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ISOLATION AND SCREENING OF BIOSURFACTANT- PRODUCING BACTERIA FROM SOIL CONTAMINATED WITH DOMESTIC WASTE WATER.

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ABSTRACT: *Bacterial isolates from waste water oil-contaminated soil sample were screened and evaluated for biosurfactant production in this study. Using culture –dependent technique, twenty two (22) bacterial isolates were recovered from eight (8) different sites. The dominant species were Bacillus and Pseudomonas; with an occurrence rate of 22.73% each. The isolates were subjected to conventional biosurfactant screening tests: qualitatively (drop collapse and microplate assay) and quantitatively (oil speading and emulsification activity). In all, Bacillus and Pseudomonas species were positive for all the tests and they had a clearing zone of 4mm each and an emulsification capacity of 51.61% and 53.13% respectively. This confirms their ability to produce biosurfactants that reduce interfacial and surface tension thereby leading to increase in solubility and emulsification of these oils.*

KEYWORD: Biosurfactants, Index Emulsification Test, Surface tension, drop collapse, oil waste water

INTRODUCTION

Biosurfactants are amphiphilic biological compounds produced extracellularly or as part of the cell membranes by a variety of yeast, bacteria and filamentous fungi from various substances including sugars, oils and wastes (Chen *et al*., 2007). They are generally lipid compounds whose features are related to two ends present in the molecule, one end is hydrocarbon part which is less soluble in water (hydrophobic end).The hydrophobic part of the molecules is a long- chain of fatty-acids, hydroxyl fatty acid or α-acyl hydroxyl-fatty acids. The other end is hydrophilic, more soluble in water and consists of carbohydrate, amino acid, cyclic peptide, phosphate and carboxylic acid or alcohol (Mata- Sandoval *et al*., 1999; Maier and Soberon-Chavez, 2000; Chayabutra *et al*., 2001; Volchenko *et al*., 2007; Chen *et al*., 2007).

Interest in microbial surfactants has been steadily increasing in recent years, as they have numerous advantages compared to chemical surfactants including a lower toxicity, higher biodegradability (Woo and Park, 2004) higher foaming, better environmental compatibility (Banat, 1995) and effective properties at extreme temperature, pH levels and salinity (Cho *et al.*, 2005). They have extended applications in petrochemical and oil industries, pharmacy, medical, cosmetics, food and pharmaceutical (Makkar and Cameotra, 2002; Van Ginkel, 1996; Babu *et al*., 1996). Thus among all Oil industry is the greatest market of these compounds (Dyke *et al*., 1993). Biosurfactant producing microorganisms were naturally present in oil contaminated soil. Oil contaminated environment contain large amount of hydrocarbons i.e., aliphatic and aromatic hydrocarbons. Microorganisms exhibit emulsifying activity by producing biosurfactants and utilize the hydrocarbons as substrate often mineralizing them or converting them into harmless products.

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Bacteria are the main group of biosurfactant-producing microorganisms, although it is also produced by some yeast and filamentous fungi. A number of studies have reported the potential of *Bacillus* species as biosurfactant producers and they produce lipopeptide type of biosurfactant (Nitschke and Pastore, 2004). The objective of the present study is to screen the biosurfactant- producing bacteria from soil contaminated with domestic waste water.

MATERIAL AND METHODS

Collection of samples

Waste water oil- contaminated soil samples were collected from eight (8) different sites in the Ekiti State University, Ado Ekiti (EKSU) premises. Five grams (5g) of the different samples were collected in sterile polythene bags, labelled appropriately and taken to the laboratory for further study.

Method of isolation

Six- fold serial dilution was carried out, pour plate method was used for inoculation; One (1ml) of 10^{-6} dilution was inoculated on sterile Petri dishes, after which the sterilized media was poured aseptically on the inoculated plates. The plates were incubated at 37^{o}C for 24hours. After incubation, morphologically different colonies observed on the plates were subcultured on a nutrient agar plates to obtain pure culture of the organisms and subsequently transferred into a nutrient agar slants. The slants were kept in the refrigerator at 4^{0}C as stock culture.

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Characterization and Identification of isolates

Pure cultures of the isolates were identified based on their cultural, morphological and physiological characteristics in accordance with the taxonomic scheme of Barrow and Feltham (1993) and reference to Holt *et al.* (1992). The tests performed include Gram stain, spore stain, motility test, catalase test, oxidase, coagulase, urease, indole production, hydrogen sulphide production, nitrate reduction, methyl red, Voges-Proskauer, oxidative/fermentative test and utilization of carbon sources.

Screening of Isolates for biosurfactant production

Prior to the screening for biosurfactants, the isolates were inoculated into 10ml of broth medium each and the incubated at 37^{o}C for 72 h. The culture media were centrifuged at 3000 revolutions per minute (r.p.m.) for 30 minutes. The supernatant was collected and the cells discarded. The supernatant was used for the various biosurfactant screening tests or assays.

Drop Collapse Assay

The assay was carried out as described by Jain *et al*. (1991). A drop of the culture supernatant was placed carefully on an oil coated glass slide and observed after one minute. If the drop of supernatant collapsed and spread on the oil coated surface, it signifies the presence of biosurfactant (positive). But if the drop remains after one minute, it was documented as negative. This test was simultaneously carried out on distilled water as control.

Oil Spreading Assay

Using a micropipette, ten microliter (10µl) of vegetable oil was added to the surface of 40ml of distilled water into a peril dish to form a tin oil layer. 10µl of the culture supernatant was gently dropped on the centre of the oil layer. After one minute, if biosurfactant was present in

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the supernatant, the oil is displaced and a clearing zone was formed as described by Morikawa *et al.* (2000).

Microplate Assay

Using a micro pipette, 100µl of the culture supernatant was taken and put into a microwell of a 96-microwell plate. The plate was viewed using a backing sheet of paper with a grid. Pure water in a hydrophobic well has a flat surface but when surfactant is present, it appears concave, taking the shape of the wells (Vaux and Cottingham, 2001).

Emulsification Capacity

Two (2ml) of kerosene was added to 2ml of the culture supernatant and the mixture was vortexed at high speed for 2 minutes. The mixture was then left for 24 h; the height of the stable emulsion layer was measured. The emulsification capacity of biosurfactant was developed by Cooper and Goldenberg (1987) and the emulsion index E_{24} was calculated as the ratio of the height of the emulsion layer and the total height of liquid.

$$E_{24} = \frac{h_{emulsion}}{h_{total}} \text{ X } 100\%$$

RESULTS

A total of twenty two (22) bacterial isolates were obtained from eight different sites of soil contaminated with domestic oil of waste water within Ekiti State University Ado-Ekiti satellite area. The frequency of isolation of the bacterial isolates is shown in Table 1. Nine (9) different bacterial types were encountered. They include *Bacillus* spp. (22.72%), *Pseudomonas* spp. (22.73%), *Enterococcus faecalis* (18.80%), *Listeria* spp. (9.10%), *Micrococcus luteus* (9.10%), *Escherichia coli* (4.55%), *Enterobacter* sp. (4.55%), *Staphylococcus aureus* (4.55%) *and Streptococcus* sp. (4.55%).

Table 1: Frequency of Bacterial Isolates

Isolates	Frequency (%)
Bacillus spp.	22.73
Pseudomonas spp.	22.73
Enterococcus faecalis	18.80
Listeria sp.	9.10
Micrococcus luteus	9.10
Escherichia coli	4.55
Enterobacter sp.	4.55
Staphylococcus aureus	4.55
Streptococcus sp.	4.55

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Table 2: Biosurfactants Screening Tests (Qualitative)

Isolates	Drop Collapse Assay	Microplate Assay
Bacillus spp.	+	+
Pseudomonas spp	+	+
Escherichia coli	-	-
Staphylococcus sp.	-	-
Streptococcus sp.	-	-
Listeria sp.	-	-
Enterobacter sp.	-	-
Enterococcus sp.	-	-
Micrococcus sp.	-	-

Key: + = Positive, - = Negative

Table 3a: Oil Spreading Assay Using Vegetable Oil (Quantitative Screening)

Isolates	Clearing Zone (mm)
Bacillus spp.	4.0
Pseudomonas spp.	4.0

Table 3b: Emulsification Activity Using Kerosene (Quantitative Screening)

Isolates	Total Height of Solution (mm)	Height of Emulsion (mm)	% Emulsification
Bacillus spp.	31	16	51.61
Pseudomonas spp.	32	17	53.13

DISCUSSION

Biosurfactant- producing bacteria were isolated from soil contaminated with domestic oil waste water of food restaurants. A total of 22 isolates were isolated from the different soil samples (eight sites) out of which 15 of the isolates were gram positive and the remaining seven (7) isolates were gram negative. The qualitative assay methods used in this study revealed that species of *Bacillus* and *Pseudomonas* isolated were positive for biosurfactant production. Further study to ascertain the potential of both *Bacillus* sp. and *Pseudomonas* sp. confirmed that they are producers of surface active agents as shown in Table 2, 3a, and 3b. This is in accordance to Tabatabaee *et al.* (2005) and Jaysree *et al.* (2011).

The high incident rate of *Bacillus* sp. and *Pseudomonas* sp. in domestic oil contaminated waste water when compared to other organisms isolated, may be a direct correlation to their ability to produce emulsifiers to degrade oil rather than just a coincidence. As shown in Table 1, these two organisms had similar frequency of isolation of 22.73% respectively. They have a combine incident rate of 45.46% which is almost half of the other isolated organisms combined. The two species; *Pseudomonas* and *Bacillus* were positive for the qualitative screening (Table 2), had a clearing zone of 4mm each in Table 3a and an emulsification capacity of 53.13% and 51.61% respectively in Table 3b.

Bacillus species has proved to occur mainly in soil and because of their spore forming ability; the spores have the ability to survive harsh conditions in soil environment. Although, *Bacillus*

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is found on other matrices (air, water etc.), its occurrence in soil samples were found to be relatively high when compared to other matrices. Other organisms with high incident rates develop mechanism for surviving harsh conditions of the soil. *Bacillus* species, most especially *Bacillus subtilis* produces surfactin and lipopeptides (Nitschke and Pastore, 2004), while *Pseudomonas aeruginosa* produces glycolipids which act as emulsifiers or surface-active agents; consequently reducing the surface tension of hydrophobic molecules and leading to their breakdown (Bodour and Miller-Maier, 2002; Yin *et al.*, 2009).

In conclusion, this study and previous reports has revealed that genera *Pseudomonas* and *Bacillus* are potential surface active agent producer and useful tools for various environmental and industrial processes.

REFERENCES

Babu, P.S., Vaidya, A.N. Bai, A.S. Kapur, R. and Juwarkar, A. (1996). Kinetics of biosurfactant production by *Pseudomonas aeruginosa* strain BS2 from industrial wastes. *Biotechology Letters*., 18: 263- 268.

Barrow, G. I. and Feltham, R. K. A. (1993). Cowan andSteel's manual for identification of Medical Bacteria. 3rd Edition Cambridge University Press London.

Bodour, A. A. , R.M. Miller-Maier,R.M. (2002). Biosurfactant: types, screening methods and applications. Encyclopedia of environmental microbiology. NY. Wiley and sons. Pp.750-770.

1998. 32:273-280.

Chayabutra, C., Wu, J. and Ju, L.K. (2001). Rhamnolipid production by *Pseudomonas aeruginosa* under denitrification: Effects of limiting nutrients and carbon substrates. Biotechnol. Bioeng., 72: 25-33

Chen, S.Y., Wei, Y.H. and Chnd, J.S (2007). Repeated pH-stat fed-batch fermentation for rhamnolipid production with indigenous *Pseudomonas aeruginosa* S2. Applied Microbial. Biotechnol., 76: 67-74.

Cho, W.S., Lee, E.H., Shim, E.H., Kim, J.S., Ryu, H.W. and Cho, K.S. (2005). Bacterial communities of biofilms sampled from seepage groundwater contaminated with petroleum oil, *J Microbiol Biotechnol*, 15: 52-964.

Cooper, D. G. and Goldenberg, B. G. (1987). Surface-active agents from two *Bacillus* species. *Applied Microbiology and Biotechnology* 53(2): 224-229.

Dyke, M.I.V., Gulley, S.L. Lee, H. and Trevors, J.T. (1993). Evaluation of microbial surfactants for recovery of hydrophobic pollutants from soil. J. Indus. Microbol. Biotechnol., 11: 163-170.

Holt, J. G., Krieg, N. R., Sneath, P. H. A., Staley, J. T. and Williams, T. (1994). Bergey's Manual of Determinative Bacteriology, 9th Edition. Williams & Wilkins, Baltimore.

Jain, D., Collins-Thompson, D. and Lee, H. (1991). A collapsing test for sceening surfactant-producing microorganisms.Journal of Microbiology Methods; 13(4): 271-279.

Jaysree, R.C., Basu, S., Singh, P.P., Ghosal, T., Patra, A.P., Keerthi, Y. and Rajendran, N. (2011). Isolation of biosurfactant producing bacteria from environmental samples. *Pharmacologlyonline* 3:1427-1433.

Makkar, R.S. and Cameotra, S.S. (2002). An update on the use of unconventional substrates for biosurfactant production and their new applications. Applied Microbiol. Biotechnol., 58:428-434.

ISSN 2055-0219(Print), ISSN 2055-0227(online)

Maier, R.M. and G. Soberon-Chavez, G. (2000). *Pseudomonas aeruginosa* rhamnolipids: Biosynthesis and potential applications. *Applied Microbiol. Biotechnol.*, 54: 625-633.

Mata-Sandoval, J.C., Karns, J. and Torrents, A. (1999). High-performance liquid chromatography method for the characterization of rhamnolipid mixtures produced by *pseudomonas aeruginosa* UG2 on corn oil. *J. Chromatogr. A.*, 864: 211-220.

Morikawa M, Hirata Y, Imanaka T. (2000). A study on the structure-function relationship of lipopeptide biosurfactants. *Biochim. Biophys. Acta (BBA) -Molecular and Cell Biology of Lipids*; 1488 (3):211-218.

Nitschke M and Pastore G.M (2004). Biosurfactant production by *Bacillus subtilis* using cassava-processing effluent. *Appl Biochem. Biotechnol.*, 112: 163-172.

Tabatabaee. A., Assadi. M.M., Noohi. A.A., Sajadian, V.A., 2005. Isolation of Biosurfactant Producing Bacteria from Oil Reservoirs. *Iranian Journal Environment Health Science Engginering*. 2: 6-12.

Vaux, D. and Cottingham, M. (2001). Methods and apparatus for measuring surface configuration.Patent number WO 2007/039729 A1.

Volchenko, N.N., Karasev, S.G. Nimchenko, D.V. and Karaseva, E.V. (2007). Cell hydrophobicity as a criterion of selection of bacterial producers of biosurfactants. Microbiology, 76: 112-114.

Woo, S.H. and Park, J.M. (2004). Biodegradation of aromatic compound from soil by drum bioreactor system, *J Microbiol Biotechnol*, 14: 435-441.

Yin, H., Qjang, J., Jia, Y., Ye, J., Peng, H., Qin, H., Zhang, N. & He, B. 2009. Characteristics of biosurfactant produced by *Pseudomonas aeruginosa* S6 isolated from oil-containing wastewater. *Process Biochemistry* 44: 302-308.

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