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ISOLATION AND PRELIMINARY SCREENING OF BIOSURFACTANT PRODUCING BACTERIA FROM OIL CONTAMINATED SOIL

Research article

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ABSTRACT

Introduction: Biosurfactants have turned into a potential contrasting option to synthetic surfactants inferable from their low toxicity, safety, biodegradability, specificity and so on. Biosurfactants are known to lower surface tension of both aqueous and hydrocarbon solutions. ***Objectives:*** The goal of embraced study was to isolate and screen the biosurfactant producing microscopic organisms from oil defiled soil. ***Materials and Methods:*** For this reason, soil samples were gathered from oil contaminated site. The isolated microorganisms from the soil samples were characterized on the premise of conventional microbiological methods. These isolates were further screened for biosurfactant production by hemolytic test, emulsification test (E24), oil spread technique (OSM), and modified drop collapse (MDC) method. ***Results:*** The isolates distinguished were *Salmonella species* (12.5%), *Enterobacter species* (12.5%), *Streptococci species* (12.5%), *Pseudomonas species* (25%) and *Klebsiella species* (37.5%). The screening results showed that 25% of the isolates displayed hemolytic action, 87.5% isolates indicated emulsification and oil spread movement while 100% of the isolates showed modified drop collapse activity. ***Conclusion:*** The best isolate identified for biosurfactant production was *Pseudomonas species*, as it was seen to be certain for all the screening tests. The outcomes recommended that these segregates have potential for hydrocarbon degradation and they can further be utilized as a part of future for biosurfactant extraction, in bioremediation and different applications.

Keywords: Biosurfactant; Drop collapse; Emulsification; Hydrocarbon degradation; Oil spread method.

INTRODUCTION

Anthropogenic activities are the primary wellspring of ecological contamination generously soil **(Baker, 1976; Tijani *et al.*, 2005).** Keeping in mind the end goal to diminish the contamination, surfactants are utilized which are chemicals that are obtained from crude materials. They have applications in petroleum and sustenance industry and in addition in environmental engineering. Practically the greater parts of the surfactants get from petroleum artificially. They ascribes to high harmfulness and non-degradability, subsequently contributing as a source towards the natural pollution **(Franzetti *et al.*, 2006).** Because of the natural concern and progression in biotechnology, biosurfactants have turned into a potential option for bioremediation to monetarily accessible synthetic surfactants **(Banat *et al.*, 2000; Hamme *et al.*, 2006; Henkel *et al.*, 2012).** Thus, keeping in mind the end goal to bioremediate the soil and to take back to its unique shape, microorganisms equipped for production of surface dynamic agents are utilized, which are predominantly **(Kosaric, 2001).**

Biosurfactants are the surface-active agents that are amphipathic or ampiphilic in nature. They have different properties, for example, frothing, wetting, infiltration, hyrdrophobicity, hydrophilicity, biodegradability, bioavailability, role in quorum detecting, biofilm development, improvement of microbial growth, heavy metal binding, antimicrobial activity and diminishment of surface and interfacial tension, which makes them a potential competitor of bioremediation, hydrocarbon degradation, MEOR (microbial enhanced oil recovery) and different other applications **(Maneerat, 2005; Thavasi *et al.*, 2011).**

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Conversion of hydrocarbon by indeginous flora to non-harmful structures through a procedure of biodegradation serves as one of the essential systems by which petroleum items can be expelled from the soil cheaply. Emulsification is one of the vital attributes of biosurfactant producing microbes in which hydrocarbons are dispersed in micro-droplets, micelles or water emulsions which are then transported into the cell **(Gautam and Tiagi, 2006).**

Biosurfactants has a tendency to connect with the boundary between two phases in a blended or heterogeneous framework that is characterized as the interface where they shape a conditioning film that will change the properties of the original surface **(Makkar and Cameotra, 2008).** This conditioning brings down the surface tension and in addition interfacial pressure between various fluid stages on the interfacial limit existing between immiscible stages and hence can affect the interfacial rheological conduct and mass exchange **(Gautam and Tiagi, 2006; Franzetti *et al.,* 2010; Perfumo *et al.,* 2010; Satpute *et al.,* 2010).** Understanding the capacity of microorganisms to deliver biosurfactants, different screening techniques have been created to be specific; hemolytic activity, oil spread method, modified drop collapse technique, tilted glass slide, emulsification index, blue agar plate, emulsification measure, hydrocarbon degradation on agar plates and so on.

The production of biosurfactant has turned out to be relatively exigent for industry because of their low yield and high generation cost **(Thampayak *et al.,* 2008).** This offered ascend to more examinations for the potential microorganisms to produce biosurfactants **(Ibrahim *et al.,* 2013).** Hence, the undertaken study meant to segregate and assess different screening techniques to recognize biosurfactant producing microscopic organisms from oil contaminated soil sample.

MATERIALS AND METHODS

Soil sample collection and enrichment of microorganisms: Oil contaminated soil sample was collected from a car workshop. Soil sample was enriched by inoculating in the MSM (mineral salt medium). 1 g of soil sample was added in 50 mL MSM broth containing (g/L); 15 g $NaNO_3$, 1.1 g KCl, 1.1 g NaCl, 0.00028 g $FeSO_4.7H_2O$, 3.4 g KH_2PO_4, 4.4 g K_2HPO_4, 0.5 g $MgSO_4.7H_2O$, 0.5 g yeast extract at 37 °C and incubated in shaking water bath at minimum speed. After 48 h the broth was serially diluted using saline and plated on nutrient agar. After 24 h of incubation the isolates obtained were identified and characterized based on their morphological, biochemical and cultural characteristics. The isolates were then processed using different screening test for the detection of biosurfactant production.

SCREENING METHODS FOR BIOSURFACTANTS

Hemolytic assay: The pure culture isolate were streaked on blood agar. Incubated for 24 h at 37 °C. The results were recorded based on the type of clear zone observed, i.e. α-hemolysis, β and γ-hemolysis **(Bicca *et al.,* 1999).**

Emulsification assay E24: Each isolate of pure culture was inoculated in their respectively labeled tube containing 2 mL of MSM. Incubated for 48 h at 37 °C in shaking water bath. After 48 hours equal amount of oil was added in tube as that of culture broth. The tubes were then vortexes at high speed for 1 min and were left for 24 hours at room temperature. The emulsion index (E24) is calculated by the height of the emulsion layer (mm) divided by total height (mm), multiplied by (100) **(Cooper and Goldenberg, 1987).**

Modified drop collapse (MDC) method: 96 well plates was coated with 2 µL of diesel oil and left for 15 min to equilibrate. Then 48 h of pure culture before and after centrifuge was inoculated in the wells and observed after 1 min for the appearance of flat drop. Flat drop is indicative of positive result and beaded drop is indicative of negative in comparison to control **(Cooper and Goldenberg, 1987).**

Oil spread method (OSM): 20 mL of distill water is taken in Petri dish for each pure culture isolateover which 10 µL of oil was spread uniformly. Then 10 µL of culture of each isolate is inoculated in their on oil film whereas 10 µL of water is used as a control. The plates were observed for zone of clearance that is indicative of positive results **(Cooper and Goldenberg, 1987).**

RESULTS

Isolation and identification: 8 bacterial isolates were obtained from oil contaminated soil sample. Soil was collected from car workshop. The isolates identified were *Klebsiella pneumoniae*, *Salmonella paratyphi* B, *Pseudomonas aeroginosa*, *Streptococci*sp and *Enterobacter* sp (Table 1 and 2).

Biosurfactant activity: The study revealed among all the isolates tested for hemolytic activity, 2 isolates were positive for hemolysis (Figure 1).

The isolates were further screened for biosurfactant production in order to examine their

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ability. Among 8 isolates, 7 were positive for emulsification (Figure 2), and 5 isolates were positive for oil spread activity forming clear and stable zone where as all the isolates were positive for modified drop collapse test (Figure 3).

The results of the screening tests for the biosurfactant production of isolated bacteria are summarized in Table 3.

The prevalence rate of the isolates exhibiting biosurfactant production is illustrated in Figure 4. All of the isolates were found positive for modified drop collapse activity, majority of them showed positive results for emulsification assay as well as oil spread method while few of the isolates displayed hemolytic activity.

DISCUSSION

The isolates reported in the study were observed to be sure for biosurfactant production and can be of extraordinary potential for bioremediation of diesel oil sullied soil. It has been recommended that the sole screening technique is not appropriate for recognizing a wide range of biosurfactants, along these lines, it is proposed that more than one screening strategy ought to be considered amid essential screening to distinguish potential biosurfactant producers **(Kiran *et al.*, 2010).**

Pseudomonas spp was observed to be sure in each of the 4 screening techniques utilized. This makes the *Pseudomonas spp* an appealing and potential possibility for biosurfactant production. Hemolytic test is broadly utilized as prelimeinary screening technique for biosurfactant generation and for the isolation of lipopeptides **(Mulligan *et al.*, 1984; Iqbal *et al.*, 1995)** and rhamnolipids biosurfactants **(Maneerat and Phetrong, 2007).** Since other microbial products or virulence factors lyses blood agar biosurfactants that are ineffectively diffusible may not lyse blood cells **(Lin *et al.*, 1989; Maneerat and Phetrong, 2007).** In this manner, it is not clear whether blood agar lysis ought to be utilized to screen for biosurfactant production **(Maneerat and Phetrong, 2007).** Corroborative detection of biosurfactants by the bacterial isolates was likewise made utilizing oil spreading strategy, emulsification test and drop collapse test. For Emulsification activity, it was expected that if biosurfactant is available in the culture broth utilized as a part of this measure, then it will emulsify the hydrocarbons exhibit in the test solution. 2 strains of *Klebsiella sp* and 1 strain of *Pseudomonas aeroginosa* were certain for high emulsification movement and the rest indicated low emulsifiaction action. Emulsification action is one of the criteria among others to bolster the choice of potential biosurfactant producers. Emulsifying activities (E24) demonstrates profitability of bioemulsifier **(Bonilla *et al.*, 2005).** Estimation of emulsification units assist in choosing the carbon and energy source for biosurfactant/bioemulsifier production. Zone of clearance on oil film was demonstrative of positive results in Oil spread method (OSM); among 8 isolates, 5 isolates were seen to be certain for the formation of clear and stable zone with a diameter of (15 mm, 11 mm, 11 mm, 13 mm, 22 mm). **Morikawa *et al.*, (2000)** reported that the range of oil uprooting in oil spreading test is specifically identified with the concentration of the biosurfactant that is available in the solution. Consequently these outcomes are pinpointing the production of biosurfactants. In modified drop collapse assay flat drop on micro titter well plate or drop with an expanded diameter in contrast with control was indicative of positive result, which was displayed by all isolates. In the accompanying test both cell free culture broth and cell culture broth were used. Isolates that were positive for cell free culture broth demonstrated that biosurfactant was released extracellularly in the broth, and the isolates that were positive for cell culture broth indicated that surface activity was associated their cell surface **(Thavasi *et al.*, 2011).**

Microorganisms deliver biosurfactants when hydrophobic substrates are accessible in their characteristic natural habitats. Their low critical micelle concentration (CMC), high biodegradibility, low toxicity, specificity makes them a potential candidateto supplant any artificially blended surfactants which expands the ecological concerns. Yet high crude material and manufacturing cost concerns their substantial scale production. Their physio-chemical properties makes them essential contender for different applications such as microbial enhanced oil recovery, bioremediation, pharmaceutical, beauty care products, biomedical and therapeutic application and so on.

CONCLUSION

In conclusion, the present study in addition past research studies has demonstrated that genus Pseudomonas is a potential biosurfactant producer and valuable tool for various environmental and industrial processes. Commercially, biosurfactants are used in various industries such as pharmaceutical, oil, food and cosmetics industry in light of their capacity to settle emulsions. Further investigation concerning the composition of the biosurfactants and phylogenetic determination of the biosurfactant producing bacteria is recommended.

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Table 1. Biochemical identification of gram negative organisms.

Isolate	TSI				Citrate	Urease	Oxidase	Motility	Isolated organism
	Butt	Slant	Gas	H_2S					
1	Acidic	Acidic	+ve	-ve	+ve	+ve	-ve	-ve	*Klebsiella pneumoniae*
2	Acidic	Alkaline	+ve	+ve	+ve	-ve	-ve	-ve	*Salmonella paratyphi* B
3	Alkaline	Alkaline	-ve	-ve	+ve	-ve	+ve	+ve	*Pseudomonas aeruginosa*
4	Acidic	Acidic	+ve	-ve	+ve	+ve	-ve	-ve	*Klebsiella pneumonia*
5	Acidic	Acidic	+ve	-ve	+ve	+ve	-ve	-ve	*Klebsiella pneumonia*
6	Acidic	Acidic	+ve	-ve	-ve	+ve	-ve	+ve	*Enterobacter* sp
7	Alkaline	Alkaline	-ve	-ve	+ve	-ve	+ve	+ve	*Pseudomonas aerugenosa*

Table 2. Biochemical identification of gram positive organism.

Isolate	Sugar test				Catalase Test	Coagulase Test	Isolated organism
	Glucose	Sucrose	Manose	Lactose			
8	++	+	+	++	**-ve**	**+ve**	*Streptococci* sp

Table 3. Screening results of isolates for biosurfactant production.

Isolates	Hemolytic test	Emulsification assay E24 (%)	Oil spread method (mm)	Modified drop collapse method (mm)
Klebsiella sp	-ve	45.23	15	+ve
Salmonella sp	-ve	12.50	11	+ve
Pseudomonas aeruginosa	+ve	23.25	7	+ve
Klebsiella sp	-ve	46.51	9	+ve
Klebsiella sp	-ve	14.50	11	+ve
Sterptococci sp	+ve	12.70	13	+ve
Enterobacter sp	-ve	0.00	0	+ve
Pseudomonas aeruginosa	-ve	16.66	22	+ve

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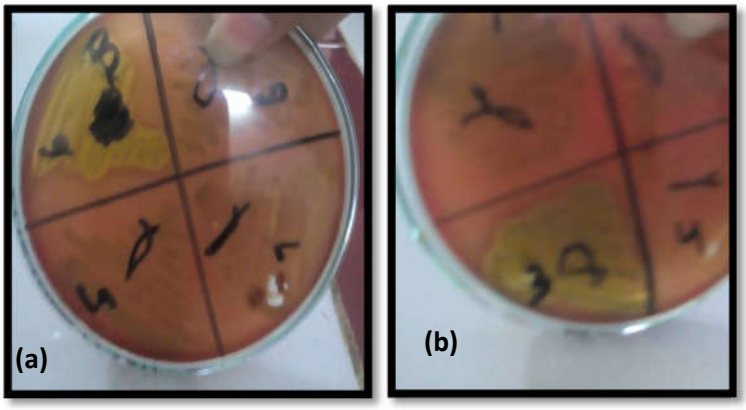

Figure 1. (a) Beta and (b) alpha hemolysis produced by *Streptococci* spp and *Pseudomonas aeroginosa*.

Figure 2. Emulsion layer formation by bacterial isolates.

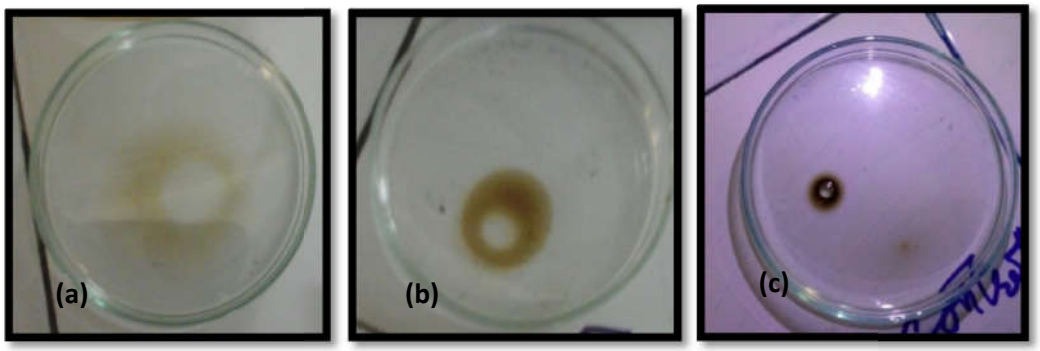

Figure 3 (a) and (b). Zone formation showing oil displacement activity by the isolates, whereas **(c)** is a negative control.

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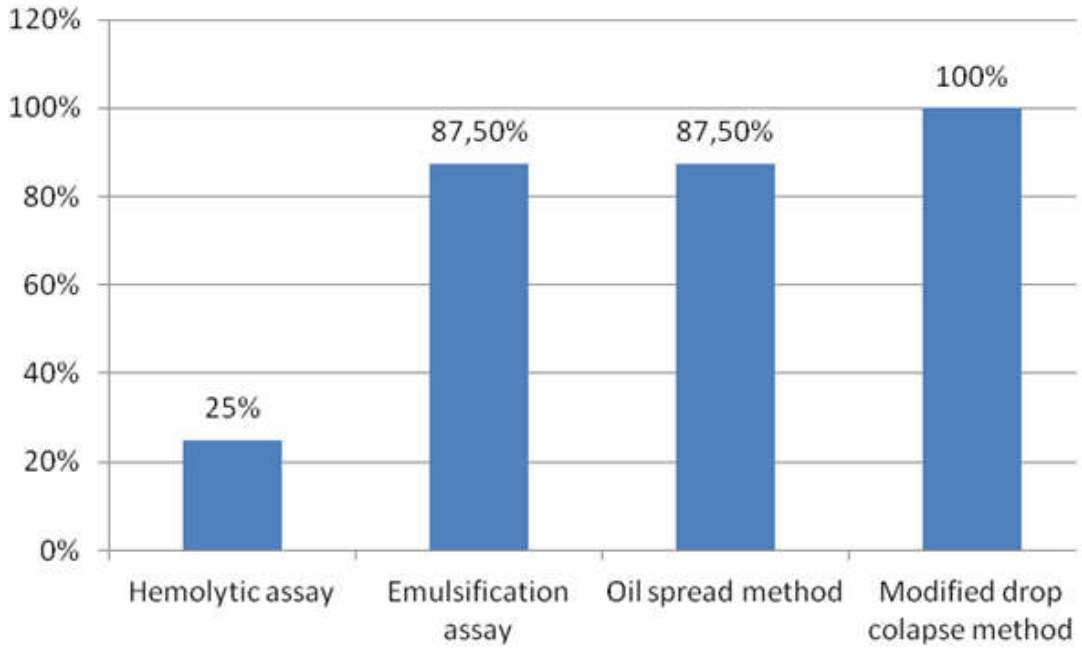
Figure 4. Percentage of isolates demonstrating positive screening test for biosurfactant production.



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