Screening for Biosurfactant Producing Bacteria from Petroleum Contaminated Soil of Automobile Workshops in Ago-Iwoye, Ogun State, Nigeria

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Abstract: Biosurfactants from microorganisms are promising agents in the bioremediation of crude oil pollution due to their low toxicity and biodegradability. The study was aimed at screening bacteria isolated from petroleum contaminated soil for biosurfactant production. Soil samples were collected from 12 automobile workshops in Ago-Iwoye, Ogun State. Bacteria were isolated and characterized using pour plate technique and standard biochemical tests. Bacterial strains identified were screened for biosurfactant production using blood haemolysis test, drop collapse test, oil spreading test and foaming activity .accordingly. Of the forty five bacteria isolated and characterized. 60% were Gram-negative (27 strains) while (40%) were Gram-positive (18strains). *Bacillus* spp (24%) was the most dominant isolate followed by *Pseudomonas aeruginosa* (22%), *Staphylococcus aureus* (16%), *Serratia marcescens* (11%), *Escheriachia coli* (11%), *Enterobacter aerogenes* (9%) and *Proteus mirabilis* (7%) was the least. Results for biosurfactant screening showed that 69% of the isolates displayed haemolytic activity, 67% were positive for the drop collapse test, 75% were positive for the oil spread test and 36 % showed high foaming activities *Pseudomonas aeruginosa* and *Bacillus* spp showed high positive values in all the tests conducted. These organisms can be employed for future environmental friendly uses in bioremediation of oil contaminated environment.

Keywords: Biosurfactant, Oil contaminated soil, Automobile workshop

INTRODUCTION

Environmental contamination by petroleum and its products remains a major threat to the sustainability of natural ecosystems (Udeani *et al*., 2009; Shoeb *et al*., 2015). This can be attributed to the massive production and constant use of petroleum products as sources of energy in domestic and industrial applications (Bayoumi *et al.,* 2011;Bolliger, 2015).

In Nigeria, the increasing demand for automobiles has resulted in the continuous growth of automobile workshops in major cities and towns (Ndubuisi-Nnaji *et al.,* 2015). These automobile workshops may be considered as an essential part of the service industry with significant impact on the environment (Ndubuisi-Nnaji *et al.,* 2015). Petroleum fractions such as petrol, diesel, lubricants (engine oil) and kerosene are used daily in various forms (Udeani *et al.,* 2009; Mandri and Lin, 2007). As a result of poor management practices of automobile vehicle mechanics many petroleum fractions are indiscriminately disposed to the natural environment which affect the metabolic functions of plants, animals and microorganisms thereby causing environmental pollution (Ebakota *et al.,* 2017).

Surfactants are amphiphilic compounds possessing both hydrophobic and hydrophilic groups as the tails and heads respectively (Okore *et al.,* 2013). These compounds are either synthetic or natural (biosurfactants). The synthetic surfactants are obtained via chemical processes from either petrochemical or oleo chemical sources while natural biosurfactants are derived as extracellular extracts of microorganisms (Letizia *et al.* 2014; Jayrees *et al*., 2011).The synthetic surfactants are expensive and quite toxic to the environment (Geetanjali *et al*., 2017).

Research attention was given to develop an ecofriendly means of completely eradicating organic pollutants from the environment (Dadrasnia and Ismail, 2015). Biosurfactants offered such possibility due to their desirable properties that are environmentally friendly.

Such properties include effective biodegradability, low toxicity, biocompatibility, higher tolerance to environmental factors, availability of cheap raw materials, higher foaming as well as specific activity under different extreme conditions of temperature, pH, and salinity (Makkar *et al.* 2011; Roy, 2017).

Biosurfactants are made from naturally occurring molecules of lipopeptides, glycolipids, fatty acids, and lipoproteins which they are more suitable for environmental and petrochemical applications (Al-Bahry *et al.,* 2013 and Al-Sulaimani *et al.,* 2011). Biosurfactants are classified based on molecular weight and chemical composition as those low molecular- weight include glycolipids and lipopeptides (Rihab, 2010). The glycolipids are the rhamnolipids, trehalolipids and sophorolipids which are disaccharides that are acylated with long- chain fatty acids or hydroxyl fatty acids (Rihab, 2010). Those glycolipids are efficient in reducing the surface and interfacial tensions at the air and water interfaces. The high- molecular weight biosurfactants are referred to as bioemulsan which are more effective at stabilizing oil-in-water emulsions and are highly efficient emulsifiers that work at low concentrations (Shah *et al.,* 2016).

Based on the chemicals compositions, biosurfactants are sub-classified as Glycolipids (such as rhamnolipids), trehalolipids, and sophorolipids, lipoproteins and lipopeptides cyclic compounds (consisting of a lipid attached to a polypeptide chain), and Phospholipids (Desai and Banat, 1997; Rosenberg and Ron 1999; Gautam and Tyagi 2006). Others sub-classifications include Cross linked Fatty, Polymeric microbial surfactants and particulate biosurfactants (Desai and Banat, 1997; Chakrabarti, 2012). Therefore, biosurfactants are important in combating the problem of soil contamination resulting from petroleum fractions. This study was carried out to screen bacteria isolates from the petroleum contaminated soil of automobile workshops for biosurfactants production.

MATERIALS AND METHODS

Sample Collection

Contaminated Soil samples were collected randomly from the twelve different automobile workshops in Ago-Iwoye, Ogun state, Nigeria using the sterilized auger at the depths of 0-10cm. The sample bags were labeled appropriately and taken to the Department of Microbiology, Olabisi Onabanjo University, Ago-Iwoye for the laboratory for analyses.

Isolation and characterization of bacteria isolates

Enrichment of the soil samples and isolation of bacteria were carried out using the method of Dukhande and Warde, (2016). One gram (1g) of each sample was inoculated in 100ml of mineral salt medium (MSM) which consist of $NaNO_3$5g/L, KH_2P0_4 1.0g/L, $MgSO_4$ $7H_2O$ 0.5g/L, $MnSO_4$ 1.5g/L, $CaC1_2$ 0.02g/L, $(NH_4)2SO_4$ 1.5g/L and $FeSO_4$ 0.01 g/L supplemented with 1% of engine oil and incubated at 30^{o}C for 72 hours. Bacteria isolation was done by spreading 0.1ml of each culture to the MSM agar plate containing 1% of engine oil. After 48 hours of incubation, morphologically distinct colonies were selected and characterized based on cellular and biochemical characteristics as described by the Bergey's manual of systematic bacteriology (Holt *et al.*, 1994).

Screening for biosurfactant production from isolated bacteria

Each bacteria isolate was inoculated into 10ml of nutrient broth medium and incubated at 37^{0}C for 72 hours thereby centrifuged at 3000 rpm for 30 minutes where the supernatant was used for some biosurfactant screening assays (Femi-Ola *et al.,* 2015).

Blood haemolysis test

Pure culture of each isolate was streaked on blood agar plate and incubated at 37°C for 48 hrs The presence of clear zone around the colony is an indication of haemolysis (Shoeb *et al. ,* 2015).

Drop collapse test

A drop of the culture supernatant was carefully placed on an engine oil coated glass slide and observed after one minute. The test is positive if the drop of supernatant collapsed and spread on the oil coated surface and negative if the drop still remains after one minute. The control experiment was simultaneously carried out using distilled water (Odalys *et al.,* 2017).

Oil spreading test

Using a micropipette, 10µl of used engine oil was added to the surface of 40 ml distill water in sterile Petri dish. As the oil formed a thin layer, the 10µl supernatant volume was gently placed at the center of oil layer. If the oil displaces and clear zone forms after one minute, then the presence of biosurfactant is confirmed. The displaced diameter was measured after 30 seconds (Morikawa *et al.,* 2000).

Foaming activity

Each bacterial isolate was cultured in 250 mL Erlenmeyer flasks containing 100 mL of nutrient broth which was incubated at 30°C on shaker incubator at 200 rpm for 72 h. Foaming activity was detected as the duration of the foam stability and height in the graduated cylinder (Dukhande and Warde, 2016). Foaming was calculated based on the equation:

$$\text{Foaming} = \frac{\text{Height of foam}}{\text{Total height}} \times 100$$

RESULTS AND DISCUSSION

A total of forty five bacterial strains belonging to seven genera were isolated and identified in this study. *Bacillus* spp (11±3.05) was the most dominant organism isolated followed by *Pseudomonas aeruginosa* (10±3.05), *Staphylococcus aureus* (7±3.05), *Serratia marcescens*(5±3.05), *Escheriachia coli* (5±3.05), *Enterobacter aerogenes* (5±3.05), and *Proteus mirabilis* (3±3.05) (Fig 1).

A greater percentage (60%) of the bacteria isolated in this study were Gram-negative while 40% were Gram-positive (Odalys*et al.,*2017). These organisms have ability to utilize hydrocarbons (Ndubuisi-Nnaji*et al.,* 2015). Their high occurrence rate in oil contaminated environments as seen in this study when compared to other organisms isolated has been linked with the ability of these organisms to produce emulsifiers which degrade oil (Femi-Ola *et al.,* 2015).

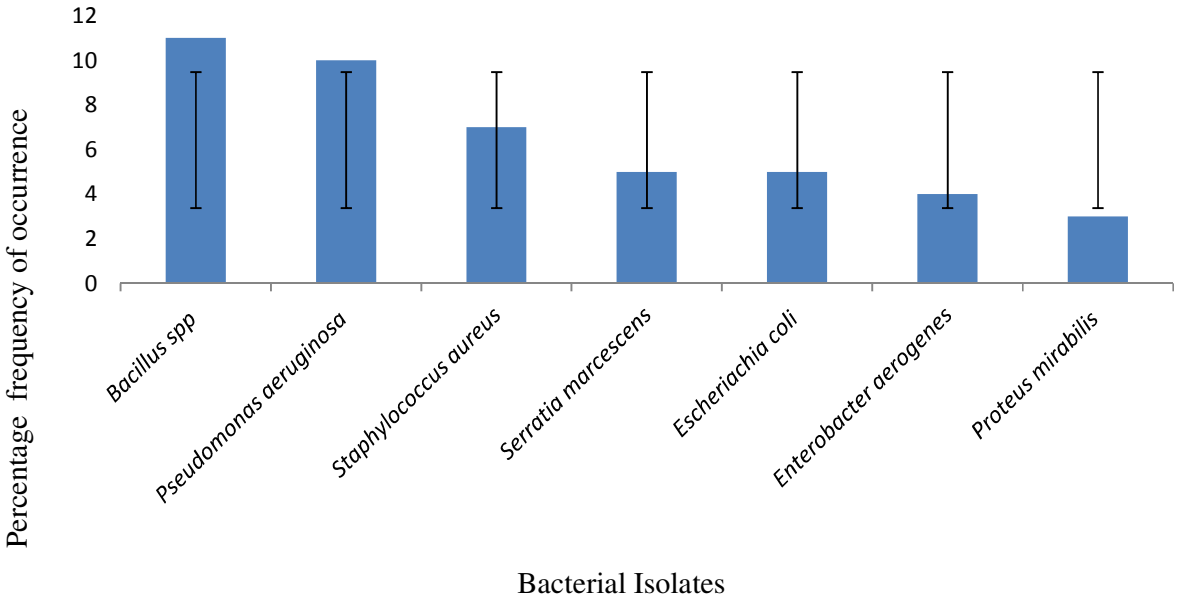

Figure 1: Occurrence of various genera of bacteria isolated from automobile workshops in Ago-Iwoye, Ogun State, Nigeria.

Results showed that 31±10.5 of the bacterial isolates displayed positive hemolytic activity of which 21±10.5 were beta (β)hemolytic, 10±10.5 alpha (α)hemolytic while 14±10.5 were negative (Fig 2). Haemolytic activity has been suggested to be a good screening criterion as well as the primary method used to screen bacteria for biosurfactant production (Carrillo *et al* 1996;Thavasi *et al*, 2011; Shoeb*et al.* 2015). Various researchers had also isolated bacteria with various degrees of haemolysis (Thavasi *et al.*, 2011; Shoeb *et al.*,2012;Shoeb *et al.*,2015; Adebajo *et al.,* 2017). Haemolysis, however does not always mean biosurfactant production as other non biosurfactant producing bacteria may also cause hemolysis (Youssef *et al*.,2004). Hence, haemolysis test should be backed up with other tests such as oil spreading technique, drop collapse assay and foaming test (Shoeb*et al.,* 2015).

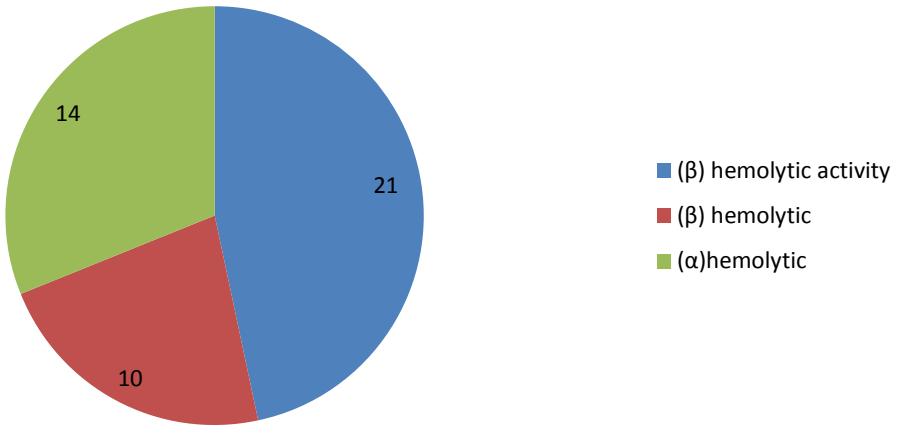

Figure 2: Haemolytic activity of the bacterial isolates from automobile workshops in Ago-iwoye, Ogun State, Nigeria.

Results of the drop collapse and oil spread tests showed that 31±2.12 of the isolates were positive for the drop collapse test (Fig 3) while 34±2.12 of the isolates were positive for the oil spreading test and 22 of these 34 displayed high oil spreading ability. (Fig 4). These two tests were employed as quantitative measures of biosurfactant production by microorganisms (Patil *et al*., 2012). Thavasi *et al*, (2011) reported a correlation between drop collapse assay and oil spreading tests in which 78.1% of the bacteria screened tested positive for the oil dropping assay and 77.1% were positive for the oil spreading assay. Oil spreading technique was found to be more sensitive than other methods of biosurfactant production screening (Youssef *et al*., 2004; Ariech and Guechi,2015) and more suitable in detecting low levels of biosurfactant production (Joshi and Shekhawat,2014; Jayasree and Latha,2018). These findings stated above was corroborated by the higher percentage of isolates that exhibited higher oil spreading ability compared to other screening tests in this study.

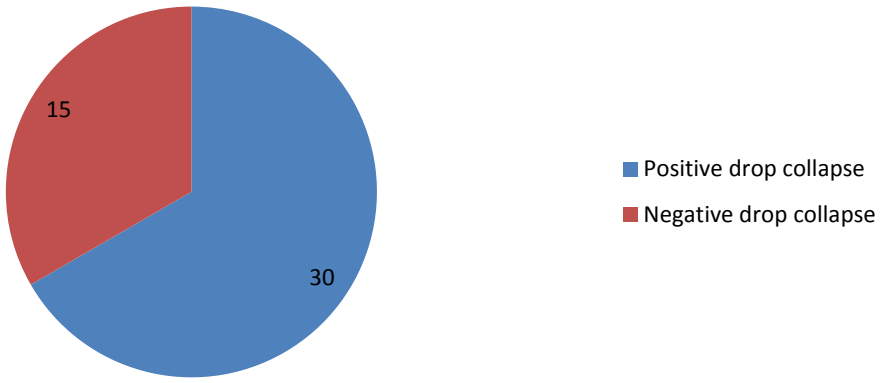

Figure 3. Drop collapse activity of the bacterial isolates from automobile workshops in Ago-Iwoye, Ogun State, Nigeria.

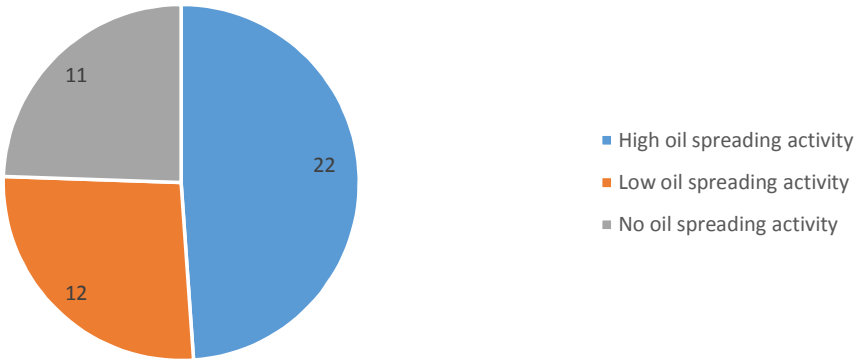

Fig 4:.Oil spreading activity of bacterial isolates from automobile workshops in Ago-Iwoye, Ogun State, Nigeria.

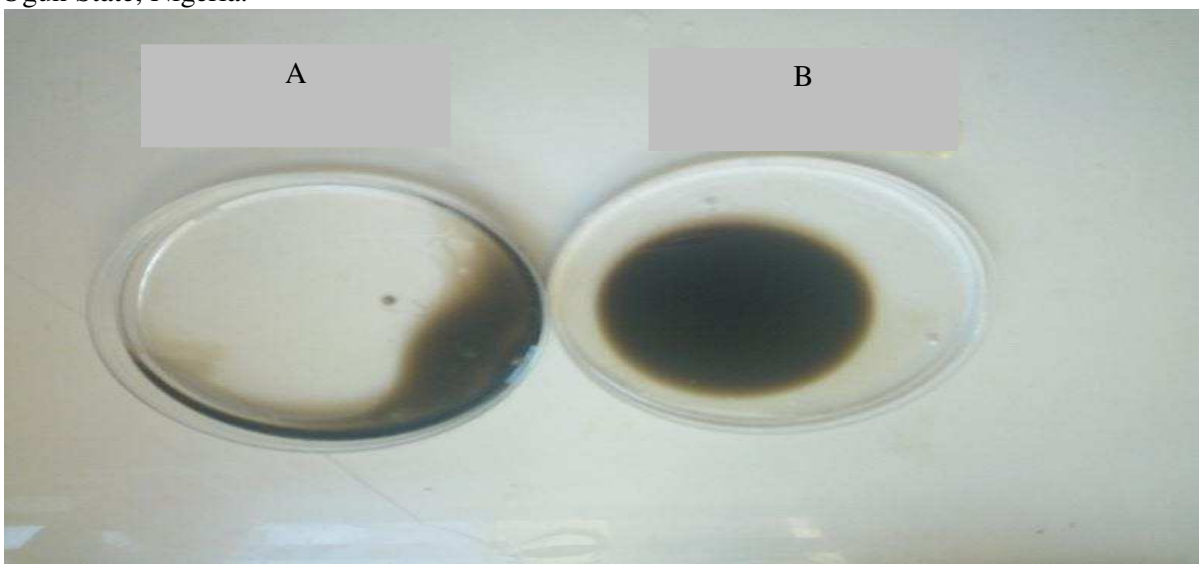

Plate 1: High oil spreading activity of *Bacillus subtilis* IJA25 (A) Negative oil spreading result of the control experiment (B).

A higher number of the isolates (36±11.4) exhibited foaming abilities out of which 16±11.4 displayed high foaming activity, 10±11.4 showed average foaming activity, 10±11.4 exhibited weak foaming activity while 9 ± 11.4 of the isolates did not show foaming activity (Fig 5).Forming is an indication of the presence of biosurfactant in the medium (Jayasree and Latha,2018).

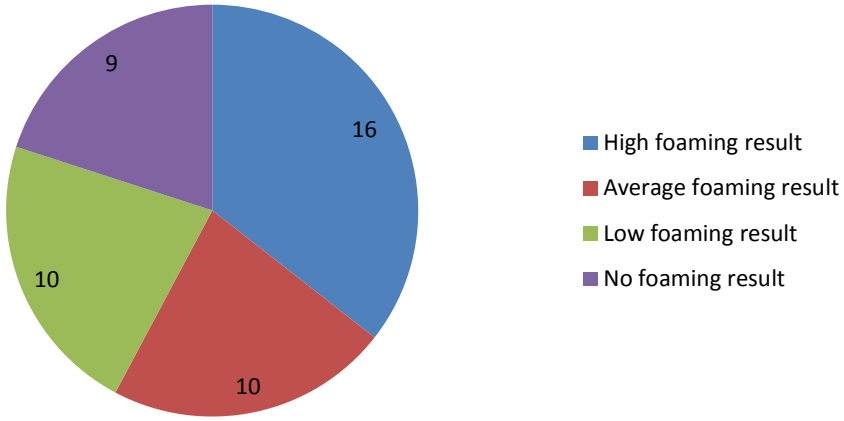

Figure 5: Foaming activity of bacterial isolates from automobile workshops in Ago-iwoye, Ogun State, Nigeria.

The bacterial strains (IJA2, IJA13, IJA25 and IJA39) that produced high levels of surfactants detectable by all tests belonged to the genera *Pseudomonas* and *Bacillus* (Table 1). These bacteria have been described to produce biosurfactants that lower the surface tensions (Raaijmakers *et al*., 2010). The isolation of such previously recognized surfactant-producing taxa not only emphasizes their ability to survive maximally in heavily contaminated environments but also confirmed their suitability of the tests used in this research for biosurfactant producers.

Table 1: Bacterial isolates with high biosurfactant producing abilities from automobile workshops in Ago-iwoye, Ogun State, Nigeria.

s/n	Organism code	Identified Bacteria Strain	Hemolysis test	Drop collapse	Oil spreading test(cm^2)	Foaming activity
1	IJA25	*Bacillus subtilis*	β	+	9.34	+++
2	IJA13	*Pseudomonas aeruginosa*	β	+	9.38	+++
3	IJA39	*Bacillus subtilis*	β	+	9.22	+++
4.	IJA2	*Pseudomonas aeruginosa*	B	+	9.65	+++

Key: β =Beta haemolyis, + positive , +++ High foaming activity,

CONCLUSION

The experimental data obtained repeatedly indicates the biosurfactant producing property of the isolates belonging to the genera *Bacillus and Pseudomonas.* These organisms can as valuable sources of the novel environmental friendly biosurfactants suitable for the future replacement of synthetic surfactants and also as highly useful tools for various environmental and industrial processes.

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