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ISOLATION, SCREENING AND CHARACTERIZATION OF BIOSURFACTANTS-PRODUCING BACTERIA FROM PETROLEUM PRODUCTS-IMPACTED SOIL

[1]Temitope, O. [1]Oyeleke, S. B, [1]Abioye, O.P, [2]Tijani, J.O.

[1] Department of Microbiology, School of Life Sciences, Federal University of Technology, Minna, Niger State-Nigeria

[2]Department of Chemistry, School of Life Science, Federal University of Technology, Minna, Niger State-Nigeria

temyfemy@gmail.com

ABSTRACT

Microbial surfactants are a diverse and heterogeneous microbial metabolites synthesized by bacteria, yeasts, and fungi. They are equally biodegradable and resilient to pH, temperature, and ionic quality. This study was carried out to isolate, characterize and screen bacterial isolates for abilities to produce biosurfactants. Soil samples were collected in 2018 from refined petroleum products contaminated soil in Ladipo Market, Lagos, Nigeria and transported to the Biotechnology Laboratory, Federal Institute of Industrial Research, Oshodi, Lagos state –Nigeria. The total petroleum hydrocarbon utilizing bacteria was determined, and pure cultures were subjected to biosurfactant screening assays like haemolytic indices assay, blue plate assay, lipase assay, and emulsification index (E24) assay. The hyper-producers of biosurfactants were further identified using biochemical tests. The heterotrophic bacterial counts and total hydrocarbon utilizing bacteria in the crude oil-contaminated soil were between the ranges of 1.46×105-1.0×108 and 1.8×104-5.2×106cfu/g of the soil. The emulsification index of the hydrocarbon utilizing isolates. Emulsification activity carried out showed that while the supernatants obtained from isolates B(Bacillus spp), C(Bacillus spp) D(Proteus spp), E(Corynebacterium spp), J (Bacillus spp), K(Micrococcus spp), L(Serratia spp), S315(Staphylococcus spp) showed no emulsification

activity, supernatants obtained from isolates E301(Bacillus spp), SS206(Klebsiella spp), I(Acinetobacter spp), G(Acinetobacter spp) and H(Pseudomonas spp) were able to emulsify crude oil to some extent with the values of 60.6%, 60.8%, 58.5%, 44.65% and 58.5% respectively. Out of the total 22 bacterial isolates, 13(59%) showed the presence of anionic surfactants, while 9(44%) showed the presence of cationic surfactants. Isolates H (Pseudomonas spp), SS327 (Burkholderia ambifera), G (Acinetobacter spp), I(Acinetobacter spp)SS206(Klebsiella spp) which are hyper-producers of biosurfactants were biochemically identified as Pseudomonas species, Burkholderia species, Acinetobacter species, Acinetobacter species, and Klebsiella species. There is a need to intensify research attention on the area of biosurfactants to sustain local industries and save foreign reserves through local production.

Key words: Biosurfactants, bacteria, emulsification, sustainable Development

INTRODUCTION

Surface-active compounds of biological origin, mostly microorganisms, have attracted much attention, and their popularity has steadily increased recently. This may be due to new or innovative processes in industrialization and the quest for continuous improvement in sustainability (Ibukun and Thring, 2018). The wide range of benefits of biosurfactants compared to their synthetic counterparts (chemical surfactants) are reasons for increasing research in this area. These include the ability to thrive and function within a wide range of temperatures, pH, and environmental degradability. They are environmentally friendly with confirmed abilities for removing petroleum hydrocarbon contaminants from drill cuttings and hydrocarbon-contaminated waste streams (Souza *et al*., 2014). In addition, they are highly favourable because of their high biodegradability, high specificity, high stability and activity at extremely low environmental impact, low toxicity, a wide range of industrial applications, and structural diversity (Luna *et al.,* 2015). This reassures us about their potential to be a sustainable and environmentally friendly alternative to chemical surfactants. Microbial surfactants, a diverse and heterogeneous group of microbial metabolites, are synthesized by a variety of microorganisms, including bacteria, yeasts, and fungi. They share a chemical relationship with compounds such as rhamnolipids and surfactin (Brumano *et al.,* 2016; Varjani *et al.,* 2017), contributing to their diverse nature.

They are classified into various groups based on chemical structure: lipoproteins, glycolipids, phospholipids, lipopeptides, fatty acids, neutral acids, etc (Mulligan, 2005). Microbial surfactants have been applied on both a laboratory scale and pilot scale for numerous biotechnological and industrial applications (Satpute, 2010). Surfactants are broadly utilized for mechanical, farming, nourishment, beauty care products and pharmaceutical applications. However, the vast majority of these mixes are orchestrated artificially and possibly cause ecological and toxicology issues due to these substances' refractory and determined nature (Ron and Rosenbery, 2002; Ibukun and Thring, 2018). The properties of biosurfactants viz-a-viz their chemically synthesized counterparts and broad substrate availability made them suitable for commercial applications. Microbial surfactants exhibit surface movement, resilience to pH, temperature and ionic quality, and biodegradability (Desai and Banat, 1997). This investigation aims to isolate, screen and characterize biosurfactants-producing bacteria from petroleum hydrocarbon-contaminated soil

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MATERIALS AND METHODS

Collection of Samples

Oil-contaminated soil samples from mechanic workshops were collected at Mechanic Workshops in Ladipo market, near Oshodi, and transported to the laboratory for analysis.

The Enumeration of Hydrocarbon Utilizing Bacteria (HUB)

This was done by using the mineral salt Agar/ vapour phase method, as described by Amanchukwu *et al.* (1989). The Mineral salt agar contained (g/l) 15 g $NaNO_3$, 1.1 g KCl, 1.1 g NaCl, 0.00028 g $FeSO_4.7H_2O$, 3.4g KH_2PO_4, 4.4 g K_2HPO_4, 0.5g $MgSO_4.7H_2O$, 15g Agat Technical. The Petri dish lid was loaded with filter paper (Whatman no 1) impregnated with bonny light crude oil. The incubation was done at 37°C for 5 days. Subculture was done using nutrient agar to obtain discrete colonies.

Screening for Biosurfactant Producing Bacteria

The isolated colonies were tested for their biosurfactant production using four methods.

Blood Haemolysis Test

Each isolate was streaked on blood agar medium and incubated at 37°C for 24 - 48 hours to assay for haemolytic activity. The plates were visually inspected for zones of clearing around the colonies, indicative of biosurfactant production (Mulligan, 2005)

Emulsification Stability Test

This was done by homogenizing an equal volume of kerosine and cell-free supernatant by vortexing at 1000rpm for 2 minutes, after which the mixture was allowed to stand for 24 hours before calculating the emulsification index using the formula stated in Equation 1 (Cooper *et al*., 2002). The emulsification index at 24 hours was calculated by

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$$\%E24 = \frac{\text{Height of emulsified layer}}{\text{Height of liquid layer}} x100 \ldots\ldots \text{Equation 1.}$$

Blue Plate Assay Method

The Cetyltrimethylammonium bromide (CTAB) agar plate method is a semi-quantitative assay for detecting extracellular glycolipids or other anionic surfactants. Siegmund and Wagner developed it. Blue agar plates containing cetyltrimethylammonium bromide (CTAB) (0.2 mg ml-1) and methylene blue (5 mg ml-1) were used to detect extracellular glycolipid production. Biosurfactants were observed by the formation of dark blue halos around the colonies (Satpute *et al.,* 2010).

Lipase Screening Method

The hydrolytic activity of each of the bacterial isolates was done on a lipase screening medium (Trybutyrin agar) with the following composition: 20 (g/L): peptone, 10; NaCl, 5; CaCl2.2H2O, 0.1; Trybutyrin, 10 mL (v/v). The agar was freshly prepared, and pure cultures were spot-inoculated in the central position of the Petri dish. After incubation, the clearance zones were carefully examined and measured with a ruler in millimetres (mm). (Okoli *et al.,*2019).

Biochemical Characterization of the Isolates

Biochemical tests were carried out on isolates capable of producing biosurfactants. These included a catalase test, a urease test, nitrate reduction, hydrogen sulphide production, casein, and gelatin liquefaction.

RESULTS AND DISCUSSION

The heterotrophic bacterial counts and total hydrocarbon utilizing bacteria in the hydrocarbon-contaminated soil ranged between 1.46×105-1.0×108and 1.8×104-5.2x×106cfu/g respectively. Statistics showed a significant difference in the population size of hydrocarbon-utilizing bacteria

compared to the heterotrophic bacterial population. The high heterotrophic bacterial population reflects an environment with relatively abundant nutrients/carbon sources besides petroleum hydrocarbon. In addition, the relatively high population of hydrocarbon-utilizing bacteria reflects an environment in which the bacterial communities are adapted to survive through the metabolism of crude oil.

In a similar report in Niger Delta soil polluted by crude oil, Ibiene *et al* (2011) reported that soils of Mogho and Aluu (Port Harcourt, Nigeria) had total heterotrophic bacteria counts between 6.56×x103 and 1.94×107cfu/g, while the hydrocarbon utilizing bacteria counts in Mogho and Aluu soils were in the range of 3.11×103-2.56×104cFu/g. Chikere and Ekuuabu (2014), in a culture-dependent approach, reported similarly that crude oil-polluted sites in the Bodo community in Gokana LGA of Rivers State-Nigeria, had a very low population of hydrocarbon utilizing bacteria [0.1-8.0×106] and heterotrophic bacterial counts (0.1x107-0.2×108cfu/g) Uba *et al.* (2019) observed in a diesel oil contaminated soil that the total heterotrophic bacterial counts were within the range of 8.3-8.9×105cgu/g while the hydrocarbon utilizing bacterial counts still at the range of 8.65-9.2(Log10).

The identities of the hydrocarbon-utilizing bacteria and the biosurfactant producers are reported in Table 2. These include *Pseudomonas, Bacillus*, *proteus*, *Corynebacterium*, *Acinetobacter, Micrococcus, Klebsiella, Staphylococcus*, and *Burkholderia* spp (Table 2). Edlund and Jansson (2006) found that members of the class Gammaproteobacteria *(Pseudomonas* spp. inclusive) and *Flavobacterium* spp. were the most dominant bacteria in a highly PAH-- and polychlorinated biphenyl-polluted sediment before and after dredging. Said *et al.* (2008) isolated *Bacillus, Staphylococcus, Pseudomonas and Acinetobacter spp.* capable of degrading PAHs from a polluted sediment. The works of Margesin *et al.* (2003) and Quatrini *et al.* (2008) demonstrated that Actinobacteria play an important role during petroleum hydrocarbon degradation. Adebusoye *et al.* (2008) had previously repeated the biosurfactant potential of some wild strains of C*orynebacterium* spp DDVI, *Flavobacterium*sp, *Micrococcus roseus* DDV3, *Pseudomonas aeruginosa* DDV4.

Table 1: Counts of Total Heterotrophic Bacteria (THB) and Total Hydrocarbon Utilizing Bacteria (THUB) in Hydrocarbon Contaminated Soil

BACTERIA	Cfu/g
THB	$1.46\pm1.53\times10^{5}$
THB	$1.01\pm0.06\times10^{8}$
THB	$7.4\pm0.72\times10^{8}$
THUB	$1.8\pm0.25\times10^{4}$
THUB	$8.5\pm0.47\times10^{5}$
THUB	$5.2\pm0.66\times10^{6}$

Values are Mean ±SEM of duplicate determinations. Values with different alphabets along a row are significantly different at $p<0.05$

Table 2: The Morphology and Selected Biochemical Characteristics of Bacterial Isolates

ISOLATE CODE	COLONIAL APPEARANCE	GRAM STAIN AND MORPHOLOGY	CATALASE TEST	OXIDASE TEST	UREASE TEST	H_2S PRODUCTION	CASEIN HYDROLYSES	GELATIN HYDROLYSIS	PRESUMPTIVE IDENTITY
A	Yellow, dry, raised with serated edges. 9-10mm in diameter	Gram negative rods in cluster and in chains	Positive	+	+	+	+	+	*Pseudomonas spp*
B	Creamy yellow, wet, raised with entire edge. 3-5mm in diameter	Gram positive rods	Positive	-	+	+	+	-	*Bacillus spp*
C	Cream, wet, raised with entire edge. 3-5mm in diameter	Gram positive cocci in twos and multiples of twos	Positive	_	+	+	+	+	*Bacillus spp*
D	Swimming organism, cream with brownish pigments, entire, 3-5mm in diameter	Gram negative rods in irregular clusters	Positive	+	+	-	-	-	*Proteus species*

E	Cream, wet, raised with entire edge. 3-5mm in diameter	Gram positive rods in irregular clusters with club like ends	Positive	+	_	+	+	+	*Corynebacterium spp*
F	Swimming organism, cream with light green pigments, entire, 3-5mm in diameter	Gram NEGATIVE rods in clusters	NEGATIVE	-	+	-	-	-	*Pseudomonas spp*
G	Colonies were creamish, entire, 3-5mm in diameter	Gram negative rods in irregular clusters	-	-	+	-	+	+	*Acinetobacter spp*
H	cream with brownish pigments, entire, 3-5mm in diameter	Gram negative rods in irregular clusters	Negative	-	+	-	-	-	*Pseudomonas spp*
I	Cream, wet, raised with entire edge. 3-5mm in diameter	Gram negative rods in irregular clusters	Negative	_	+	-	+	+	*Acinetobacter spp*
J	Cream, wet, raised with entire edge. 2-3mm in diameter	Gram positive rods in irregular clusters	Positive	+	+	-	+	-	*Bacillus spp*
K	Cream, wet, raised with entire edge. 3-5mm in diameter	Gram positive rods in irregular clusters	Positive	+	+	-	-	-	*Micrococcus spp*
L	Cream, wet, raised with entire edge. 3-5mm in diameter with reddish pigmentation	Gram Negative rods in clusters	NEGATIVE	-	+	-	-	-	*Serratia species*
SS206	Cream, wet, raised with entire edge. 3-5mm in diameter and appearing	Gram negative rods in singles and in pairs	Negative	-	-	-	-	-	*Klebsiella* sp.

	sticky on the wire loop								
SS314	Cream, wet, raised with entire edge. 3-5mm in diameter	Gram positive rods in irregular clusters	Positive	-	+	+	+	-	*Bacillus species*
SS315	Cream, wet, raised with entire edge. 2-3mm in diameter	Gram positive cocci in pairs	Positive	-	-	-	+	-	*Staphylococcus species*
SS325	Moderate heart like organism, entire, colourless and raised. 10-12mm in diameter	Gram positive rods in irregular clusters	Positive	-	+	+	+	+	*Bacillus spp*
SS327	Cream, wet, raised with entire edge. 3-5mm in diameter	Gram negative rods in irregular clusters	NEGATIVE	-	+	-	+	+	*Burkholderia ambifera*
SG140	Yellow, dry, raised with serated edges. 9-10mm in diameter	Gram positive rods in irregular clusters	Positive	-	+	+	+	+	*Bacillus spp*
SG146	Swimming organism, cream with crystal violet pigments, entire, 3-5mm in diameter	Gram negative rods in irregular clusters	Positive	+	+	-	-	+	*Chromobacterium spp*
F047	Swimming organism, cream with brownish pigments, entire, 3-5mm in diameter	Gram positive rods in clusters	Positive	-	+	+	+	+	*Bacillus species*
E301		Gram positive rods in clusters	Positive	-	+	-	-	+	*Bacillus species*

The reports documented that *Corynebacterium* sp DDVI and *Pseudomonas aeruginosa* had emulsification indices of 63 % and 78 %, respectively. This is within the range of emulsification indices observed in bacterial isolates in this study. In a related study, Ndibe *et al.* (2018) observed that similar groups of bacteria (*Bacillus*/*Corynebacterium, staphylococcus auerus*) from River Rido, Kaduna State, Nigeria exhibited an emulsification index between 42 and 64 %. The emulsification index of the hydrocarbon utilizing isolates. Emulsification activity carried out revealed that while the supernatants obtained from isolates B (*Bacillus* spp), C(*Bacillus* spp), D (*Proteus*spp), E (*Corynebacterium*spp), J(*Bacillus*spp), K(*Micrococcus* spp), L(*Serratia* spp), SS315(*Staphylococcus* spp), SS325 (*Bacillus*spp), SG140 (*Bacillus* spp) and F047(*Bacillus* spp) showed no emulsification activity, supernatants obtained from isolates E301(*Bacillus* spp), SS206(*Klebsiella* spp), I(*Acinetobacter* spp), G(*Acinetobacter* spp) and H(*Pseudomonas*spp) were able to emulsify crude oil to some extent with the values of 60.6 %, 60.8 %, 58.5 %, 44.65 % and 58.5 % respectively. Emulsification assay is an indirect method used to screen biosurfactant production. This assumption is that if the cell-free culture broth used in this assay contains biosurfactant, it will emulsify the hydrocarbons in the test solution. Emulsification assay is an indirect method used to screen biosurfactant production. This assumption is that if the cell-free culture broth used in this assay contains biosurfactant, it will emulsify the hydrocarbons in the test solution. Bonilla *et al*. (2005) showed that emulsifying activities (E24) determine the productivity of biosurfactants, and those are given as a percentage of the height of the emulsified layer divided by the total height.

Feniboet *al.* (2019) opined *that Pseudomonas aeruginosa*, *Bacillus subtilis, Acinetobacter calcoceticus* and *Candida albicans* represent major genera of microorganisms with high ability to produce emulsions, rhamnolipids, surfactin, sophorolipids, mannosylerithritol lipids. *Klebsiella pneumonia* (strain IVN51) isolated from hydrocarbon–polluted soil in Ogoniland (Niger Delta) soil showed an emulsification index of 60 % as compared with the emulsification index of sodium dodecyl sulphate (SDS) (Anna and Marinella, 2022).

The blue-plate assay was introduced into the screening to ascertain the types of charges released by microbial surfactants (Table 4). The plates with deep bluish colouration after the growth of the bacteria tentatively showed that the surfactant produced by the bacteria is negatively charged. However, the Non--bluish growth pattern tentatively indicates that the surfactant produced by the bacteria is positively charged. In this current study, out of a total of 22 bacterial isolates, 13(59%) showed the presence of anionic surfactants, while 9(44 %) showed the

presence of cationic surfactants. Hussain and Khan (2018) had previously reported the presence of anionic surfactant-producing *Pseudomonas aeruginosa* strain from soils of automobile workshops in Aligash, India.

.**Table 3: The Emulsification Index of the Hydrocarbon-utilizing Bacterial Isolates Screened for Biosurfactants Production**

Isolate code/Bacteria	Emulsification Index (%)
A(*Pseudomonas* spp)	5.80±2.5
B(*Bacillus* spp)	0.00±0.00[a]
C(*Bacillus* spp)	0.00±0.00[a]
D(*Proteus* species)	0.00±0.00[a]
E(*Corynebacterium* spp)	0.00±0.00[a]
F(*Pseudomonas* spp)	3.00±0.30[a]
G(*Acinetobacter* spp)	44.65±10.15[bc]
H(*Acinetobacter* spp)	41.00±6.40[bc]
I(*Acinetobacter* spp)	58.45±0.35[c]
J(*Bacillus* spp)	0.00±0.00[a]
K(*Micrococcus* spp)	0.00±0.00[a]
L(*Serratia* species)	0.00±0.00[a]
SS206(*Klebsiella* sp)	60.80±0.10[c]
SS314(*Bacillus* species)	3.75±1.25[a]
SS315(*Staphylococcus* species)	0.00±0.00[a]
SS325(*Bacillus* spp)	28.84±28.84[a]
SS327(*Burkholderia ambifera*)	56.50±0.30[c]
SG140(*Bacillus* spp)	0.00±0.00[a]
SG146(*Chromobacterium* spp)	10.1±1.80[b]
F047(*Bacillus* species)	0.00±0.00[a]
E301(*Bacillus* species)	60.55±4.45[c]

Values are Mean ±SEM of duplicate determinations. Values with different alphabets along a row are significantly different at $p<0.05$

Table 4: Blue Plate Assay on Hydrocarbon-Utilising Bacterial Isolates

Isolate Code/Bacteria	Observation	Remarks
A(*Pseudomonas* spp)	Bluish coloration and moderate growth	Anionic surfactant
B(*Bacillus* spp)	Bluish coloration and moderate growth	Anionic surfactant
C(*Bacillus* spp)	Non bluish coloration and moderate growth	Cationic surfactant
D(*Proteus* species)	Bluish coloration and moderate growth	Anionic surfactant
E(*Corynebacterium* spp)	Non bluish coloration and moderate growth	Cationic surfactant
F(*Pseudomonas* spp)	Bluish coloration and moderate growth	Anionic surfactant
G(*Acinetobacter* spp)	Bluish coloration and moderate growth	Anionic surfactant
H(*Acinetobacter* spp)	Bluish coloration and moderate growth	Anionic surfactant
I(*Acinetobacter* spp)	Bluish coloration and moderate growth	Anionic surfactant
J(*Bacillus* spp)	Bluish coloration and moderate growth	Anionic surfactant
K(*Micrococcus* spp)	Non bluish coloration and moderate growth	Cationic surfactant
L(*Serratia* species)	Bluish coloration and moderate growth	Anionic surfactant
SS206(*Klebsiella* sp)	Bluish coloration and moderate growth	Anionic surfactant
SS314(*Bacillus* species)	Bluish coloration and moderate growth	Anionic surfactant
SS315(*Staphylococcus* species)	Non bluish coloration and moderate growth	Cationic surfactant
SS325(*Bacillus* spp)	Bluish coloration and moderate growth	Anionic surfactant
SS327(*Burkholderia ambifera*)	Bluish coloration and moderate growth	Anionic surfactant
SG140(*Bacillus* spp)	Non bluish coloration and moderate growth	Cationic surfactant
SG146(*Chromobacterium* spp)	Non bluish coloration and moderate growth	Cationic surfactant
F047(*Bacillus* species)	Non bluish coloration and moderate growth	Cationic surfactant
E301(*Bacillus* species)	Non bluish coloration and moderate growth	Cationic surfactant

In this study, the ability to express lipase enzymes was one of the integral screening procedures, as reported in Table 5. The zone of clearance by the bacteria on trybutyrin agar showed that isolates C (*Bacillus* spp), H (*Pseudomonas* spp), SS206 (*Klebsiella* sp), SS314 (*Bacillus* spp),

SS315(*Staphylococcus species*), SS327(*Burkholderia ambifera*), SG146 (*Chromobacterium spp*), F047 (*Bacillus* spp), and E301 (*Bacillus spp*) had the highest zone of 15.50 cm, 16.50 cm, 25.0 cm, 18.50 cm, 24.00cm, 18.50 cm, 16.00cm, and 13.00 cm respectively (Table 5).

The microorganisms that degrade or utilize petroleum hydrocarbons mostly possess genes for breaking down complex lipid molecules through lipase (Enzyme). These same groups of hydrocarbon utilizers are also biosurfactant producers. Pendse and Aruna (2018) also reported that hydrocarbon-utilizing bacterial isolates with the ability to produce biosurfactants also showed strong abilities to produce lipase. Zarinviarsagh *et al.* (2017) showed *Ochrobactrum intermedium strain* MZV101 with potentials for lipase and biosurfactants producing potentials. The ability of isolates to produce haemolysis on a blood agar plate indicates their ability to produce biosurfactants. Three types of haemolysis are known to occur: α, β, and γ. Alpha (α) haemolysis is said to occur when a greenish colouration is produced around the colony. Beta (β) haemolysis occurs when a clear zone is produced around the colony, while Gamma (γ) haemolysis (γ) occurs when no change occurs around the colony. The blood hemolysis pattern of the hydrocarbon-utilizing bacteria was studied as part of the screening program to identify the hyperproducers of biosurfactants. Isolates A (*Pseudomonas* spp), D (*Proteus species*), E (*Corynebacterium* spp), F (*Pseudomonas* spp), G (*Acinetobacter*spp), H (*Pseudomonas* spp), I (*Acinetobacter* spp), SS315 (*Staphylococcus* spp), SS 325 (*Bacillus* spp), SS 327 (*Burkholderia ambifera*), SG 140(*Bacillus* spp), SG 146(*Chromobacterium* spp), F047(*Bacillus* species), and E301(*Bacillus* species), had haemolytic zones of 33.00 mm, 25 mm, 32.00 mm, 59.50 mm, 14.50 mm, 35.00 mm, 41.00 mm, 35.50 mm, 35.00 m, 77 m, respectively (Table 6). Bacterial isolates coded A (*Pseudomonas* spp), D (*Proteus* species), F (*Pseudomonas* spp), G (*Acinetobacter* spp), SS314 (*Bacillus* species), SS315 (*Staphylococcus* species), SS327(*Burkholderia ambifera*) had complete β-haemolysis and are identified as best or hyper-producers of biosurfactants. Haemolytic activity appears to be a good screening criterion in the generic search for biosurfactants in microorganisms, as Saravanakumari *et al.* (2010) mentioned. In another related study, Astuti *et al.* (2019) also documented high haemolytic indices of *Pseudoxanthomonas* sp strain G3, which had a high emulsification index of 72% and reduced interfacial tension between 12.6-9.7 dynes/cm. Isolates H (*Pseudomonas* spp), SS327 (*Burkholderia ambifera*), G (*Acinetobacter* spp), I (*Acinetobacter* spp), SS206 (*Klebsiella* sp) had proved to be the best of the biosurfactants producing bacteria based on the screening results as discussed in this study

Table 5: Effect of Lipase Producing Potential

Isolate Code/Bacteria	Mean (Zone Of Clearance)
A(*Pseudomonas* spp)	0.00±0.00[a]
B(*Bacillus* spp)	0.00±0.00[a]
C(*Bacillus* spp)	15.50±2.50[cd]
D(*Proteus* species)	7.50±2.50[b]
E(*Corynebacterium* spp)	0.00±0.00[a]
F(*Pseudomonas* spp)	0.00±0.00[a]
G(*Acinetobacter* spp)	5.00±0.00[b]
H(*Acinetobacter* spp)	16.50±1.50[cd]
I(*Acinetobacter* spp)	5.00±0.00[b]
J(*Bacillus* spp)	5.00±0.00[b]
K(*Micrococcus* spp)	0.00±0.00[a]
L(*Serratia* species)	0.00±0.00[a]
SS206(*Klebsiella* sp)	25.00±3.00[f]
SS314(*Bacillus* species)	18.50±0.50[de]
SS315(*Staphylococcus* species)	24.00±2.00[f]
SS325(*Bacillus* spp)	0.00±0.00[a]
SS327(*Burkholderia ambifera*)	22.00±2.00[ef]
SG140(*Bacillus* spp)	6.00±1.00[b]
SG146(*Chromobacterium* spp)	18.50±1.50[de]
F047(*Bacillus* species)	16.00±2.00[cd]
E301(*Bacillus* species)	13.00±1.00[c]
A(*Pseudomonas* spp)	

Values are Mean± SEM of duplicate determinations. Values with different alphabets along a row are significantly different at $p<0.05$

Table 6: Effect of Blood Haemolytic Indices

Isolate Code	Mean (Zone Of Clearance In Mm)	Type Of Haemolysis
A(*Pseudomonas* spp)	33.00±2.00^{de}	β(complete zone)
B(*Bacillus* spp)	0.00±0.00^{a}	-
C(*Bacillus* spp)	25.00±2.00^{c}	β(complete zone)
D(*Proteus* species)	32.00±2.00^{cd}	α(incomplete zone)
E(*Corynebacterium* spp)	59.50±1.50	β(complete zone)
F(*Pseudomonas* spp)	14.50±2.50^{b}	β(complete zone)
G(*Acinetobacter* spp)	35.00±4.00^{de}	α(incomplete zone)
H(*Acinetobacter* spp)	41.00±2.00^{e}	α(incomplete zone)
I(*Acinetobacter* spp)	0.00±0.00^{a}	-
J(*Bacillus* spp)	0.00±0.00^{a}	-
K(*Micrococcus* spp)	0.00±0.00^{a}	-
L(*Serratia* species)	0.00±0.00^{a}	-
SS206(*Klebsiella* sp)	0.00±0.00^{a}	β(complete zone)
SS314(*Bacillus* species)	35.50±4.50^{de}	β(complete zone)
SS315(*Staphylococcus* species)	35.00±4.50^{de}	α(incomplete zone)
SS325(*Bacillus* spp)	77.00±8.00^{g}	β(complete zone)
SS327(*Burkholderia ambifera*)	0.00±0.00^{a}	-
SG140(*Bacillus* spp)	0.00±0.00^{a}	-
SG146(*Chromobacterium* spp)	0.00±0.00^{a}	α(incomplete zone)
F047(*Bacillus* species)	62.50±2.50^{f}	-

Values are Mean± SEM of duplicate determinations. Values with different alphabets along a row are significantly different at $p<0.05$

CONCLUSION AND RECOMMENDATIONS

In summary, this study successfully isolated and characterized 22 strains of soil-dwelling bacteria, of which 13(59%) showed the presence of anionic surfactants, while 9(44%) showed the presence of cationic surfactants. Hyper-producers of biosurfactants were biochemically

identified as *Pseudomonas* species, *Burkholderia*species, *Acinetobacter* species, and *Klebsiella* species. It is highly recommended that these organisms be subjected to molecular characterization as the biochemical characterization method used in this study is inadequate to ascertain the identity of the organisms. Also, there is a need to intensify research attention on biosurfactant production, which is a better alternative to synthetic surfactants in diverse industries.

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