International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
DOI: 10.22192/ijarbs Coden: IJARQG (USA) Volume 8, Issue 6 -2021

Research Article

DOI: http://dx.doi.org/10.22192/ijarbs.2021.08.06.008

Isolation of Biosurfactant Producing Bacteria from Crude Oil Polluted Soil

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Abstract

Biosurfactants are amphiphilic compounds produced extracellularly by microorganisms on cell surfaces, or excreted extracellularly. They contain hydrophile and hydrophobic moieties that reduce surface and interfacial tension between molecules at the surface and interface respectively. The present study was focused on isolation of biosurfactant producing bacteria from crude oil polluted soil in a site over time and assessing the ability of these isolates by various standard method. These are oil spreading method, emulsification capacity and hemolytic activity methods, were used to screen the capability of isolates for producing biosurfactant. Studies were also carried out using hydrocarbon/crude oil as source of carbon. The identified bacteria such like *Bacillus subtilis*, *Bacilluscereus Pseudomonas* sp and *Staphylococcus aureus* isolated from the soil were found to possess biosurfactant producing capabilities. The biosurfactants produced by *Bacillus subtilis* had an emulsification capacity (E 24) of 2.4% *Bacillus cereus* 2.5%, *Pseudomonas*sp 2.0% and *Staphylococcus aureus*1.7% respectively whereas the Oil spreading and hemolytic activity tested positive to all the biosurfactants. Results of the present study suggested that all the biosurfactant isolates have potential for application in oil degradation studies.

Keywords: Biosurfactants, oil spread, emulsification capacity, hemolytic activity

Introduction

Surfactants are chemical compounds which lower the surface tension of a liquid, the interfacial tension between two liquids, or that between a liquid and a solid. Surfactants are produced by yeasts, bacteria and filamentous fungi and thus are called biosurfactants. Biosurfactants lower surface and interfacial tensions and also bind tightly to surfaces. Microorganisms producing biosurfactants help to amplify the bioavailability of hydrocarbons by enhancing the contact between pollutants and the microorganisms and thus accelerates bioremediation of hydrocarbon contaminated sites (Desai and Banat, 1997; Jaysre *et al*., 2011).

Microorganisms produce surfactants to facilitate emulsification of petroleum hydrocarbons (Banat *et al*., 2000).

Biosurfactants increase bioavailability of contaminants hence speeding up uptake and biodegradation process (Deziel*et al*., 1996). *Pseudomonas aeruginosa* are best known for their ability to produce glycolipid type biosurfactants (Sharma *et al*., 2015) which have been applied in bioremediation of oil sludge contaminated soils (Cameotra and Singh. 2008).

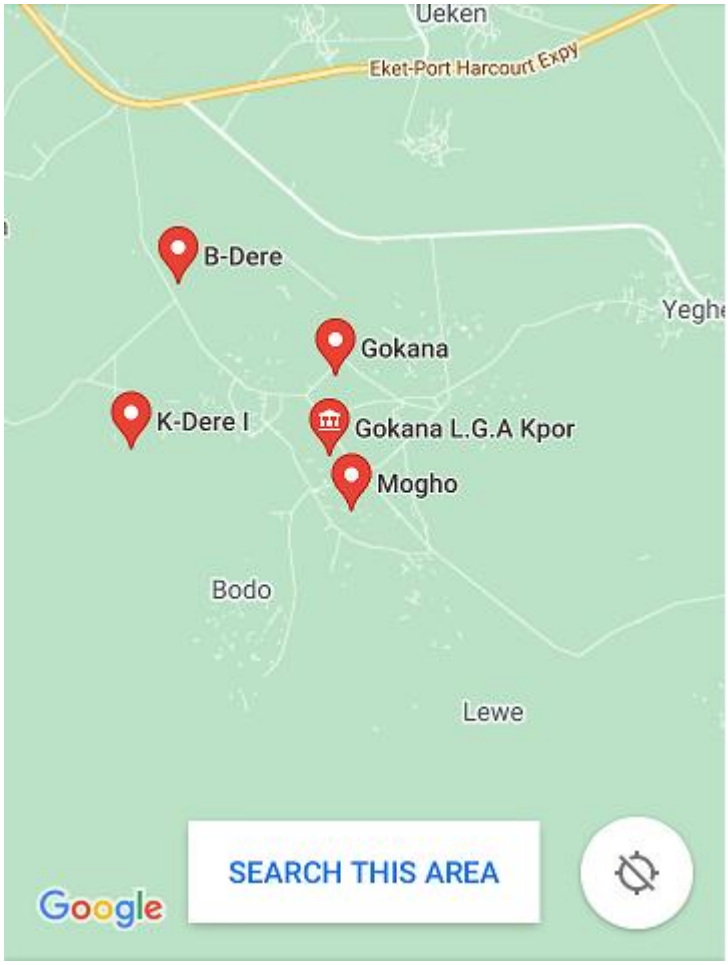
Biodegradation of hydrocarbons in soil can also be efficiently enhanced by addition or *in situ* production of biosurfactants. It was generally observed that the degradation and adaptation time for microbes was shortened. Studies with chemical surfactants showed that the degradation of phenanthrene by an unidentified isolate could be increased by a nonionic surfactant based on ethylene (Boopathy *et al*., 1999).

This study reports on potentials of biosurfactant producing bacteria isolated from two environmental samples.

Materials and Methods

Description of sampling Location and collection

Petroleum contaminated soils were collected from Bodo city in Gokana Local Government Area of Rivers State, Nigeria. Topsoil was collected from the depth of 0 -10 cm with a soil augur collected into a sterile 250 ml Erlenmeyer flasks and the mouth covered with sterile cotton wool. The sample was transported to the laboratory in an ice bucket for isolation ofbiosurfactant producing bacteria.



GPS map showing location of Bodo Community in Gokana Local Government Area of Rivers State.

GPS map showing location of Elebele community in Ogbia Local Government Area of Bayelsa State.

Enrichment cultural isolation of biosurfactants

One gram (I g) of the contaminated soil sample was inoculated into the mineral salt medium (Bushnell Haas medium). This mineral salt medium lacks a carbon source and is chiefly made up of KH_2PO_4 (g/l), K_2HPO_4 (0.5g/l), $MgSO_4.7H_2O$ (0.2 g/l), $FeCL_3$ (0.05 g/l), $(NH_4)_2SO_4$ (1.0g/l), Agar (20g/l) and $CaCl_2$ (0.02 g/l) (Bushnell and Haas, 1941).

Inocula preparation

One gram of the Soil samples were serially diluted and 0.1ml was spread plated on 7.5g Bushnell Haas agar medium prepared and was incubated under aerobic conditions at 37^{o}C for 24– 96 hours. Crude oil was used as hydrocarbon source in each of the plate. The medium was enriched with hydrocarbon source using vapor phase method and was incubated (Ekpenyong *et al*., 2007).

Screening for Biosurfactant producing Bacteria

The following methods were used for the screening of biosurfactant.

Haemolytic activity (Vanessa, 2013)

Soil microorganisms were screened on blood agar containing 5% (v/v) sheep blood. Incubated was maintained at 37^{o}C for 48 hrs. Haemolytic activity was detected by the presence of a clear zone around bacterial colonies.

Emulsification capacity (Vanessa, 2013)

Two millilitres (2 ml) hydrocarbon and 1 ml cell free extract was obtained after the centrifugation of the sample culture,2 ml hydrocarbon and 1ml cell free extract were homogenized by vortexing for 2 minutes. The emulsion activity was investigated after 24 hours. The emulsification index (B24) was calculated by the total height of the emulsion divided by the total height of the aqueous layer and multiplying by 100.

$$EC = \frac{\text{height of the emulsion} \times 100}{\text{Height of the aqueous layer}}$$

Oil spreading method

Fifty millilitres of distilled water was added to the petri dish and100ml of crude oil was added to the surface water, Then 10ml of cell free culture was dropped into the crude oil surface. The diameter of clear zone on the surface was measured and was compared to 10ml distilled water as negative control.

Tables 1 and 2 shows the reaction of the biosurfactant producing organisms to biochemical tests and Emulsification capacity, oil spreading and hemolytic activity test from isolated biosurfactants respectively.

Results

Table 1: Biochemical characteristics of hydrocarbon degrading bacteria from soil

Cell morphology	Cell shape	Rods	Rods	Cylindrical rods	Coccus
Microorganism		*Bacillus cereus*	*Bacillus subtilis*	*Pseudomonas* sp	*Staphylococcus aureus*
Colony	Shape	Irregular	Circular flat	Irregular large	circular
Gram reaction	gram reaction	+	+	-	+
	nitrate reduction	+	+	+	+
	oxidase test	-	-	+	-
	Catalase	+	+	+	+
Biochemical Test	M.R	-	-	+	+
	V.P	+	+	-	-
	Indole	-	-	-	-
	Citrate utilization	+	+	+	+
	H_2S reduction	-	-	-	-
	Urease activity	-	-	-	+

Table 2: Emulsification capacity, oil spreading and hemolytic activity test from isolated biosurfactants

Biosurfactants isolate	Emulsification capacity E24%	Oil Spreading	Hemolytic activity
Bacillus cereus	2.5	+	+
Bacillus subtilis	2.4	+	+
Pseudomonas sp	2.0	+	+
Staphylococcus aureus	1.7	+	+

Discussion

Biosurfactant producing bacteria isolated from crude oil polluted soil are *Bacillus subtilis, Bacillus cereus, Pseudomonas* sp and *Staphylococcus aureus* (Jaysre *et al.*, 2011). The isolates were characterized and identified with reference to standard methods and manuals. *Bacillus cereus* showed higher emulsifying ability compared to other organisms as shown on Table 2. All the isolates showed good emulsification ability except *Staphylococcus aureus* which was the least (Vanessa, 2013). Also, all isolates are positive for bothoil spreading and haemolytic activity (Desai, and Banat, 1997).The result shows the highest Emulsification capacity of 2.5 and lowest 1.7. Therefore, under favourable conditions; *Bacillus* spends to degrade hydrocarbons in relatively high rate as long as the concentration is not toxic to the organisms (Al-Awahdi *et al.*, 1994; Boopathy and Maning, 1999; Cameotra and Singh, 2008).

Conclusion

The biosurfactant producing bacteria such as *Bacillus cereus* and *Bacillus subtilis* are found to produce more biosurfactants. Both isolates were obtained from crude oil contaminated soil and hence was expected to be a biosurfactant producer. All of the isolates showed biosurfactants activity due to inducement of the pollutant.

Isolation of biomarker gene and subsequent PCR amplification may prove useful to artificially produce environmentally friendly surfactants. For future studies involving biosurfactants and bioremediation, the two isolates *Bacillus cereus* and *Bacillus subtilis* are desirable biosurfactants capable of degrading hydrocarbon pollutants in the environment.

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Quick Response Code	Website: www.ijarbs.com
	Subject: Microbiology

DOI:10.22192/ijarbs.2021.08.06.008

How to cite this article:
Ayebabogha, M. N., Akujobi, C.O and Braide, W. (2021). Isolation of Biosurfactant Producing Bacteria from Crude Oil Polluted Soil. Int. J. Adv. Res. Biol. Sci. 8(6): 58-62.
DOI: http://dx.doi.org/10.22192/ijarbs.2021.08.06.008