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# STABILITY OF BIOSURFACTANT PRODUCED BY *PSEUDOMONAS TAENENSIS*

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## ABSTRACT

Biosurfactants are one of the microbial bioproducts that are naturally synthesized and are applicable for many industrial purposes. In this study, antibacterial, stability and antibiotic susceptibility of biosurfactant was evaluated. Biosurfactants produced from different substrates (groundnut cake, cassava flour waste, pome, cooking oil, engine oil, cassava waste water, molasses, cassava peel, potato) by *Pseudomonas taenensis* were evaluated for antibacterial activity using agar well diffusion method. Antibiotics susceptibility of *Pseudomonas taenensis* was carried out using different antibiotics (augmentin, ofloxacin, tetracyclin and ciprofloxacin, cotrimoxazole, pefloxacin, amoxylin, ceftriazone, nitrofuranton and gentamycin). The stability of the biosurfactant was evaluated by adjusting the biosurfactant to: pH (2, 4, 6, 8, 10 and 12) using 1M NaOH and 1M HCl, temperature (4, 30, 37, 55, 75 and 100 °C) and NaCl (0, 5, 10, 15, 20 and 25 %). Results showed that only biosurfactant produced using cassava waste water as substrate was sensitive to *Escherichia coli* while biosurfactant produced using cassava flour waste, pome and molasses were sensitive to *Staphylococcus aureus*. Biosurfactant-producing isolate (*Pseudomonas taenensis*) was sensitive to four antibiotics (augmentin, ofloxacin, tetracyclin and ciprofloxacin) and resistant to six antibiotics (cotrimoxazole, pefloxacin, amoxylin, ceftriazone, nitrofuranton and gentamycin). Biosurfactant was stable over all the wide ranges of pH, temperature and sodium chloride concentrations investigated. This study therefore revealed that biosurfactant have good stability, thus, could survive environmental stress; Not all biosurfactant and biosurfactant producers have antimicrobial and antibiotic property.

**Keywords:** Emulsification Index, Antibiotics, Well diffusion, *Escherichia coli, Klebsiella pneumonia* and *Staphylococcus aureus.*

## INTRODUCTION

The independent growth of microrganisms under several conditions could lead to the production of huge quantities of beneficial products rapidly from cheap and renewable materials in large quantities known as microbial bioproducts which are biodegradable and possessed low toxicity (Khire, 2010; Asuti *et al*., 2019). Microorganisms that synthesize amphiphilic compounds with emulsifying and surface activities are known as biosurfactant (Singh *et al*., 2007). Surfactants could be synthesized from cells growing on water-immiscible hydrocarbons as well as on water-soluble compounds (Mukherjee *et al*., 2006). Pseudomonas (Gram-negative) and Bacillus (Gram-positive), amidst the bacterial genera, have received the interest on account of their ability to synthesize effective biosurfactants that are efficacious in pharmaceuti-

cal, agriculture, chemical and food industries (Roongsawang *et al.,* 2011).

Over the synthetic counterparts (i.e. chemical), merits of biosurfactants include: stability at different physical and chemical conditions, good biocompatibility, synthesis under user-friendly conditions, higher biodegradability and lower toxicity (Zhang *et al.,* 2004; Ruggeri *et al.,* 2009). Emulsification, wetting, foaming, cleansing, phase separation, surface activity and reduction in viscosity of crude oil are diverse functional properties exhibited by biosurfactants which makes them amenable for the application in diverse niche areas such as agriculture, food industries, oil recovery, pharmaceuticals, cosmetics and environmental remediation (Mulligan 2005; Campos *et al.,* 2013; Sachdev and Cameotra, 2013; Gudina *et al.,* 2016; Foukia *et al*., 2016).
Das *et al.* (2008) documented that the first antimicrobial marine biosurfactant was reported from *Bacillus circulans* isolated from the Andaman and Nicobar Islands, India, which exhibited antimicrobial potential against several multidrug resistant human pathogens with non-hemolytic property.

Moreso, high cost of production of biosurfactant is a major challenge but could be solved through improvement in the fermentation technology, optimization of environmental conditions, screening for overproducing strain to attain the maximize productivity strain, use of cheaper and renewable substrates (Helmy *et al.*, 2011; Marchant and Banat, 2012; Marchant *et al*., 2014).

A variety of carbon (water soluble and water insoluble) and nitrogen sources including some unusual carbon sources such as ethanol, blended gasoline, hydrocarbons like heptadecane, hexadecane have been employed for biosurfactants production depending upon the substrate composition (Cunha *et al.*, 2004). Makkar *et al.* (2011) and Banat *et al*. (2014) reported on the production of biosurfactant involving the use of cheaper, renewable substrates such as: molasses, plant oils, oil wastes, lactic whey, distillery wastes, animal fat, soybean, potato, sweet sorghum, canola meal, coconut cake, peanut cake and soybean cake by several researchers.

Products such as bran, straw of wheat, straw of rice, hull of soy, corn, rice, sugar cane molasses, beet molasses, bagasse of sugarcane, cassava flour and its wastewater are good substrates of agro-industrial waste which contain high amount of carbohydrates, lipids and hence, can be used as a rich carbon source for microbial growth (Benincasa, 2007; Thavasi *et al*., 2014). Corn steep liquor and ground-nut oil refinery residue were also stated as low cost nutrients for the production of biosurfactant from *Candida sphaerica* strain UCP 0995 (Luna *et al.*, 2012).
However, evaluation of the effectiveness of the biosurfactants produced from cheap and readily available substrates of agricultural and industrial wastes is necessary for their application in a wide range of environmental and industrial processes. Currently, there is limited research on determination of biosurfactant properties produced by *Pseudomonas taenensis.* Therefore, this study investigates the antibacterial and stability properties of the biosurfactant as well as the antibiotic susceptibility pattern of the biosurfactant-producing microorganism.

## MATERIALS AND METHODS

### *Production of biosurfactant using different substrates*

The fermentation medium contains (g/L): 1.0 $K_2HPO_4$, 0.2$MgSO_4.7H_2O$, 0.05 $FeSO47H_2O$, $0.1CaCl_2.2H_2O$, 0.01$Na_2MoO_4.2H_2O$, 30 NaCI and 5g each of the solid waste (cassava flour waste, potato waste, peanut cake, molasses and cassava waste peel or 2% liquid waste (cassava waste water, palm oil mill effluent, waste lubricant oil and waste cooking oil) as carbon source was autoclaved at 120◦C for 15 mins. The pH was maintained at 7. The sterilized medium was inoculated with 5mL of culture broth and the content was mixed properly and incubated at 35$^{O}$Cin an orbital rotary shaker set at 120rpm for 7 days (Govidammal and Parthasarathi, 2013; Tambekar and Gadakh, 2013).

### *Bacterial Isolates*

*Bacillus subtilis*, *Staphyloccoccus aureus*, *Escherichia coli* and *Klebsiella pneumonia* used for the antibacterial study was obtained from the College of Veterinary Medicine, Federal University of Agriculture, Abeokuta. Nigeria.

### *Antibacterial Analysis of Biosurfactant on Bacterial Isolates*

Antibacterial activity of biosurfactant produced from different liquid and solid wastes by *Pseudomonas taenensis* was carried out on *Bacillus subtilis*, *Staphyloccoccus aureus*, *Escherichia coli* and *Klebsiella pneumonia*. Biosurfactant was tested for antibacterial activity using agar well diffusion method. Twenty milliliters of sterile Mueller-Hinton agar plates were swabbed with each of the isolates. Wells were made using a sterile cork-borer (6 mm) on the swabbed plates, biosurfactant solution in methanol and methanol (control) were inoculated into the wells and incubated at 37°C for 24 hrs, the diameter of inhibition zones was measured (Khopade *et al.,* 2012).

### *Antibiotic Susceptibility of Biosurfactant -Producing Isolates*

Prepared sterile Mueller Hinton Agar plate was swabbed with *Pseudomonas taenensis*. Multiple Gram negative antibiotics disc containing; augmentin, ofloxacin, tetracyclin and ciprofloxacin, ceftriazone, nitrofuranton, gentamycin, amoxylin, cotrimoxazole and pefloxacin were placed aseptically on the sterile swabbed plates each and plates were incubated at 37 °C for 24 hrs. After incubation, plates were checked for the appearance of zone of inhibition, then the diameter of the zone was recorded (Chakrabarti, 2012).

### *Stability of Biosurfactant*

The stability of biosurfactant was performed according to the method of Foukia *et al*. (2016). Stability of the surface-active compound was determined by using crude biosurfactant produced from the substrate with the highest antibacterial potential. The biosurfactant was adjusted to: pH (2, 4, 6, 8, 10 and 12) using 1M NaOH and 1M HCl, temperature (4, 30, 37, 55, 75 and 100°C) and NaCl (0, 5, 10, 15, 20 and 25 %) for 30 mins. Emulsification index (E.I) was used to investigate the stability activity of the biosurfactant by adding 2mls of the biosurfactant that had been subjected to the different conditions of pH, temperature and sodium chloride to 2mls of kerosene, vortexed for 2 minutes and allowed to stabilize for 24 hrs. Emulsification Index was determined by measuring height of emulsified layer against its total height multiplied by 100.

## RESULTS

### *Production of Biosurfactant*

The production of biosurfactant by *Pseudomonas taenensis* using the different solid and liq-

uid substrates had been reported in the previous study of Akintokun *et al.* (2017).

### *Activities of Different Biosurfactants on Bacterial Isolates*

The biosurfactant demonstrated slight antibacterial activities against the test pathogens. Zones of inhibition indicated a quantitative measurement for the antibacterial property against the pathogens. Resistance observed by the test isolates (*Bacillus subtilis*, *Staphyloccoccus aureus*, *Escherichia coli* and *Klebsiella pneumonia*) to the biosurfactant was an indication of the inability of the biosurfactant to prevent the growth of the test isolates thereby remaining very viable, whereas, the appearance of clear zone after 24 hrs indicated the potential of those biosurfactants on the test pathogens. Antibacterial analysis revealed that *Escherichia coli* only was sensitive to biosurfactant produced from cassava waste water while *Staphylococcus aureus* only was sensitive to biosurfactant produced from pome, cassava flour waste and molasses substrates. *Bacillus subtilis* and *Klebsiella pneumonia* were resistant to all the biosurfactant produced from all the substrates (Table 1).

**Table 1: Antibacterial Analysis of Biosurfactant on Bacterial Isolates**

| Different wastes as substrates for biosurfactant production | Zones of inhibition (mm) | | | |
|---|---|---|---|---|
| | *Bacillus subtilis* | *Staphyloccoccus aureus* | *Escherichia coli* | *Klebsiella pneumonia* |
| Groundnut cake Biosurfactant | Resistance | Resistance | Resistance | Resistance |
| Cassava flour waste Biosurfactant | Resistance | 7.0 ± 0.5 | Resistance | Resistance |
| Pome Biosurfactant | Resistance | 4.5 ± 1.0 | Resistance | Resistance |
| Cooking oil Biosurfactant | Resistance | Resistance | Resistance | Resistance |
| Engine oil Biosurfactant | Resistance | Resistance | Resistance | Resistance |
| Cassava water Biosurfactant | Resistance | Resistance | 2.1± 0.4 | Resistance |
| Molasses Biosurfactant | Resistance | 1.1± 0.1 | Resistance | Resistance |
| Cassava peel waste Biosurfactant | Resistance | Resistance | Resistance | Resistance |
| Potato waste Biosurfactant | Resistance | Resistance | Resistance | Resistance |

Values are Mean ± Standard Error of Means

### *Antibiotic Activity of Biosurfactant-producing Organism*

The antibiotic assay showed that the biosurfactant producing isolate was sensitive to only few antibiotics in the multiple antibiotics disc. *Pseudomonas taeanensis* was sensitive to augmentin, ofloxacin, tetracyclin and ciprofloxacin and resistant to Cotrimoxazole, Pefloxacin, Amoxylin, Ceftriazone, Nitrofuranton and Gentamycin suggesting the inactivity of *Pseudomonas taeanensis* to the antibiotics with zone of inhibition, thus, the antibiotics could prevent their growth while resistance of *Pseudomonas taeanensis* to certain antibiotics showed the inefficiency of the antibiotics on *Pseudomonas taeanensis* (Table 2).

**Table 2: Susceptibility Pattern of *Pseudomonas taeanensis* to Antibiotics**

| Antibiotics | Diameter of zone of inhibition (mm) |
|---|---|
| Augmentin | 10.2± 1.76 |
| Ofloxacin | 10.0± 2.03 |
| Ceftriazone | Resistance |
| Nitrofuranton | Resistance |
| Gentamycin | Resistance |
| Amoxylin | Resistance |
| Tetracyclin | 9.0± 1.02 |
| Cotrimoxazole | Resistance |
| Ciprofloxacin | 6.0± 1.22 |
| Pefloxacin | Resistance |

Values are Mean ± Standard Error of Means

***Stability of Biosurfactant Produced by Pseudomonas taenensis Using Cassava flour waste***

The biosurfactant produced from the substrate with the highest antibacterial potential (cassava flour waste) was analysed. The result showed that the biosurfactants are stable over wide pH, temperature and sodium chloride concentration.

An increase in pH from 2 to 12 significantly showed that the biosurfactant are stable. pH lower than 6 showed decrease in stability and the best pH was 8 (Figure 1). Results showed that the biosurfactant are stable from temperature of 4°C to 100°C but increase in temperature from 55°C to 100°C reduces the stability of the biosurfactant (Figure 2).

In addition, effect on sodium chloride concentration on biosurfactant showed that biosurfactant are more stable at NaCl concentration of 0 to 20 while the stability of biosurfactant reduces at a concentration higher than 20 (Figure 3).

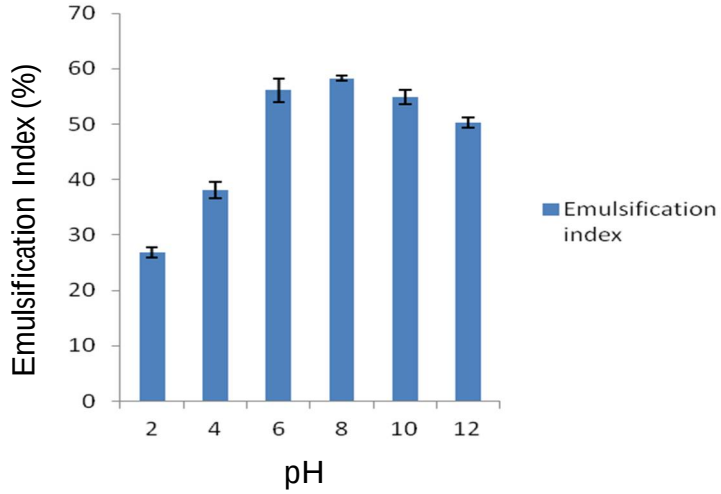


**Figure 1: Effect of pH on cassava waste biosurfactant produced by *Pseudomonas taenensis***

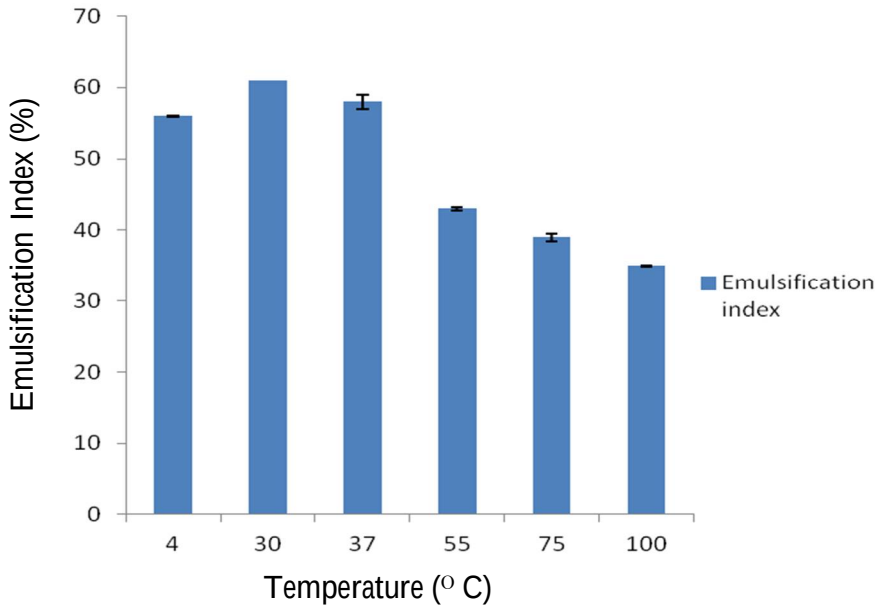


**Figure 2: Effect of Temperature on cassava waste biosurfactant produced by *Pseudomonas taenensis***

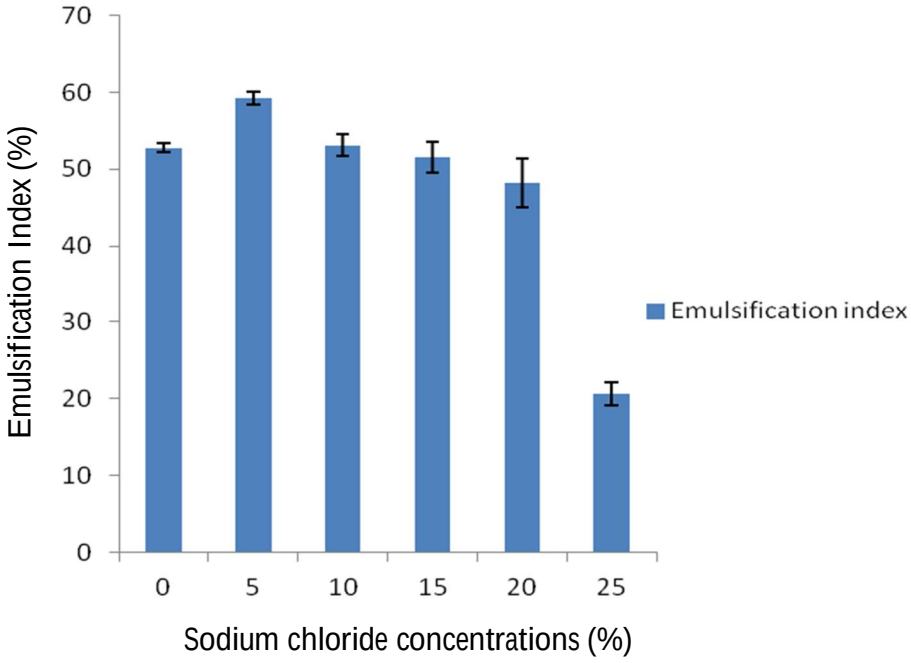


**Figure 3: Effect of NaCl on cassava waste biosurfactant produced by *Pseudomonas taenensis***

## DISCUSSION

Suitability of surfactants produced by microorganisms for application in several industrial areas depends on its stability against extreme or varying conditions of temperature, pH and salinity (Araujo *et al*., 2019). Singh and Cameotra, (2004) reported that biosurfacant that possessed antibacterial activity could be used as anti-adhesives on solid surfaces for the prevention of pathogenic bacterial strains. The antibacterial activities of the biosurfactant in this study revealed that certain biosurfactants were sensitive to few pathogens, although, several studies have highlighted the antimicrobial properties of different biosurfactant produced from different substrates.

The findings in this study disagree with those published by Mouafo *et al*. (2018) who affirmed the antimicrobial activity of biosurfactants synthesized by lactobacilli against Gram-positive and Gram-negative bacteria. Gudina *et al.* (2010) showed that biosurfactants produced by *Lactobacillus paracasei* were active against *E. coli, P. aeruginosa, S. aureus, S. epidermidis, Streptococcus agalactiae* and *Streptococcus pyogenes* and Naughton *et al.* (2019) reported the use of biosurfactants as antimicrobial molecules in medicine for applications against pathogenic microorganisms for infections and diseases.

Variation or inefficiency of few biosurfactants to inhibit the growth of the test pathogens compared to the findings of some researchers, could be attributed to the fact that different isolates were used for the biosurfactant production. The antimicrobial activities of the biosurfactant recorded showed that not all biosurfactants could be used as an alternative antimicrobial agents because most of the pathogenic microbes are resistant to the biosurfactant except biosurfactant produced from pome, cassava flour waste and molasses.

The antimicrobial property of biosurfactants rely on different mechanisms to destroy target organisms as compared to conventional antibiotics (Banat *et al*., 2010) and they primarily destroy bacterial cells by directly disrupting the integrity of the plasma membrane or cell wall (Sang and Blecha 2008; Yount and Yeaman 2013). Cameotra and Makkar (2004) suggested that the ability of the biosurfactants with antimicrobial activities could be due to the interaction with the cytoplasmic membrane of the pathogenic isolates by binding to the phospholipid surface through electrostatic forces that are then absorbed in the hydrophobic core of the membrane perturbing the packing of the lipids that in turn leads to the dissolution of the proton motive force and leakage of essential molecules. The antimicrobial activities observed could also be an outcome of the adhesion property of these surface-active agents to the cell membrane, leading to subsequent collapse of the nutrition cycle (Ines and Dhouha, 2015).

Biosurfactant produced from cassava flour waste had the highest antibacterial activity suggesting the use of the substrate in industrial scale. The antibiotic test showed that the biosurfactant-producing isolate was resistant to some antibiotics thus their handling should be under caution and necessary awareness should be promoted. The applicability of biosurfactants in several fields depends on their stability at different temperatures, pH values and salt concentrations. Physiological factors were considered to be the most critical environmental parameter in many industries before the biomolecules could be applied (Al-Sulaimani *et al*., 2011).

The stability of the biosurfactant corroborates with the findings of Singh and Cameotra (2004) where biosurfactant produced by *Arthrobacter protophormiae* was found to be both thermostable (30-100°C) and stable at pH (2 to 12). The stability of biosurfactants at pH (2 to 12) indicates that biosurfactants have activity in both acidic and alkaline condition. In the same vein, El-Sersy (2012) indicated that biosurfactant from *B. subtilis* was found to have high surface activity predominantly in the alkaline condition. Meanwhile, Techaoei *et al.*, (2011) documented that the effects of lower biosurfactant activity in acidic condition to be due to its negatively charged molecules. Similar results had been reported for biosurfactant production from other microorganisms such as *P. aeruginosa* which was stable at a wide range of pH and sodium chloride concentrations (Yin *et al*., 2009; Vijayakumar and Saravanan, 2011; Techaoei *et al*., 2011; Khopade *et al.*, 2012).

Resistance to low and extreme temperature shows the thermal stability of the biomolecule; the ability to preserve a moderate salinity environment indicates that the biosurfactant is halophilic (Chandankere *et al.* 2013) and the moderately strong ionic tolerance suggests its suitability for microbial enhanced oil recovery under high-salinity conditions (Shavandi *et al*., 2011). However, this study contradicts the findings of Sarubbo *et al.* (2007) who reported loss of biosurfactant thermal stability after heating for 1 hr at 70 °C.

The stability of the biosurfactants suggest their ability to withstand harsh or unfavourable conditions which are promising for the industrial application because most of the biosurfactants are resistant towards environmental factors such as NaCl, temperature and pH.

## CONCLUSION

The unique tolerance of the stability of biosurfactant produced by *Pseudomonas taenensis* makes this bacterium a good candidate for different industrial applications. Other biosurfactant from different substrate by various isolates should also be investigated for high antimicrobial activity. This study revealed the possible application of the biosurfactant in the industrial biotechnological area but not as an antibacterial agents.

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