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Isolation and characterization of biosurfactant producing bacteria from Mile 2 and Ologe Lagoons, Nigeria

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ABSTRACT

Aims: This study was aimed to isolate and characterize biosurfactant producing bacteria from Mile 2 and Ologe Lagoon which are sinks for domestic and industrial waste waters and potential source of value added bioresources such as biosurfactants, hydrocarbon degraders and organisms with potential for biotechnological applications.
Methodology and results: Physicochemical parameters of the two lagoon waters were analyzed using standard procedures. Bacteria were isolated using enrichment techniques on 1% Escravos light crude oil, palm oil and groundnut oil on mineral salt medium (MSM). Biosurfactant production by the isolates was assayed by hemolytic activity, oil spread test, blue agar test and emulsification activity. Isolates were identified using their colony morphologies and biochemical characteristics, while the antibiotic susceptibility of the isolates was determined using multidisc. The physicochemistry of the lagoon water showed high nitrate content of 15.7 mg/L and 19.6 mg/L for Mile 2 and Ologe Lagoon, respectively. Total hydrocarbon content (THC) of both lagoon waters was low, with values 0.53 mg/L for Mile 2 Lagoon and 0.44 mg/L for Ologe Lagoon. The predominant genera of bacteria identified include *Micrococcus*, *Bacillus*, *Pseudomonas*, *Acinetobacter*, *Stomatococcus* and *Moraxella*. A total of 23 bacterial isolates were tested for hemolytic activity, of which 13 showed β-hemolysis which is presumptive for biosurfactant production, 5 showed α-hemolysis and the remaining 5 exhibited γ-hemolysis. Majority of the isolates were positive for oil spread assay and blue agar test (19) indicating production of anionic biosurfactant. The isolates showed good emulsification activity; AGG3 (67.7%), AGG1 (62.3%), AGG2 (60%), AGG4 (60%), MTP2 (56%), AGC4 (54%) and the least emulsification value of 23.3% for strain AGP1. Most of the isolates were susceptible to ciprofloxacin, perfloxacin and showed resistance to septrin and erythromycin.
Conclusion, significance and impact of study: This study showed that Mile 2 and Ologe Lagoon are a potential source of biosurfactant producers with diverse emulsification properties and prospective industrial applications. This would have implication for economic empowerment, as well as sustainable and environmentally friendly clean-up technology in both locally and globally.

Keywords: Bioremediation, biosurfactants, emulsifying bacteria, emulsification index, lagoon water

INTRODUCTION

Biosurfactants are surface active molecules with hydrophilic and hydrophobic moieties that segregate at liquid/solid, liquid/gas, or liquid/liquid interfaces (Liu *et al.*, 2015; da Silva Araújo *et al.*, 2020). These surface-active compounds with emulsifying activities are usually produced by microbes due to presence of hydrocarbons in their environment. Hence, biosurfactants are mostly produced by hydrocarbon-degrading microorganisms during their lag phase of growth through excretion or adhesion to the cells especially when cultivated on substrates that are insoluble in water. Biosurfactants allow microorganisms to grow on hydrophobic substrate by reducing surface tension between phases thereby, making the substrates accessible for uptake and metabolism (Aparna *et al.*, 2011; Patowary *et al*., 2017). The process allows bioavailability of contaminants for microbial degradation through better solubilization of hydrocarbons in water (Banat *et al.*, 2014).

Biosurfactants are generally categorized by their microbial origins and chemical structures into six classes. These are glycolipids, lipopeptides (lipoproteins), neutral lipids, phospholipids, polymeric and particulate biosurfactants (Banat *et al.*, 2010; Vijayakumar and Saravanan, 2015). A good number of biosurfactants are anionic or neutral while the amine containing groups are cationic. Their hydrophobic moiety consists of long chain

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fatty acids, while the hydrophilic moiety can be carbohydrate, cyclic peptide, amino acid, phosphate carboxyl acid or alcohol. The molar mass of biosurfactants generally ranges from 500 to 1500 Da (Campos *et al.*, 2013).

A great number of studies have reported the production of biosurfactants, consisting of diverse chemical compositions, by different groups of microorganisms originating from aqueous environments, polluted and unpolluted soils with crude oil*. Bacillus* and *Pseudomonas* are the most prolific producers of biosurfactant reported in literature (Obayori *et al.*, 2009; Silva *et al.*, 2014). Ilori and Amund (2001) reported production of peptidoglycolipids bioemulsifier by *P. aeruginosa* isolated from crude oil polluted soil in Nigeria. Patowary *et al.* (2017) also reported isolation of a potent strain of *Pseudomonas aeruginosa* from hydrocarbon polluted soil with potential to produce biological surface-active molecules, thereby utilizing the contaminants as carbon and energy source leading to complete mineralization of the pollutant. Different strains of fungi and yeasts have also been reported to produce biosurfactants. Adebusoye *et al*. (2008) described the isolation of two yeast strains, *Saccharomyces cerevisiae* and *Candida albicans*, from polluted lagoon water in Lagos.

Biosurfactants are high-value microbial products gaining interest owing to their eco-friendliness. They have gained application in medicine, industrial and biotechnological sector (Nitschke and Costa, 2007; Makkar *et al.*, 2011). They have also been used as moistening, dispersing, emulsifiers and foaming agents in various sectors. Another area of applications is microbial enhanced oil recovery (MEOR) technique that utilizes microbial surfactants to release crude oil from binding surfaces such as rocks and crevices (Banat *et al.*, 2010; Perfumo *et al.*, 2010). Mulligan (2005) and Thavasi (2011) have reported successful use of biosurfactants in facilitating the degradation of organic pollutants in soil and for dispersion of oil from oil spills.

The remarkable properties of biosurfactants and their wide range of applications have necessitated the need to screen for more potent producers in the environments. This paper reports the isolation and characterization of biosurfactants producing bacteria from Mile 2 and Ologe Lagoon in Lagos State, Nigeria. Although some biosurfactant producers have been reported from other sources in Nigeria, there is dearth of information on biosurfactant producers from these lagoons water.

MATERIALS AND METHODS

Samples collection

Water samples were collected in sterile conical flasks (250 mL) from two different lagoons in Lagos State, Nigeria, namely: Mile 2 (6°30'N 3°23'E) and Ologe Lagoon (6°27'N 3°02'E) and compounded. Sample from each lagoon was pooled from 20 points (100 mL each) in the lagoon. Microbiological analysis of samples was carried out immediately upon arrival in the laboratory. On the other hand, samples for physicochemical analysis were stored in the refrigerator at 4 °C and analyzed later.

Physicochemical analysis

The pH and temperature of samples were determined using tabletop Adwa pH meter (AD1040 pH/mV Szeged, Hungary) and mercury bulb thermometer, respectively. Other parameters like total organic carbon, total hydrocarbon content, nitrate, phosphate and sulphate were determined using standard analytical procedures (AOAC, 1990; Chopra and Kanwar, 1998). The total hydrocarbon was extracted from the water using n-hexane:dichloromethane solvent systems (1:1) as previously described by Adebusoye *et al.* (2007). Gas chromatograph (Hewlett Packard 5890 Series II) equipped with flame ionized detector (GC-FID) was used to analyze the extract. The extracts (1 µL) each was injected into GC-FID, with column OV-3 and nitrogen as the carrier gas. The injector and detector temperatures were maintained at 220 °C and 280 °C, respectively. The column was programmed at initial temperature of 50 °C for 2 min then ramped at 5 °C/min to 250 °C and held for 5 min. Air flow rate was 450 mL/min, hydrogen 45 mL/min and nitrogen 22 mL/min.

Microbial enumeration

The total microbial population densities in the water samples were enumerated using standard plate count method. Total heterotrophic bacteria (THB) were enumerated on nutrient agar, while total heterotrophic fungi (THF) were enumerated on potato dextrose agar. Enumeration of total hydrocarbon utilizing bacteria (THUB) was done using the mineral salt medium (MSM) described by Haba *et al.* (2003). The medium is composed of 0.22 g/L Na_2HPO_4, 0.8 g/L KH_2PO_4, 3.0 g/L NH_4NO_3, 0.2 g/L $MgSO_4{\cdot}7H_2O$, 0.05 g/L $FeCl_3{\cdot}6H_2O$, 0.01 g/L $CaCl_2{\cdot}2H_2O$ and 0.05 g/L yeast extract. pH was adjusted to 7.2 and bacteriological agar (20 g) was added. Trace elements solution (1 mL/L) were filter sterilized using membrane filter 0.45 µm and added aseptically to the medium as described by Bauchop and Elsden (1960). Fungal growth was suppressed using 50 µg/mL nystatin. The plates were incubated at room temperature (27 ± 2 °C) and counted after 5-7days.

For enumeration of total hydrocarbon utilizing fungi (THUF), the pH was adjusted to 5.6 and filter-sterilized streptomycin (10 µm/mL) was incorporated into the MSM. For both bacteria and fungi, the spread plate technique was used, in which aliquot (0.1 mL of serially diluted sample) were plated out on appropriate MSM and 9 cm Whatman filter paper soaked with Escravos light crude oil was inverted on the plate according to the vapour phase transfer method described by Raymond *et al.* (1976). The plates were incubated at room temperature (27 ± 2 °C) for 5-14 days after which the colonies were counted.

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Isolation of potential biosurfactant producers using continuous enrichment technique

The isolation of biosurfactant producing bacteria was done by continuous enrichment technique on Escravos light crude oil, palm oil and groundnut oil in MSM as described by Haba *et al.* (2003). Each flask contained 50 mL MSM and 1% of crude oil, palm oil or groundnut oil were added and sterilized. Inoculation of sample was done by introducing 1 mL of water sample from Mile 2 and Ologe Lagoon, respectively. The inoculated flasks were subjected to shaker at room temperature (27 ± 2.0 °C) for 3 weeks in the dark until there was growth (turbidity). Following 3 consecutive transfers at shorter intervals (7 to 15 days), hydrocarbon degraders were isolated by plating out the final flask on Luria-Bertani (LB) agar. Several colonies that appeared were further purified by subculturing repeatedly on LB agar.

Identification and characterization of isolates

Selected isolates were characterized and identified on the basis of their colonial morphology, cellular morphology and biochemical characteristics according to the taxonomic schemes of Cowan and Steel's (Barrow and Feltham, 1995). The biochemical tests carried out on individual isolate include oxidase, catalase, indole production, endospore staining, citrate utilization and motility test. Besides, the ability of respective isolate to ferment sugar (glucose, fructose, sucrose and maltose) was also determined.

Screening assay for biosurfactant production

Hemolytic activity

The method described by Satpute *et al.* (2008) was used in determining the hemolytic activity of the isolates. Bacterial isolates were streaked on Luria-Bertani (LB) agar supplemented with 5% fresh human blood and incubated at (27 ± 2 °C) for 48-72 h. Plates were observed for α, β and γ-hemolysis. Greenish clearing zones around the colonies indicated α-hemolysis, while complete clearing zone around the colonies indicated β-hemolysis. γ-hemolysis was characterized by lack of changes around the colonies. Hemolysis was presumptive for biosurfactant production.

Oil spread method

The oil spread method was carried out as described by Satpute *et al.* (2008). Petri-dish base was filled with 50 mL of distilled water. Using a micro pipette, 20 µL of crude oil was layered uniformly on the water. Thereafter, 10 µL of culture grown on Luria-Bertani (LB) broth was added at different spot on the crude oil which is coated on the water surface. Observation of clear zone after 1 min indicated biosurfactant production.

Emulsification index (E_{24})

Emulsification index (E_{24}) was measured as described by Ilori *et al*. (2005). Isolate grown on MSM supplemented with 1% of either crude oil, palm oil or groundnut was centrifuged at 5000 *g* for 10 min to pellet out the cells. Biosurfactant activity was measured by adding 2 mL of crude oil, palm oil and groundnut oil separately to 2 mL of cell free extract (supernatant) and vortexing at high speed for 2 min. The tube was left for 24 h, after which the height of emulsion was measured. The E_{24} is the height of the emulsion divided by the total height of the tube content (aqueous phase, oil and emulsion) and multiplied by 100.

Blue agar plate (BAP) method

Anionic biosurfactants, especially rhamnolipid, can be detected by the blue agar plate method. Basal salt agar (BSA) medium supplemented with carbon source (2%) and cetytrimethyl ammonium bromide (0.5 g/L)-methylene blue (0.2 g/L) was prepared. The test organism was inoculated on the medium and incubated at room temperature (27 ± 2 °C) for 2-7 days. A dark blue halo around the culture was considered as positive for biosurfactant.

Antibiotic susceptibility test

Bacterial susceptibility to antimicrobial agent was performed by the disk agar diffusion method using guidelines established by Bauer *et al*. (1966). The isolates were tested for their susceptibility or resistance to Gram-positive and Gram-negative antibiotic disc. The antibiotics and their respective concentrations include Ampliclox (30 µg), Amoxacillin (30 µg), Augmentin (30 µg) Chloramphenicol (30 µg), Ciprofloxacin (10 µg), Ofloxacin (10 µg), Ceftriaxone (30 µg), Septrin (30 µg), Streptomycin (30 µg), Sparfloxacin (10 µg) and the antibiotic discs were placed on Mueller-Hinton agar already seeded with 0.1 mL inoculum of isolates. Plates were incubated at room temperature (27 ± 2 °C) 48 h and observed for zones of inhibition. Antibiotic susceptibility patterns were interpreted according to the Clinical and Laboratory Standards Institute Guidelines (CLSI, 2010).

RESULTS

Physicochemical and biological characteristics of water samples

Table 1 shows the physicochemical and biological characteristics of the water samples from Mile 2 and Ologe Lagoon. The results showed high nitrate content of 15.7 mg/L in Mile 2, while 19.6 mg/L was recorded for Ologe Lagoon. Moderately high phosphate contents of 9.8 mg/L and 12.8 mg/L were also detected in the samples. The concentrations of the total hydrocarbon (THC) of both lagoon waters were low, with values 0.53 mg/L and 0.44 mg/L, respectively. The total heterotrophic

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Table 1: Physicochemical and microbiological characteristics of lagoon water.

Parameter	Mile 2	Ologe
Appearance	Darkish-yellow	Brownish
pH	7.9	8.3
Nitrate (mg/L)	15.7	19.6
Phosphate (mg/L)	9.8	12.8
Sulphate (mg/L)	7.0	8.0
THC (mg/L)	0.53	0.44
TOC (%)	0.43	0.32
THB (× 10^{12} CFU/mL)	2.4	2.6
THUB (× 10^{9} CFU/mL)	3.8	3.4
THF (× 10^{9} CFU/mL)	1.2	1.0
THUF (× 10^{7} CFU/mL)	1.2	1.6

THC: Total hydrocarbon content, TOC: Total organic carbon, THB: Total heterotrophic bacteria, THUB: Total hydrocarbon utilizing bacteria, THF: Total heterotrophic fungi, THUF: Total hydrocarbon utilizing fungi.

bacteria (THB) counts were 2.4 × 10^{12} CFU/ mL and 2.6 × 10^{12} CFU/mL, respectively, while total heterotrophic fungi (THF) counts were 1.2 × 10^{9} CFU/mL and 1.0 × 10^{9} CFU/mL, respectively. Total hydrocarbon utilizing bacteria (THUB) were 3.8 × 10^{9} CFU/mL and 3.4 × 10^{9} CFU/mL, while total hydrocarbon utilizing fungi (THUF) were 1.2 × 10^{7} and 1.6 × 10^{7} CFU/mL, respectively.

Isolation and Identification of biosurfactant producing bacterial

Enrichment of samples with different substrates resulted in the isolation of different biosurfactants producers as illustrated in Table 2. A total of 23 bacterial isolates were identified using cultural, morphological and biochemical characteristics from both lagoons. Out of the total 23 bacteria isolates identified, 16 were Gram-positive bacteria, while 7 were Gram-negative. The strains isolated from Ologe Lagoon (n=13) were designated as strain AGC1, AGC2, AGC3, AGC4, AGG1, AGG2, AGG3, AGG4, AGP1, AGP2, AGP3, AGP4, AGP5, while the remaining 10 isolates MTC1, MTC2, MTG1, MTG2, MTG3, MTP1, MTP2, MTP3, MTP4 and MTP5 were isolated from Mile 2 Lagoon. Biochemical characterization of the isolates showed that the isolates belong to 6 genera: *Micrococcus* (n=7), *Bacillus* (n=6), *Pseudomonas* (n=3), *Acinetobacter* (n=3), *Stomatococcus* (n=3) and *Moraxella* (n=1).

Assay for biosurfactant production

Different methods were used to screen for biosurfactant production of the isolates, and the results are shown in Table 3. All isolates were obtained from three different substrates which include crude oil, palm oil and groundnut oil. The results showed that 6 bacterial were isolated from crude oil (MTC1, MTC2, AGC1, AGC2, AGC3 and AGC4), 7 bacterial from groundnut oil (MTG1, MTG2, MTG3, AGG1, AGG2, AGG3, AGG4) and 10 from palm oil (MTP1, MTP2, MTP3, MTP4, MTP5, AGP1, AGP2, AGP3, AGP4 and AGP5). Out of the 23 bacteria screened for hemolytic activity, 5 (MTP3, MTP4, AGC1, AGC3 and AGP1) showed greenish zones of clearing around the inoculum (α-hemolysis),13 (MTC1, MTC2, MTG1, MTP1, MTP2, MTP5, AGC2, AGC4, AGG1, AGG2, AGG4, AGP3 and AGP5) showed zone of complete clearing (β-hemolysis) and 5 (AGG3, AGP2, AGP4, MTG2 and MTG3) showed no clear zone (γ-hemolysis).

Table 3: Biosurfactant producing properties of the isolates.

Isolate	Hemolytic activity	Oil spread	Blue agar plate	E_{24} (%)
MTC1	β	-	+	40.00
MTC2	β	+	+	42.90
MTG1	β	-	+	40.00
MTG2	γ	+	-	50.00
MTG3	γ	+	+	36.70
MTP1	β	-	+	40.00
MTP2	β	+	+	56.70
MTP3	α	+	+	43.30
MTP4	α	+	+	46.70
MTP5	β	+	+	37.00
AGC1	α	+	+	51.30
AGC2	β	-	+	54.30
AGC3	α	-	+	42.90
AGC4	β	+	-	54.30
AGG1	β	-	-	62.30
AGG2	β	-	+	60.00
AGG3	γ	-	-	66.70
AGG4	β	-	+	60.00
AGP1	α	+	+	23.30
AGP2	γ	+	+	53.30
AGP3	β	+	+	00.00
AGP4	γ	+	+	16.70
AGP5	β	+	+	36.70

E_{24}: Emulsification index, α: Alpha-hemolysis, β: Beta-hemolysis, γ: Gamma-hemolysis.
+: High emulsification activity, -: No reaction.
MTC1, MTC2, AGC1, AGC2, AGC3, AGC4: Isolates obtained from crude oil; MTG1, MTG2, MTG3, AGG1, AGG2, AGG3, AGG4: Isolates obtained from groundnut oil; MTP1, MTP2, MTP3, MTP4, MTP5, AGP1, AGP2, AGP3, AGP4, AGP5: Isolates obtained from palm oil.

Fourteen (14) isolates showed clear zones for the oil spread test and 9 isolates showed negative result for this test. Nineteen (19) of the isolates were positive for blue agar test. Emulsification activity is also an important technique to select for potential biosurfactant producing bacteria. Most of the isolates that showed β-hemolysis also showed good emulsification index. These include strain AGG1 (62.3%), AGG2 (60%), AGG4 (60%), MTP2 (56%) and AGC4 (54.3%). Strain AGG3 also showed good emulsification index of 66.7% despite showing γ-hemolysis.

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Table 2: Cultural, morphological and biochemical characteristics of isolates.

Isolate	Pigmentation	Cell shape	Gram staining	Spore staining	Acid fast	Oxidase	Motility	Catalase	Citrate	Indole	H_2S	GLU	SUC	MAL	FRU	Putative identity
MTC1	Cream	Rod	-	-	-	-	+	+	+	+	+	+	+	+	+	*Pseudomonas*
MTC2	Brownish	Coccus	+	+	+	+	+	+	+	+	-	+	+	+	-	*Micrococcus lactis*
MTG1	Green blue	Coccus	+	+	+	-	+	+	+	+	+	-	-	-	+	*Micrococcus cristinae*
MTG2	Cream	Coccus	-	-	-	-	+	+	+	+	+	+	+	+	-	*Acinetobacter lwofii*
MTG3	Cream	Coccus	+	-	-	+	+	+	+	-	+	+	+	+	-	*Micrococcus cristinae*
MTP1	Yellow	Coccus	+	+	+	-	+	+	+	+	+	+	-	-	+	*Stomatococcus mucilaginous*
MTP2	Cream	Coccus	-	-	-	-	-	+	+	+	+	+	-	+	+	*Acinetobacter antratus*
MTP3	Brown	Rod	+	+	+	+	+	+	+	+	-	+	+	+	+	*Bacillus licheniormis*
MTP4	Whitish	Coccus	+	+	+	-	-	+	+	+	-	+	+	+	+	*Stomatococcus mucilaginous*
MTP5	Grey	Rod	+	+	+	-	+	+	+	+	-	+	+	+	+	*Bacillus subtilis*
AGC1	Blue green	Rod	-	-	-	-	+	+	+	+	+	+	+	+	+	*Pseudomonas fluorescens*
AGC2	Cream	Coccus	+	-	-	+	+	+	+	+	-	+	+	+	+	*Micrococcus agilis*
AGC3	Yellow	Coccus	+	-	-	-	+	-	-	+	+	+	+	+	-	*Stomatococcus mucilagenous*
AGC4	Pink	Rod	+	-	-	+	+	+	+	+	-	+	+	+	+	*Bacillus alvei*
AGG1	Yellow	Coccus	+	-	-	+	+	+	+	+	+	+	+	+	-	*Micrococcus agilis*
AGG2	Brown	Rod	-	-	-	+	+	+	+	-	-	+	+	+	+	*Pseudomonas pseudomallei*
AGG3	Orange	Coccus	+	-	-	+	+	+	+	-	-	-	+	-	+	*Micrococcus agilis*
AGG4	Cream	Coccus	-	-	-	+	+	+	+	-	+	+	+	+	+	*Acinetobacter lwofii*
AGP1	Yellow	Coccus	+	-	-	-	-	+	+	+	+	+	-	+	+	*Micrococcus mucillagenous*
AGP2	Pink	Rod	+	-	-	-	-	+	+	+	+	+	+	+	+	*Bacillus megaterium*
AGP3	Brown	Rod	+	-	-	-	+	+	+	-	-	+	+	+	+	*Bacillus sterothrmophilus*
AGP4	Brown	Coccus	-	-	-	-	+	+	+	-	-	+	+	+	+	*Moraxella lwofii*
AGP5	Cream	Rod	+	-	-	+	+	+	+	-	-	+	+	+	+	*Bacillus pumilus*

Key:
H_2S: Hydrogen sulphide, GLU: Glucose, SUC: Sucrose, MAL: Maltose, FRU: Fructose.
+: Positive reaction, -: Negative reaction.

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Table 4: Antibiotics susceptibility patterns of Gram-positive isolates.

Isolate	AM	APX	GN	CPX	E	PEF	CTX	STR	SXT	Z
MTC2	R	R	R	S	R	S	R	S	R	R
MTG1	S	R	R	S	R	S	R	S	R	R
MTG3	R	R	R	S	R	S	R	S	R	R
MTP1	R	R	R	S	R	S	R	S	R	R
MTP3	R	R	R	S	R	S	R	S	R	R
MTP4	R	R	R	S	R	S	R	S	R	R
MTP5	R	R	R	S	R	S	R	S	R	R
AGC2	R	R	R	S	R	R	R	R	R	R
AGC3	S	R	R	S	R	S	R	R	R	R
AGC4	R	R	R	S	R	S	R	R	R	R
AGG1	R	R	R	S	R	S	R	R	R	R
AGG3	R	R	R	S	R	S	R	R	R	R
AGP1	R	R	R	R	R	S	R	R	R	R
AGP2	R	R	R	S	R	S	R	R	R	R
AGP3	R	R	R	S	R	S	R	R	R	R
AGP5	R	R	R	S	R	S	R	R	R	R

AM: Amoxacillin, APX: Ampiclox, GN: Gentamycin, CPX: Ciprofloxacin, E: Erythromycin, PEF: Pefloxacin, CTX: Ceftriaxone, STR: Streptomycin, SXT: Septrin, Z: Zinnacef
R: Resistant, S: Susceptible

Table 5: Antibiotics susceptibility patterns of Gram-negative isolates.

Isolate	AM	AU	CH	GN	CPX	OFX	PEF	STR	SP	SXT
MTCI	S	R	R	S	S	S	S	S	R	R
MTP2	R	R	S	R	S	S	S	R	R	R
MTG2	R	R	S	S	S	S	S	S	S	S
AGC1	R	R	R	S	R	S	R	R	R	R
AGG2	R	R	R	S	R	R	R	R	R	R
AGG4	R	R	R	S	R	R	R	R	R	R

AM: Amoxacillin, AU: Augmentin, CH: Chloramphenicol, GN: Gentamycin, CPX: Ciprofloxacin, OFX: Ofloxacin, PEF: Perfloxacin, STR: Streptomycin, SP: Sparfloxacin, SXT: Septrin
R: Resistant, S: Susceptible

Antibiotic resistance patterns of the isolates

The antibiotic resistance patterns of the isolates using multidisc are shown in Tables 4 and 5. Most Gram-positive isolates were susceptible to Ciprofloxacin (10 µg), Perfloxacin (10 µg) and showed resistance towards Septrin (30 µg), Erythromycin (10 µg) and Amoxacillin (30 µg). Some of the Gram-negative bacteria also showed susceptibility towards Perfloxacin (30 µg), Ofloxacin ((10 µg), Ciprofloxacin (10 µg), Gentamycin (10 µg) and resistant to Augmentin (25 µg) and Amoxacillin (30 µg).

DISCUSSION

The presence of bacteria capable of producing surface-active compounds from water and soil have been reported (Patowary *et al.*, 2017; Ndibe *et al.*, 2018; Ewida and Mohamed, 2019). These organisms were capable of producing metabolites or surface-active compounds leading to solubilization of liquid hydrocarbons, forming micelles which are transported into the cell (Adebusoye *et al.*, 2008). Physicochemical factors of the environment are known to have direct influence on the type, number and metabolic activities of the microflora of any ecosystem (Adebusoye *et al.*, 2008). In this study, the results of some of the physicochemical parameters in the two lagoons were fall within the acceptable standards stipulated by Nigeria's Federal Environmental Protection Agency (FEPA, 2003). The pH and sulphate values recorded in the two lagoons are within the acceptable values of pH (6-9), and sulphate (500 mg/mL) stipulated by FEPA, while the phosphate content of 9.8 mg/L (Ologe) and 12.8 mg/L (Mile 2) are higher than the acceptable limit of 5.0 mg/mL acceptable by FEPA. The nitrate level of both lagoons on the other hand is higher than the 10 mg/L permitted in surface water. High nitrate level is dangerous to health especially in infants and pregnant women (Adesuyi *et al.*, 2015). The variation

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observed in the physicochemical characteristics of the two lagoons may be due to the differences in their nature. Ologe Lagoon is an inland lagoon that receives mainly industrial effluents from food, pharmaceutical and textile industries. On the other hand, Mile 2 Lagoon is an extension of the Lagos Lagoon which is an estuarine lagoon that is a sink for municipal wastes, industrial effluents and oil tanker jetty wastes.

The pH values (7.9 and 8.3) recorded in the two lagoon waters are within the range previously reported by other for the Lagos Lagoon (Amund and Igiri, 1990; Adebusoye *et al.*, 2008; Amaeze *et al.*, 2012). The total heterotrophic bacteria and fungi counts observed for the two water samples was relatively high, which could be due to high level of available nutrients occasioned by indiscriminate discharge of organic wastes through human activities and inorganic wastes from industry into such water body. The low population of total hydrocarbon utilizing bacterial (THUB) and fungi (THUF) from both water bodies as compared with total heterotrophic counts is not surprising as previous reports had shown that hydrocarbon degrading populations are usually several orders of magnitude lower than the total heterotrophs except in environmental compartments that are highly impacted with hydrocarbons pollutants (Amund and Igiri, 1990; Balogun and Fagade, 2010).

Some of the isolates obtained in this study belong to genera such as *Pseudomonas*, *Bacillus*, *Acinetobacter* and *Micrococcus* which have already been reported as biosurfactant producers in other environments (Satpute *et al*., 2008; Obayori *et al*., 2009; Balogun and Fagade, 2010; Agwu *et al*., 2012; Femi-Ola, 2015). Although most of the isolates reported in this study belong to genera that have been reported to be abundant in lagoon water (Adebusoye *et al*., 2008; Adamu *et al.*, 2015), to the best of our knowledge, this group of organisms have not been reported to be associated with biosurfactant production in Mile 2 and Ologe Lagoon waters. It is therefore plausible to say that the lagoon waters are potential sources of bacteria that can be used in the production of value-added products such as biosurfactants.

The oil used in this study include crude oil, groundnut oil and palm oil. Various types of edible oils have been used to stimulate biosurfactant production, but there is limited report on the use of groundnut oil (Chittepu, 2019) and palm oil for biosurfactant production (Wan Nawawi *et al.*, 2010; Saisa-Ard *et al*., 2013; Hope and Gideon, 2015). The groundnut oil and palm oil are common oils used in the Nigerian environments and usually find their way into the water systems. The fact that 17 of the 23 isolates reported here were isolated on edible oils suggests that these edible oils are veritable substrates for biosurfactant production.

The results of the various screening methods highlight the biosurfactants producing potentials of the isolates. Based on Table 3 isolates MTC1, MTC2, MTG1, MTP1, MTP2, MTP5, AGC2, AGC4, AGG1 AGG2, AGG4, AGP3 and AGP5 able to produce β-hemolysis on blood agar signifies their capacity to lyse red blood cells (Balogun and Fagade, 2010). According to Himbert *et al*. (2017), the envelope of red blood cell is composed of >40% lipid, hemolysis by biosurfactant producers could be a result of emulsification of the lipid containing bilayer that resulted in dissolution of the membrane. However, this test is a presumptive test for biosurfactant production and organisms that showed β-hemolysis may not necessarily be biosurfactant producers, while those that are negative may be potential producers.

Most of the isolates were able to emulsify crude oil, palm oil and groundnut oil, with some (AGG4, AGG2, AGG1 and AGG3) showing emulsification index of 60% and above. This showed that Ologe and Mile 2 Lagoon are potential sources of good emulsifiers (Rosenberg and Ron, 1997; Balogun and Fagade, 2010). Futuremore, 19 isolates out of the 23 isolates were positive for blue agar test which is used for the detection of anionic biosurfactants, especially rhamnolipds. This finding suggests that these isolates are potential biosurfactant producers. Biosurfactants are generally low molecular weight microbial products composed of sugars, amino acids, fatty acids and functional groups such as carboxylic acids with excellent surface activity. They are known to lower the surface and interfacial tension between different phases, possess low critical micelle concentration (CMC) as well as formation of stable emulsions (Perfumo *et al*., 2010). These key properties distinguish them from bioemulsifiers which are high molecular weight compounds made up of complex mixtures of heteropolysaccharides, lipopolysaccharides, lipoproteins and proteins (Perfumo *et al*., 2010) that have emulsifying activity like biosurfactants but are less effective at reducing surface tension. Thus, high emulsification index may not necessarily be a reflection of bioemulsifier production.

Despite the variation in responses of our isolates to the different tests, most of them showed positive results for the emulsification index. It is not surprising as previous reports have shown that some organisms could be negative for β-hemolysis and oil spread test but still showing good emulsification index. For instance, Satpute *et al.* (2008) reported that 19 out of the 44 bacterial isolates assayed using various methods showed negative results for oil spread test but were positive for emulsification index. This was also observed in isolate AGG3 that recorded emulsification index of 66.70% but showed γ-hemolysis and also negative for blue agar test which are indicator of biosurfactant production. Isolate AGG4 and AGC2 alongside showing index of 60% were also positive for β-hemolysis and blue agar test, thus making them the best isolates for biosurfactant production. The good emulsification index recorded by our isolates suggests that these bacteria may be potential candidate for biotechnological application. Balogun and Fagade (2010) also suggested that such isolates are of great potential in the oil industry to enhance oil recovery.

Antibiotics are secondary metabolites produced as a survival strategy by microorganisms. It is not surprising that most of the isolates in this study showed multi-resistance to the antibiotics tested. This may be attributed to the fact that surface waters are usually the sink for

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antibiotics and pharmaceutical products in untreated wastewater with significantly high antibiotic concentrations reported from rivers and coastal waters across regions around the world (Baquero *et al*., 2008; Richa and Kashore, 2020). As noted by several authors, these waters are some of the ecosystems most affected by antibiotics pollution which has made them harbor pools of antibiotics resistant bacteria (Managaki *et al*., 2007; Tamtam e*t al.*, 2008). Inundation of the environment with waste from diverse sources such as domestic waste, hospital and medical waste, livestock and poultry and sewage plants impose selective pressure leading to recruitment of assortment of antibiotic resistance genes and subsequent predominance of organisms endowed with resistance to diverse antibiotics (Tamtam e*t al.*, 2008; Hu *et al*., 2013; Chang *et al*., 2015).

CONCLUSION

This study shows efficient biosurfactants production activity from indigenous lagoon bacteria. A total of 23 bacterial were isolated by continuous enrichments method and screened for biosurfactant production using different screening techniques. Most of the isolated bacteria were capable of degrading crude oil, groundnut oil and palm oil when supplemented as carbon source. Most of the isolate showed β-hemolysis on blood agar and had high emulsification index value. The ability of lagoon bacteria to produce biosurfactant is very important considering the level of hydrocarbon pollution in Nigeria and the need to use ecologically friendly and indigenous products for remediation process. The potentials of the isolates to produce these surface-active compounds can be harnessed for various industrial applications as well as to curtail the menace of crude oil in the natural environments.

CONFLICT OF INTEREST

There is no conflict of interest.

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