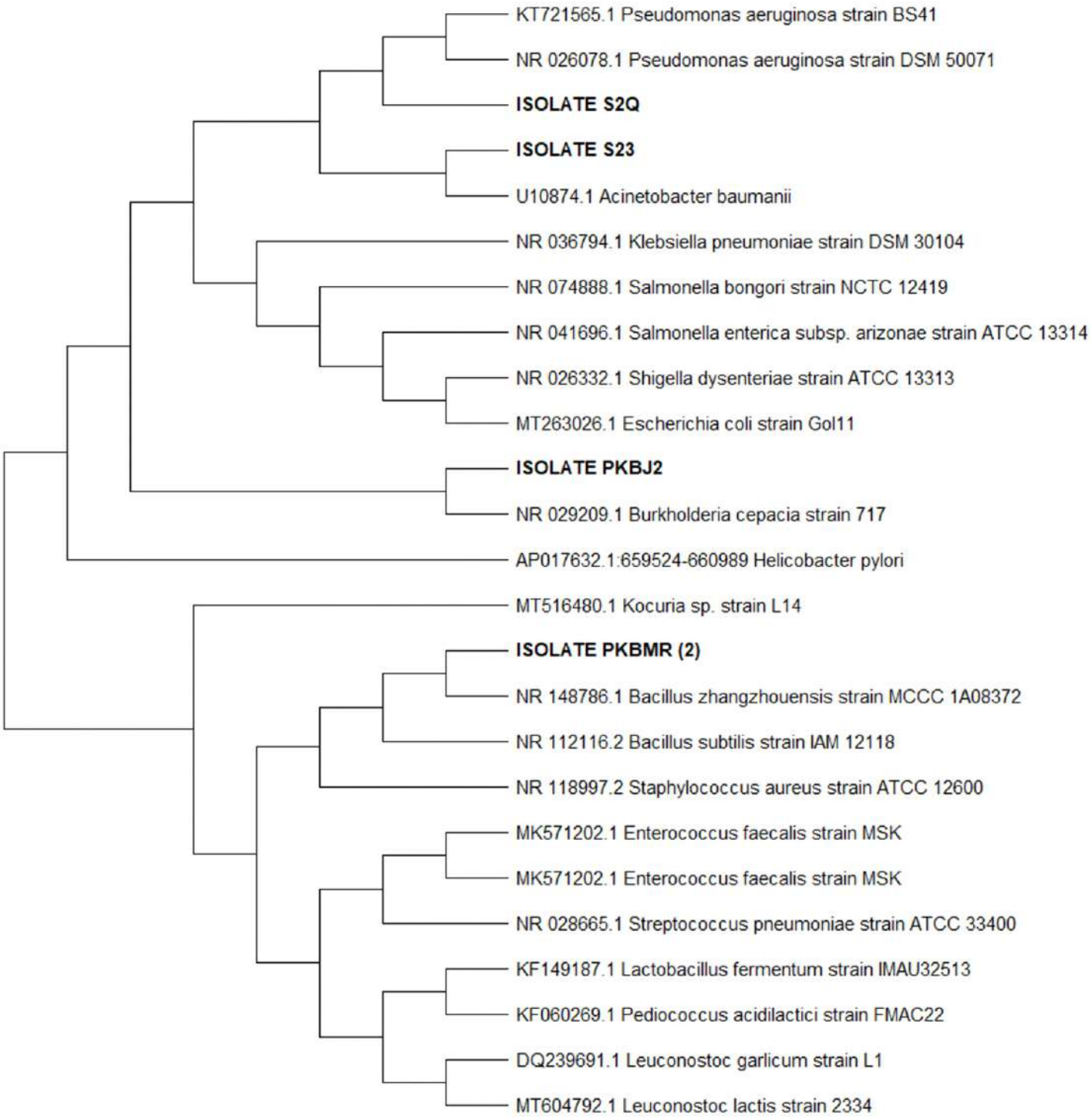


Figure 4
Phylogenetic tree of bacterial strains



Discussion

The outcomes got in the current study showed that cassava squanders recorded the biggest number of province counts, as heterotrophic microscopic organisms have more state counts over the hydrocarbon utilizers since they are critical, as recently detailed by Banat *et al.*(24). In the meantime, Bacillus species were disengaged from every one of the squanders, despite the fact that more are from cassava piece like Gharaei-Fathabad(25) that chipped away at bacterial biosurfactants in the drug industry.

Acinetobacter baumanii MS14413 was isolated from Sawdust which is similar to Lee et al. (26), who reported systems biotechnology for strain improvement using mollases. *Pseudomonas putida* strain SG1 was isolated from Sawdust, similar to Meenakshisundaram *et al.*(19), which studied future microbial surfactants. *Burkholderia cepacia* strain 717 was isolated from palm kernel baggasse, another likely novel biosurfactant producer.

Bustamante *et al.*(27) screened 68 bacterial disconnects from soil and saw just 6% of secludes with great

emulsification action of up to 61%, which is in line with the high emulsification of *Pseudomonas putida* SG1 and *Burkholderia cepacia* strain 717 in the current review. Estimating emulsification units helps pick the carbon and energy hotspots for evaluating biosurfactant creation. Adeyemi et al. (2) utilized a cassava flour handling emanating as a substrate surfactant delivered by *B. subtilis*, which concurred with the current concentrate where *Bacillus zhangzhouensis* was separated been a comparative strain.

The ability of the bacterial isolates from these wastes to produce biosurfactants is important, considering the level of pollution and contamination in the said areas and the need to use indigenous and ecologically friendly products in the remediation process. Moreover, since interest in biosurfactants as a novel research area continue to gain the attention of intending scientist, this demand the development of many methods for the screening of biosurfactant-producing strains. Emulsification capacities of *Pseudomonas putida* strain SG1, *Acinetobacter baumanii* strain MS14413, *Bacillus zhangzhouensis* strain cdsV18 and *Burkholderia cepacia* strain 717 make them a new potential candidate for biosurfactant production. It is noted to state that more of the most outstanding biosurfactant-producing bacteria came from Palm kernel bargasse and Sawdust. Although the most producing isolate *Pseudomonas putida* strain SG1 was isolated from Sawdust. This investigation supports Kalvandi *et al.* (3) theory that surfactants generated by all three isolates are responsible for the release of TPH from the soil. The SHA302 isolate was chosen as an effective isolate for molecular identification owing to its increased efficiency in TPH release. The findings of the 16S rRNA gene sequencing showed the highest degree of affinity (93.98%) between our strain and *Bacillus pumilus* strain ATCC 7061 (T), accession number NR 043242. The strain has the entry number OK285074 and was listed in the gene bank as Bacillus sp. strain SHA302 (T).

It was referred to from Sidkey *et al.* (21) that it is critical to take note that a large portion of the scientists has involved most extreme few evaluating techniques for the choice of biosurfactants makers; they proposed that a solitary strategy isn't reasonable to distinguish all sort of biosurfactants. Thusly, a mix of different techniques is expected for powerful screening. This examination concurred with the crafted by Nordiyana *et al.* (28) that likewise detached *Bacillus subtilis*. They involved Potato substrate as a carbon hotspot for biosurfactant creation.

The screening results of the present study are also in alignment with the work of Femi-Ola *et al.*(29), which screened and characterized biosurfactant-producing bacteria from soil samples in Ogun State, Nigeria. Silva *et al.*(30) and Makkar *et al.*(31) reported the application of a modified drop-collapse technique for surfactant quantization and screening of biosurfactant-producing microorganisms, which is another complimentary screening method but was not considered in the current study.

Conclusion

Cassava peel from the study reveals to have a potential production level than Sawdust and Cassava flake to the oil spreading test, while Palm kernel has a very high emulsification index. All the Agro-industrial wastes considered in this work harbor biosurfactant strains producers with the capacity of mass production. More work should be carried out on these novel biosurfactant production strains.

Author Contribution Statement

The authors confirm their contribution to the paper as follows:

Study conception and design: Ismail B. Onajobi, Jamiu O. Adeyemi.

Data collection: Ismail B. Onajobi, Jamiu O. Adeyemi, Frank A. Orji, Oyindamola J. Samson.

Analysis and interpretation of results: Haneefat O. Egberongbe, Suliamon A. Aina, Oluwabukola T. Afolabi, Lawrence O. Adebajo and Obasola E. Fagade.

Draft manuscript preparation: Ismail B. Onajobi, Jamiu O. Adeyemi, Oyindamola J. Samson.

All authors reviewed the results and approved the final version of the manuscript. All authors agreed to be responsible for all aspects of the work to ensure the accuracy and integrity of the published manuscript.

Conflict of interest

The authors declare no conflict of interest.

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Ethics statement

This work was authorized by the Research Ethics Committee (OOU-REC) of Faculty of Science, Olabisi Onabanjo University, Ago Iwoye.

Availability of data

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References

1. Vijayanand S, Divyashree M. Bioremediation of heavy metals using biosurfactant producing microorganisms. Int. J. Pharm. Sci. Res. 2015; 6(5): 840-847.
2. Adeyemi JO, Onajobi IB, Adebajo LO, Orji FA, Ilusanya OAF, Samson OJ. Biosurfactant Production Potential of some Bacteria recovered from Agro-industrial Wastes. FTSTJ. 2019; 4(3): 870-873.
3. Kalvandi S, Garousin H, Pourbabaee AA, Farahbakhsh M. The release of petroleum hydrocarbons from a saline-sodic soil by the new biosurfactant-producing strain of Bacillus sp. Sci Rep. 2022; 12, 19770. https://doi.org/10.1038/s41598-

022-24321-3
4. Salim AA, Grbavčić S, Šekuljica N, Stefanović A, Jakovetić Tanasković S, Luković N, Knežević-Jugović Z. Production of enzymes by a newly isolated Bacillus sp. TMF-1 in solid state fermentation on agricultural by-products: The evaluation of substrate pretreatment methods. Bioresour Technol. 2017 Mar; 228:193-200. doi: 10.1016/j.biortech.2016.12.081.
5. Onajobi IB, Idowu EO, Adeyemi JO, Samson OJ, Ogunyinka PI, Fagade OE. In vitro antibacterial activities and molecular characterization of bacterial species isolated from farmlands against selected pathogens. Biotechnol Rep (Amst). 2020 Aug 9;27:e00513. doi: 10.1016/j.btre.2020.e00513.
6. Marchant R, Banat IM. Microbial biosurfactants: challenges and opportunities for future exploitation. Trends Biotechnol. 2012 Nov;30(11):558-65. doi: 10.1016/j.tibtech.2012.07.003.
7. Andreollia M, Zapparolia G, Angelini E, Lucchetta G, Lampisa S, Vallini G. Pseudomonas protegens MP12: a plant growth-promoting endophytic bacterium with broad-spectrum antifungal activity against grapevine phytopathogens, Microbiol. Res. 2019; 219 123–131.
8. Sandiford SK. Current developments in antibiotic discovery for treating Clostridium difficile infection, Expert Opin. Drug Discov. 2019; 14 (1) 71–79.
9. Wang M, Zhang B, Li G, Wu T, Sun D. Efficient remediation of crude oil-contaminated soil using a solvent/surfactant system. RSC Adv. 2019; 9(5), 2402–2411. https://doi.org/10.1039/C8RA09964B
10. Marchant R, Banat IM. Microbial biosurfactants: challenges and opportunities for future exploitation. Trends Biotechnol. 2012 Nov;30(11):558-65. doi: 10.1016/j.tibtech.2012.07.003.
11. Arora P, Kshirsagar PR, Rana DP, Dhakephalkar PK. Hyperthermophilic Clostridium sp. N-4 produced a glycoprotein biosurfactant that enhanced recovery of residual oil at 96 °C in lab studies. Colloids Surf B Biointerfaces. 2019 Oct 1;182:110372. doi: 10.1016/j.colsurfb.
12. Ray M, Kumar V, Banerjee C, Gupta P, Singh S, Singh A. Investigation of biosurfactants produced by three indigenous bacterial strains, their growth kinetics and their anthracene and fluorene tolerance. Ecotoxicol Environ Saf. 2021 Jan 15;208:111621. doi: 10.1016/j.ecoenv.2020.111621.
13. Das P, Mukherjee S, Sen R. Biosurfactant of marine origin exhibiting heavy metal remediation properties. Bioresour Technol. 2009 Oct;100(20):4887-90. doi: 10.1016/j.biortech.2009.05.028.
14. Chikere BO, Chijioke–Osuji C. Microbial diversity and physicochemical properties of a crude oil polluted soil. Nig J Microbiol. 2002; 20: 1039-46.
15. Nwachukwu N, Orji A, Iheukwumere I, Ekeleme U. Antibiotic Resistant Environmental isolates of Listeria monocytogenes from Anthropogenic Lakes in Lokpa-Ukwu, Abia State of Nigeria. Aust. J. Basic & Appl. Sci. 2010; 4(7): 1571-1576.
16. Venty S, Sri H, Dewi A. Optimization of biosurfactant production in soybean oil by Rhodococcus rhodochrous and its utilization in remediation of cadmium-contaminated solution IOP Conf. Ser.: Earth Environ. Sci. 2016; 107: 1-8. doi: 10.1088/1755-1315/75/1/012013.
17. Fontes GC, Amaral PF, Nele M, Coelho MA. Factorial design to optimize biosurfactant production by Yarrowia lipolytica. J Biomed Biotechnol. 2010; 821306. doi: 10.1155/2010/821306.
18. Raza ZA, Khalid ZM, Khan MS, Banat IM, Rehman A, Naeem A, Saddique MT. Surface properties and sub-surface aggregate assimilation of rhamnolipid surfactants in different aqueous system. Biotechnology Letters 232 2010: 811–816.
19. Meenakshisundaram M, Pramila M, Rameshwari R. Isolation and screening of biosurfactant-producing bacteria from Uyyokondan river, Tiruchirappalli district, Tamilnadu. Int. J. of Adv. Res. 2016; 4(1): 107-176. www.journalijar.com
20. Ibrahim M, Ijah U, Manga S, Bilbis L, Umar S. Production and partial characterization of biosurfactant produced by crude oil degrading bacteria. Int. Biodeterior. Biodegradation. 2013; 81: 28–34. doi: 10.1016/j.ibiod.2012.11.012
21. Sidkey NM, Mohamed HF, Elkhouly HI. Evaluation of Different Screening Methods for Biosurfactant Producers Isolated from Contaminated Egyptian Samples Grown on Industrial Olive Oil Processing Waste. MRJI. 2016; 17(4):1-9.
22. Joshi M, Deshpande J. Polymerase chain reaction: Methods, principles and applications. International Journal of Biochemistry Resources 2010; 1(15): 81-97.
23. Zhang JT, Liang X. One-way ANOVA for functional data via globalizing the pointwise FF-test. Scand J Stat 2014; 41:51–71.
24. Banat IM, Franzetti A, Gandolfi I, Bestetti G, Martinotti MG, Fracchia L, Smyth TJ, Marchant R. Microbial biosurfactants production, applications and future potential. Appl Microbiol Biotechnol. 2010 Jun;87(2):427-44. doi: 10.1007/s00253-010-2589-0.
25. Gharaei-Fathabad, E. Biosurfactants in pharmaceutical industry: A Mini– Review. Am. J. Drug Discov. Dev. 2011; 1: 58-69.
26. Lee SY, Lee DY, Kim TY. Systems biotechnology for strain improvement. Trends Biotechnol. 2005 Jul;23(7):349-58. doi: 10.1016/j.tibtech.2005.05.003.
27. Bustamante M, Duran N, Diez M. Biosurfactants are useful tools for the bioremediation of contaminated soil: a review. J. Soil Sci. Plant Nutr. 2012; 12(4): 667-687.
28. Nordiyana N, Mohd R, Mohd E, Arbakariya B, Ruzniza M, Helmi W. Isolation and screening of high efficiency biosurfactant-producing bacteria Pseudomonas sp. JOBIMB. 2013; 1(1):25-31.
29. Femi-Ola TO, Oluwole OA, Olowomofe TO, Yakubu H. Isolation and screening of biosurfactant producing bacteria from soil contaminated with domestic waste water. J. Nat. Sci. Res. 2016; 4(13): 59-68.
30. Silva TR, Duarte AWF, Passarini MRZ, Ruiz ALTG, Franco CH, Moraes CB, et al. Bacteria from Antarctic environments: diversity and detection of antimicrobial, antiproliferative, and antiparasitic activities. Polar Biol. 2018; 41 1505–1519.
31. Makkar RS, Cameotra SS, Banat IM. Advances in utilization of renewable substrates for biosurfactant production. AMB Express. 2011 Mar 28;1(1):5. doi: 10.1186/2191-0855-1-5