Production of Biosurfactant from microorganism using tigernut as sole carbon source

H.S Audu[1] A. Jimoh[2] S.H.Paul[3]
Research Scholar [1], Professor [2], Professor[3] Department of Chemical Engineering [Federal] University of Technology Minna, Niger State, Nigeria

Abstract— Bio surfactants are structurally diverse group of surface-active compounds produced by microorganismsextracellularly or as part of the cell membranes by a variety of yeast, bacteria and filamentous fungi from various substances including sugars, oils and wastes. In this present study a bio surfactant producing bacterial strain was isolated, screened for bio surfactant production capabilities.The bio surfactant production was studied using a Minimal Salt Medium (MSM) with Tigernut Waste (1 %) as the carbon source. The produced bio surfactant was characterized by measuring the emulsification index; forming characteristics Drop collapse and produced biosurfactant Fourier Transform Infrared radioscopy (FTIR).The results which showed that the produced bio surfactant shares similar characteristics as to standard biosurfactant.

Index Terms— Biosurfactant, microorganism, FT-IR, Drop collapse, Emulsification index

1 INTRODUCTION

THIS document Biosurfactants are structurally diverse group of surface-active compounds produced by microorganisms extracellular or as part of the cell membranes by a variety of yeast, bacteria and filamentous fungi from various substances (Chen *et al.*, 2007). Surfactant molecules have the ability to form stable emulsions and are present in various formulations of pharmaceutical products cosmetics petroleum compounds (makkar*et al*2011).They also have potential applications in environmental uses such as organic pollutants treatment and oil recovery (Neves*et al.*, 2007).

Bio surfactants productions by microorganisms entails the utilization of hydrocarbons, oily residues during their exponential cellular growth but can also be produced from agro waste such as vegetable oils glycerol starch (Marylane de Sousaet al., 2014) and are known to exist in variety of chemical structures, such as ramnolipids, glycolipids lipopeptides polymeric and particulate structures. Biosurfactants have also been found to have essential properties which are of biomedical and therapeutic importance as well as antiviral and antifungal properties (Muhammed .I .A 2011).

Owing to their interesting properties such as lower toxicity, higher degree of biodegradability, higher foaming capacity and optimal activity at extreme conditions of temperatures, pH levels and salinity, they have been increasingly attracting the attention of the scientific and industrial community (Banat et al., 2010). Furthermore, their availability in commercial quantity is basically hindered by the economy of production and recovery processes when compared to their chemically synthesized surfactants type. So far, several renewable substrates from various sources, especially from industrial wastes have been intensively studied for microorganism cultivation and surfactant production at an experimental scale. The purpose of this study is to investigate the potential of using a novel substrates sourced from tiger nut (popularly called AYA) as a carbon source for bio surfactant production by a locally isolated microorganism (pseudomonas aeruginosa)sourced from polluted soil from mechanic workshops and fuel filling stations and garden soil.

2.0MATERIALS AND METHOD

2.1Isolation and Identification of Biosurfactant-Producing Bacteria from the Waste Oil-Contaminated Soil

Four soil samples were collected from mechanic sites and petrol stations in Tudun Fulani Bosso, in Niger State, Nigeria; and 10 grams of each soil sample was placed into 250 ml flask containing 100 ml of distilled water and incubated at 23^{o}C on a shaker at 200 rpm for about 48hours, after which samples from each slurry was serially diluted, plated on nutrient agar medium (i.e. an aliquot of 10^{-4}, 10^{-5} and 10^{-6}) and incubated for 48 hours in duplicates. After incubation, plates were enumerated and morphologically different bacterial colonies were sub cultured on nutrient agar for 24 hours to obtain the pure culture. The cultures were stored in agar slants and used for biosurfactant screening.Das, K.*et al* 2007.

2.2 Production of Biosurfactants by the Selected Bacterial Isolates

Nine purified isolates were selected and tested for biosurfactant production. The Isolated colonies were inoculated into 5 ml mineral salt medium (MSM) containing 2% tigernut flour as the sole carbon and energy source. The MSM was a mixture of two solutions. Solution A contained (per liter) 2.5 g of $NaNO_3$, 0.4 g of $MgSO_4.7H_2O$, 1.0 g of NaCl, 1.0 g of KCl, 0.05 g of $CaCl_2.2H_2O$, and 10 ml of concentrated phosphoric acid (85%). The solution was adjusted to pH 7.2 with KOH pellets.

Solution B contained (per liter) 0.5 g of $FeSO_4.7H2O$, 1.5 g of ZnSO4.7H2O, 1.5 g of $MnSO_4.H_2O$, 0.3 g of K_3BO_3, 0.15 g of $CuSO_4.5H_2O$, and 0.1 g of $Na_2MoO_4.2H_2O$. One (1) ml of solution B was added to 1000 ml of solution A to form the MSM. The broth cultures were incubated in a shaker (at 200 rpm) for 7 days at room temperature. Suspensions were then tested for the presence of surfactant according to (Das, K.*et al* 2007). After incubation, the culture media were centrifuged at 4000 rpm for 30 min to obtain a cell free supernatant. The culture free supernatant was tested for the presence of biosurfactant.

2.3Screening for Biosurfactant Production

i. Oil Spreading Test

The produced biosurfactants were separated from culture media by centrifugation to get culture supernatant and characterized by measuring diameters of clear zones produced when a drop of a biosurfactant-containing solution is placed on an oil-water surface. Fifty (50) ml of distilled water was added to a large Petri dish (15 cm diameter) followed by addition of 2 drops of condemned engine oil to the water surface. And 1 drop of culturesupernatant was gently placed on the center of the oil layer. The presence of biosurfactant was detected by the displacement of the oil and hence the appearance of clear zone after 30 sec.

ii. Emulsification Assay (Emulsification index E24)

In a screw caped tube, 4 ml of the cell free supernatant was added to 4 ml of each of the following oils: kerosene, fuel, paraffin and vegetable oil. The tubes were vortexed at high speed for 2 minutes. The mixture was allowed to settle for 24 hours and the emulsification index (E24) was measured as the ratio of the height of the emulsion layer and the total height of the mixture and then multiplied by 100

$$E_{24} = \frac{\text{Height of emulsion formed (cm)}}{\text{Total height of solution (cm)}} \times 100$$

iii. Drop collapse

Drop collapse test was performed in the 96 well microlitre plates. Petrol oil and diesel oil (2 µl each) separately were applied separately to each well, equilibrated at 23°C for 24 h. The 48 h culture was centrifuged at 5000 rpm for 20 mins, to remove the cells. Five microlitre aliquot of the supernatant was poured into oil coated region of each well. The drop size was observed after 1 min. The result was considered positive when the diameter of the drop was increased or drop collapsed as compared to the drop produced by distilled water which was taken as a negative control (Bodour*et al*. 1998).

2.5Identification of Microorganism through 16S rRNAGene

Bacterial isolate exhibiting high biosurfactant producing capability was selected and identified by partial 16S rRNAsequencing. Partial sequencing of the 16SrRNA gens was carried out at inqaba biotech lab Ibadan Nigeria.

Extraction of Biosurfactants

The culture broth was refrigerated at 4^0C and then centrifuged at 4000 rpm for 30minutes to remove the cells and filtered with sterile whatman No 1.Filter paper. The clear sterile supernatant served as the source of crude biosurfactant. The biosurfactant was recovered from the cell free culture supernatant by cold acetone precipitation; 2ml of chilled acetone was added and allowed to stand for 10 hours at 4^0C. The precipitate was collected by centrifugation and evaporated to dryness to remove residual acetone after which it was re-dissolved in sterile water.

2.4 Characterization of Biosurfactant

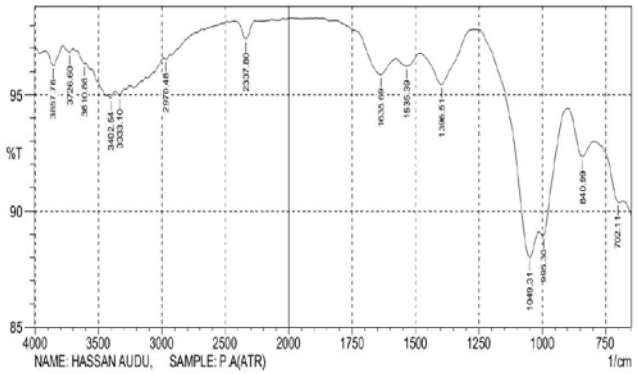
Fourier transforms infrared spectroscopy

The solid biosurfactant extracts recovered from the supernatant of the production medium was characterized by Fourier transform infrared spectroscopy (FTIR) according to the method of Jorge, *et al*., 2013. The FTIR spectra, with a resolution of 4 cm^{-1}, were collected from 400 to 4000 wave numbers ($cm^{-1)}$, and is an average of 128 scans using a Tensor 27 Infrared Spectrometer operating in the attenuated total reflection (ATR) mode (equipped with a single horizontal Golden Gate ATR cell).

S/N	BIOCHEMICAL TESTS					Bacterial isolates
	ISOLATES	Oxidase	Gram staining	Motility	catalase	indole	
1	Isolate A	+ve	-ve	-ve	+ve	+ve	Staphylococcus spp.
2	Isolate B	-ve	+ve	+ve	-ve	+ve	Bacillus *Spp*
3	Isolate C	+ve	-ve	+ve	-ve	-ve	Serratiaspp
4	Isolate D	-ve	+ve	+ve	-ve	+ve	Bacillus Spp
5	Isolate E	+ve	-ve	+ve	+ve	+ve	Pseudomonas spp
6	Isolate F	-ve	+ve	+ve	-ve	+ve	Bacillus spp
7	Isolate G	-ve	-ve	-ve	-ve	-ve	Bacillus spp
8	Isolate H	+ve	-ve	+ve	+ve	-ve	Pseudomonas *spp*
9	Isolate I	-ve	-ve	+ve	-ve	+ve	Enterobacter spp.

Microorganisms	Biosurfactant Assay	Tigernut Flour	Tigernut waste	Tigernut extract
Isolate A	Oil spreading(cm)	2.0	0.2	0.8
	Drop collapse	-ve	-ve	-ve
Isolate B	Oil spreading(cm)	1.0	1.0	0.2
	Drop collapse	-ve	-ve	-ve
Isolate C	Oil spreading(cm)	1.2	0.6	0.
	Drop collapse	-ve	-ve	-ve
Isolate D	Oil spreading(cm)	1.0	0.2	0.4
	Drop collapse	+ve	+ve	-ve
Isolate E	Oil spreading(cm)	1.0	0.2	0.4
	Drop collapse	-ve	-ve	-ve
Isolate F	Oil spreading(cm)	0.2	0.1	0.4
	Drop collapse	-ve	-ve	-ve
Isolate G	Oil spreading(cm)	1.5	0.2	0.2
	Drop collapse	-ve	-ve	-ve
Isolate H	Oil spreading(cm)	4 .0	0.6	0.6
	Drop collapse	+ve	-ve	-ve
Isolate I	Oil spreading(cm)	1.0	0.4	0.2
	Drop collapse	-ve	-ve	-ve

Table 3.1 Biosurfactant production activity of isolates when grown in mineral medium with tigernut waste as car-



3.1 Percentage transmittance versus wave numbers of partially purified Biosuractant.

3.0 DISCUSSION OF RESULTS

Serial dilution of soil sample and subsequent plating on nutrient agar resulted in isolation of fifteen isolates. A total of 9 isolates were isolated from the different soil samples(two sites) out of which of the isolates 3 were gram positive and the remaining seven (6)isolates were gram negative. The qualitative assay methods used in this study revealed thatspecies of Pseudomonasisolated was positive for biosurfactant production.

The result of the molecular characterization of the potent isolate shown in figure 3.1 in NCBI showed that the strain had a sequence length of 1132bp.a sequence comparison using BLAST it was identical to *Pseudomonas aeruginosa* (LMG 1242, 1131 bp with 99% homologyas shown in Table 3.1, In the drop-collapsing test all the three strains collapsed the oil drop thus producing a flat drop indicating that they are potentproducers of biosurfactant(carillo.,1996) Three of the isolates successfully displace the oil hence confirmation to production of biosurfactant. Displacement of oil clearly is a sign of extracellular surfactants present in the supernatant of culture.

3.1 FTIR RESULTS

The molecular composition of the crude biosurfactant was evaluated by FTIR and from the spectral result in figure 4.4 and summary of findings shown in table(4.5) fourtheen peaks were identified and the most significant bands which showed O-H stretching bands of the ramnose ring were observed in the region 3857.76cm^{-1} ,3726.60 , 3610.88 ,3402.54and 3333.1cm^{-1} a result which is in agreement with the work of (Thavasi*et al.*,2011) who discovered O-H stretching bands.The compound showed the C–H stretching vibrations in the transmittance range 2970.49cm^{-1} Carbonyl stretching band was found at 2337.80 cm^{-1} which is the characteristic peak for ester compounds as reported by Tuleva.et al., (2012). The ester carbonyl group was also proved from the band at 1635.69 cm^{-1} which corresponds to C-O deformation vibrations. Carbonyl (C=O) stretching (acidic) was observed at 1535.39cm^{-1} similar to 1398.51cm^{-1} Scissoring vibration of a CH_2 group adjoining a carboxyl ester was also observed at 1049.31 cm^{-1} to 965.30cm^{-1}. The peak in the region of 804.99cm^{-1}- 702.1cm^{-1} indicates CH stretching in the rhamnose, similar to what Tuleva.et al., (2012) achieved .Rhamnolipids produced by Pseudomonas aeruginosa were the most studied biosurfactants due to their potential applications in a wide variety of industries and the high levels of their production .

4.0 CONCLUSION

From this research the following conclusions were drawn;

A potent Strain Bacteria pseudomonas aeruginosa isolated from oil contaminated Soil grown on tigernut supplemented media lead to the production of biosurfactant.

The molecular Characterization of the potent microorganisms showed that the biosurfactant producing bacteria is a pseudomonas belonging to the genre aeruginosa and it lead to

biosurfactant production when grown in a mineral salt medium with tigernut flour as carbon source.

That Tigernut supplemented media can lead to the production of Biosurfactant.The Fourier transformation spectroscopy showed that the produced biosurfactant belonged to the glycolipids type of biosurfactant.

REFERENCES

1. BODOUR A, MILLER-MAIER R. APPLICATION OF A MODIFIED DROP-COLLAPSE TECHNIQUE FOR SURFACTANT QUANTIFICATION AND SCREENING OF BIOSURFACTANT-PRODUCING MICROORGANISMS. JOURNAL OF MICROBIOLOGICAL METHODS.1998; 32(3):273–80.

2. CHEN, C., BAKER, S., AND DARTON, R. (2007). THE APPLICATION OF A HIGH THROUGHPUT ANALYSIS METHOD FOR THE SCREENING OF POTENTIAL BIOSURFACTANTS FROM NATURAL SOURCES, JOURNAL OF MICROBIOLOGICAL METHODS,70, 503-510

3. CARRILLO, P., MARDARAZ, C., PITTA-ALVAREZ, I.S., GIULIETTI, A.M (1996). ISOLATION AND SELECTION OF BIOSURFACTANTPRODUCING BACTERIA. WORLD JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY, 12:82-84.

4. DAS P, YANG XP, MA LZ. ANALYSIS OF BIOSURFACTANTS FROM INDUSTRIALLY VIABLE PSEUDOMONAS STRAIN ISOLATED FROM CRUDE OIL SUGGEST SHOW RHAMNOLIPIDS CONGENERS AFFECT EMULSIFICATION PROPERTY AND ANTIMICROBIAL ACTIVITY. 2014; 5:1-8.

5. ERUMSHOEB, UZMABADAR, JAMEELAAKHTER, FAIZA A. ANSARI1, MAHEENWAQAR AND MAQSOOD A. ANSARI (2012).SCREENING OF SURFACTANT PRODUCING BACTERIAL STRAINS ISOLATED FROM SOIL SAMPLES OF AN AUTOMOBILE WORKSHOP.KARACHI UNIVERSITY JOURNAL OF SCIENCE, 2012, 40, 31-36 31.

6. THAVASI R, SUBRAMANYAM NM, JAYALAKSHMI S, BALASUBRAMANIAN T, IBRAHIM MB. BIOSURFACTANT PRODUCTION BY AZOTOBACTERCHROOCOCCUM ISOLATED FROM THE MARINE ENVIRONMENT. MAR. BIOTECHNOL. 2009; 11:551-556.

7. TUELVA, B.K., IVANOV, G.R. AND CHRISTOVA, N.E. (2002). BIOSURFACTANT PRODUCTION BY NEW PSEUDOMONAS PUTIDA STRAIN. ZEITSCHRIFTNATURFORSCHUNG C, 57, 356-360.

8. MUHAMMAD, I.A. (2012). USE OF PETROLEUM PRODUCTS AS SUBSTRATE FOR THE PRODUCTION OF BIO SURFACE ACTIVE AMPHIPHILES BY PSEUDOMONAS AERUGINOSA. AN UNPUBLISHED THESIS SUBMITTED TO THE DEPARTMENT OF MICROBIOLOGY, AHMADU BELLO UNIVERSITY, ZARIA

10. NEVES LCM, OLIVEIRAKS,KOBOYASHIMMJ,PENNATCV,CONVERTI A BIOSURFACTANT PEODUCTION BY CULTIVATION OF BACILLUS ATROPHAEUS ATCC 9372 IN SEMIDEFINED GLUCOSE CASEIN-BASED.MEDIAAPPLBIOCHEM BIOTECHNOL,2007;137-140;539-54