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Research Article

Isolation and Characterization of Bacteria for Bio-surfactant production from oil-contaminated and Ffarmland Soil in Dutsin-Ma L.G.A

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Abstract— Surface-active substances that are secreted by microorganisms are called biosurfactants. Although there are a number of known microorganisms that create biosurfactants, more research is needed to determine which ones could produce the chemical more quickly and profitably for commercial usage. These kinds of bacteria were identified in the Dutsin-Ma metropolitan investigation as being involved in the production of biosurfactant. Six soil samples in all were gathered from various locations within the Dutsin-Ma city. Six soil samples in all were gathered from various locations within the Dutsin Ma city. The tests included isolating the bacteria, identifying the group to which the organisms belong using gram staining, testing the isolated bacteria biochemically, and performing additional tests to identify bacteria that create biosurfactants. Prior to microscopic examination, the bacteria used in this investigation were first acquired macroscopically. It was noted that several colony characteristics included colour, texture, border, and height. It was noted that B. subtilis had an entire border, a smooth texture, and a yellowish coloration. The isolates' biochemical examination reveals *Bacillus subtilis*, *Pseudomonas sp.,* and *pseudomonas aeruginosa*. The fact that the bacteria are there indicates that the soil is a natural habitat for them. based on the biosurfactant activity screening using the oil spread, drop collapse, and haemolysis tests. Every isolate underwent a hemolysis test, and every one of them proved positive (*Pseudomonas spp*., however, had a negative result). The haemolysis result demonstrates beta hemolysis activity, a sign of the strain's capacity to generate biosurfactants. With the exception of pseudomonas sp., all isolates showed positive for the drop collapse and oil spread tests. Further research is required to determine the precise features of the type and structure of the biosurfactant that these bacteria eat, based on the results of this study.
Keywords— **Biosurfactants, Bacteria, Soil, Screening, Dutsin-Ma**

1. Introduction

Biosurfactants are surface active compounds released by microorganisms. These are several known microbes involve in the production of biosurfactants but exploration of potential biosurfactant producer is need of hours to accelerate its economic production for industrial use. These compounds are mainly classified according to their molecular weight, physic-chemical properties and mode of action[1]. They are eco-friendly, easily biodegradable and non-toxic material. Numerous applications of biosurfactants is being practiced with several advantages in laundry cleaning, food processing, cosmetic industry, petroleum, microbial enhance oil recovery, agriculture and medical[2]. It can be produced through use of cheaper, renewable substrates as potential carbon sources that came from industries such as agricultural wastes (sugars, molasses, plant oils, oil wastes, starchy substances and lactic whey), dairy industry whey, distillery wastes, and animal fat and oil industries. This leads to greater possibility of economical bio-surfactant production and eco-friendly management of unwanted industrial wastes. There had been recent developments in the extraction of microbial surfactants from cell-free supernatants and their qualitative and quantitative analysis and application[2].

2. Related Work

Although, very few literatures were available regarding the toxicity of biosurfactants, they are generally considered low or non-toxic products and are appropriate for pharmaceutical, cosmetic and food uses. demonstrated the higher toxicity of the chemical-derived surfactant (Corexit) which displayed a LC50 against Photobacterium phosphoreum and was found to be 10 times lower than of rhamnolipids. Compared the toxicity and mutagenicity profile of biosurfactant from *Pseudomonas aeruginosa* and chemically derived surfactants and indicated the biosurfactant as non-toxic and non-mutagenic. The low toxicity profile of biosurfactant,

sophorolipids from Candida bombicola made them useful in food industries[9].

Some researchers conducted shows that Microbial derived compounds can be easily degraded when compared to synthetic surfactants and are suitable for environmental applications such as bioremediation/biosorption. The increasing environmental concern forces us to search for alternative products such as biosurfactants Synthetic chemical surfactants impose environmental problems and hence, biodegradable biosurfactants from marine microorganisms were concerned for the biosorption of poorly soluble polycyclic aromatic hydrocarbon, phenanthrene contaminated in aquatic surfaces controlled the blooms of marine algae, Cochlodinium using the biodegradable biosurfactant sophorolipid with the removal efficiency of 90% in 30 min treatment[2,3,].

The disposal of frying oil waste is a serious question mark for environment ecosystem hazard; hence present research explains the increasing interest in the use of waste frying oils for microbial transformation[4]. This study was conducted to produce biosurfactant from microbial source using waste fried oil as substrate. The bacterial isolate thus obtains serves as a biofactory for production of industrially important biosurfactant. It also provides an insight for utilization of waste fried oil as cheaper alternative substrate for biosurfactant production[5] thereby contributing to waste minimization and reduction in pollution load due to fried waste oil disposal[6]

3. Experimental Method

3.1 Study area

This study was conducted in Katsina State, Dutsinma, at Federal University Dutsinma katsina state.

3.2 Sample collection

The soil sample was collected from mechanic area opposite federal university Dutsin-Ma and Farmland in some selected farms within the area using sterile nylon and sample bottle, labeled A, B, C-J and transported to laboratory for further analysis.

3.3 Serial dilution and Media preparation

The soil sample was diluted serially using 10^{-1} to 10^{-6} through a series of standard volume into fivefold to reduce the load of microorganisms present, and to get accurate result. The 2 power that is 10^{-3} and 10^{-5} diluted sample was selected for further analysis then isolated into a culture media. MacConkey Agar and Nutrient Agar was initially used and later uses compositions with agar agar as solidifying agent and uses the fractions as the sole expense of carbon. All media were prepared according to manufacturers instruction[7].

3.4 Screening test of isolate for ability to utilize hydrocarbon

Every isolate underwent a hemolysis test, the test is as follow; Nutrient agar and blood agar based was used in the preparation. 5% of blood was used in 95% of the prepared media. The haemolysis result demonstrates the alpha, beta or gamma hemolysis activity, a sign of the strain's capacity to generate biosurfactants[8].

3.5 Gram staining

A sterile wire loop was used to select a colony from the petridish that had been emulsified on the slide in order to create a smear. A drop of regular saline was applied to the clean slide. Heat repaired the stain by passing it over a flame. After a minute of crystal violet stain application, it was cleaned with distilled water. After that, the smear was covered with Lugol's iodine for one minute, cleaned with distilled water, and quickly decolored with any alcohol example acetone, ethanol and/or methanol for thirty seconds. The smear was then immediately cleaned with distilled water, flooded with safranin, and left for one minute before being cleaned again with distilled water and allowed to dry[3]. The smear's surface was covered with oil, and viewed under X100 objectives lens[9].

3.5 Biochemical Characterization

3.5.1 Catalase Test

It served as a means of distinguishing between bacteria that generate the catalase enzyme, like Staphylococcus, and those that do not. A slide was coated with a drop of hydrogen peroxide (H_2O_2), and the drop of H_2O was used to emulsify a growing culture for a full day. Bubbles are a sign of a positive reaction when they are present, and their absence indicates a negative reaction[10].

3.5.2 Urease Test

The bacteria used in this test are those that can break down urea through an enzymatic process to create ammonia. Each bottle's urea agar base medium received an inoculation of the test organism, and it was then incubated for 48 hours at 37°C. When ammonium production results in a color shift from yellow to pink, the test is considered positive[11].

3.5.3 Indole test

The test is used to identify indole positive or indole negative. The bacteria was cultured on 5 milliliters of nutritional broth and incubated. Kovac's reagent was introduced after 24 hours, as red coloration that appeared in the reagent layer above the soup in less than a minute and yellow colour in indole negative[12].

3.5.4 Citrate Test

Using a sterile wire loop, inoculum of the test organism was added to a sterile citrate medium that was slanted and solidified. For 48 hours, the test tube was incubated at 35°C. A positive test results becomes turbid and a light green to blue color shift in the medium[12].

3.7 Screening for Biosurfactant Activity

There are multiple of screening conducted, among the sellected test that was conducted are as follows; The drop collapse test, oil spread test, and hemolysis, the biosurfactant activity of the isolates from petroleum products was identified.

3.7.1 Haemolysis test

The hemolysis test is the initial screening procedure for the generation of biosurfactants. Isolated strain was injected onto blood agar (5% human blood) and incubated aerobically at 30°C for 48hrs and anaerobically at 45°C for 48hrs[12].

3.7.2 Drop collapse test

One clean glass slide was used for each of the 24 hour old bacterial cultures' supernatants, and 0.1 milliliter of diesel oil was applied. For a duration of one minute, the flattening characteristic of diesel oil was observed, and the outcomes were documented[12].

3.7.3 Oil spreading test

A 15cm diameter petridish was filled with forty 40ml of distilled water, 2ml of diesel oil was poured to the water's surface, and 10ml of the bacterial isolates' culture extract was then put on top of the oil. The diameter of the oil surface's clean zones was measured, and the findings were noted[13].

5. Results and Discussion

A total of six soil samples were collected from different sites within Dutsin-Ma metropolis and the following results were obtained and presented in the tables and chats below:

The organisms obtained in this study were first obtained macroscopically before microscopic analysis. Special colony features such, colour, texture, margin, and elevation were observed. B. subtilis was observed as having yellowish colouration, with a smooth texture, and a margin which is entire. The elevation observed from the organism is flat, whereas *P. aerugenosa* was observed to have greenish coloration, with a smooth texture and an entire margin having a flat elevation as illustrated in table.

Table 1: Morphological Characteristics of the isolates.

Sample No.	Color	Texture	Elevation	Bacteria
1	Yellowish	Smooth	Flat	*B. Subtilis*
2	Yellowish	Smooth	Flat	*B. Subtilis*
3	Yellowish	Smooth	Flat	*B. Subtilis*
4	Greenish	Smooth	Flat	*P. aeruginosa*
5	Greenish	Smooth	Flat	*P. aeruginosa*
6	Greenish	Smooth	Flat	*P. aeruginosa*

Below figure shows the haemolysis test, it has been suggested that organism must be screened for biosurfactant activity by haemolysis test, drop collapse assay and oil spread test. Haemolysis test was carried out on all the isolates, and were all tested positive except *Pseudomonas spp. which shows negative result.* The result for haemolysis shows beta haemolysis activity which is an indication of the strains ability to produce biosurfactants.

Key: Positive = *Pseudomonas aeruginosa and bacillus subtilis*

Negative = *pseudomonas aeruginosa*

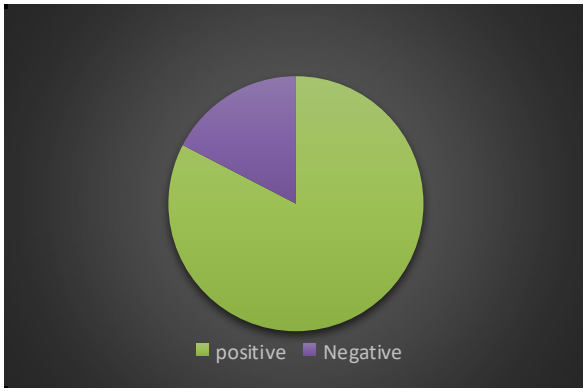
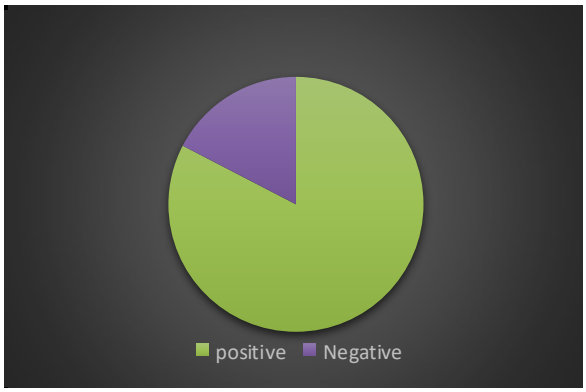


Figure 1: Drop collapse assay conducted on the isolates suspecting to be producing bacteria.

The below figure show drop collapse and oil spread test and was tested positive for all the isolates except *Pseudomonas sp.*



Key: Positive = *pseudomonas aeruginosa and bacillus subtilis*, Negative = *pseudomonas aeruginosa*

Figure 2: Oil spread test conducted on the isolates suspecting to be producing bacteria

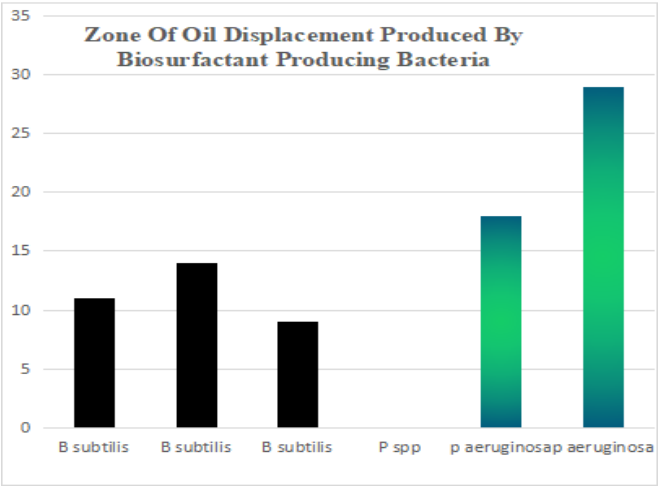


Figure 3: Zone of oil displacement

Biochemical characterization of the suspected biosurfactants-producing bacteria

The biochemical characterization of the isolates seen in table above identified the bacterial isolates as *Pseudolonas spp, pseudomonas aeruginosa* and *bacillus subtilis*. Gram Staining Coagulase Catalase Oxidase Citrate Motility Indole The presence of the organism shows that the soil is a natural habitat of bacteria.

Table 2: Biochemical characterization of the suspected biosurfactants-producing bacteria

Isolates	S 1	S 2	S3	S 4	S 5	S 6
Gram Staining	+	+	+	-	-	-
Coagulase	+	+	+	-	-	-
Catalase	+	+	+	+	+	+
Oxidase	-	-	-	+	+	-
Urease	-	-	-	-	+	+
Citrate	+	+	+	+	+	+
Motility	+	+	+	+	+	+
Indole	+	+	-	-	-	-
Inference	*B. S*	*B. S*	*B. S*	*P. a*	*P. a*	*P. a*

Key; S= Sample, - = Negative, + = Positive, P. a= Pseudomonas Aeruginosa, B. S =Bacillus Subtilis

6. Conclusion and Future Scope

This study reveals that the *Bacillus subtilis and Pseudomonas auroginosa* Isolated from hydrocarbon contaminated soil have the ability to express biosurfactants, and can be useful tools for various environmental and industrial process, especially in the area of bioremediation of oil spills. Bacteria isolated in this study could be a valuable source of novel and environmentally-friendly biosurfactants for the future replacement of synthetic surfactants. Further work should be performed especially around Katsina state and neighbouring states, for clear picture of the biosurfactant forming bacteria and the posibility of making commercially produced isolates for easy work in future.

Data Availability

All the necessary data have been captured here with the exception of preliminary data which will be available via the corresponding author email when contacted.

Conflict of Interest

There is no conflict of interest throughout the research and the research was fully funded by the researchers.

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Authors' Contributions

Author-1 Have done the laboratory work and the write-up, Author-2 involved in checking and correcting the work Author-3 wrote the first and second draft of the manuscript. All authors reviewed and edited the manuscript and approved the final version of the manuscript.

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search experience.

