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SCREENING OF BIOSURFACTANT PRODUCING MICROORGANISMS FROM THE SOIL

Anushree Suresh, Disha Nagda, Jayanthi Abraham*

School of Biosciences and Technology, VIT University, Vellore, Tamil Nadu, India - 632 014

*Corresponding author: jayanthi.abraham@gmail.com

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Abstract: The primary motive of this research was to check for the presence of microbial species which has the ability to produce biosurfactant. The soil samples were collected from petrol bunk which was contaminated with petroleum oil and enrichment technique was carried out with minimal salt medium (MSM) for isolation of microbial strain. Serial dilutions of the enriched culture were performed. Two bacterial isolates and one fungal isolate showed activity for biosurfactant production. The methods employed for screening were oil displacement, oil drop collapse, blood hemolysis, blue agar plate method and confirmatory tests like phenol-sulphuric acid method. Both, bacterial isolates (MB1 and MB2) and fungal isolate (JAD1) showed emulsification activity of 41.66 %, 64.70 % and 90.90 % respectively. Biosurfactant was extracted and screened for antimicrobial activity against Gram positive and Gram-negative clinical pathogens. These biosurfactant producing isolates can be used to enhance in situ bioremediation of soils contaminated with polycyclic aromatic hydrocarbons (PAHs).

Keywords: *antimicrobial activity, BAP test, GC-MS, hemolytic activity, oil-collapse, oil-emulsification assay*

INTRODUCTION

Biosurfactants are amphiphilic compounds, produced extracellularly or as part of the cell membrane by a variety of organisms like yeast, bacteria and filamentous fungi [1]. Biosurfactants which are basically the derivative forms of living organisms, with beneficial and various characteristics like less toxic, a high biodegradation activity, good compatibility with the environmental conditions, a highly selective nature for substrates, and lower critical micelle concentration (CMC) [2]. Biosurfactant from microbes may be of the following types: mycolic acid, glycolipids, polysaccharide–lipid complex, lipoprotein or lipopeptide, phospholipid, etc. These have their association mainly with the lipoidal compounds whose structural composition is highly concentrated at the two ends of the entire molecule. One end being the sole carbon-hydrogen part, is insoluble in water [3]. This is the portion of the molecule that is composed of fatty acid long chains, hydroxyl fatty acid or α-acyl hydroxyl-fatty acids [4]. The other end which is hydrophilic, is with high solubility in water and mainly comprises of carbohydrate, amino acid, cyclic peptide, phosphate and carboxylic acid or alcohol [5]. Some of the examples of microorganisms reported for the production of different types of biosurfactant have been listed below - glycolipids (*Pseudomonas aeruginosa*), rhamnolipids (*Rhodococcus erithropolis*), trehalose lipids (*Arthobacter sp*.), sophorolipids (*Candida bombicola, C. apicola*), mannosylerythritol lipids (C. *antartica*) and liposan (*C. lipolytica*) [6].

Biosurfactants produced from the microorganisms have been used for bioremediation. Degradation of petroleum products pollution in the environment can be achieved successfully with the help of biosurfactant producing microorganisms [7].

Major applications of biosurfactant are in the food, cosmetic and pharmaceutical industries. One of the challenges faced is that water repelling pollutants such as petroleum hydrocarbons could not be degraded by the microorganisms without proper chemical solubilization of the pollutant [8]. Reversing the absorption of hydrocarbons from soil totally govern the process of mineralization. Surfactants possess the activity of increasing the total occupancy area of hydrophobic materials, such as pesticides in soil and water environment [9]. This is involved in further increasing their water-soluble property. Due to this, it can be said that if there are any surfactants present, then the extent of degradation of pollutants by these microbial species is high [10]. However, it has to be also noted that this area of application of biosurfactants for the degradation activity of pesticides present in the soil and water environment has been highlighting importance since recent years only [11].

The present study focuses on the production of biosurfactant by *Aspergillus niger* JAD1, *Staphylococcus aureus* and *Acinetobacter sp*. isolated from soil samples contaminated with oil near petroleum bunk in Vellore. Sample was screened for biosurfactant producing isolates using oil displacement, oil spreading and emulsification stability test. Isolates were identified by morphological and molecular characterization techniques.

MATERIALS AND METHODS

Chemicals

The chemicals used in this study were purchased from HiMedia (Mumbai, India). All the other reagents used for analytical techniques were purchased from National scientific products (Pondicherry, India) with 99.9 % purity (analytical grade).

Collection of samples

Soil sample (approximately of 10 g weight) contaminated with oil from petroleum bunk (top layer), in Vellore, were collected in a polythene bag, for further biosurfactant analysis.

Isolation and enrichment of biosurfactant-producing microbial species

10 g of the soil sample were inoculated in a conical flask containing 100 mL of MSM broth. The composition of MSM ($g \cdot L^{-1}$) was (KH_2PO_4 - 0.7 g, Na_2HPO_4 - 2.0 g, $MgSO_4 \cdot 7H_2O$ - 0.4 g, $CaCl_2 \cdot H_2O$ - 0.01 g, $FeSO_4 \cdot 7H_2O$ - 0.001 g). Glucose 20.00 $g \cdot L^{-1}$ was added as the carbon source. A control for the same was also maintained. The flasks were incubated on orbital shaker conditions (130 rpm) for 72 hours at 37 °C. After incubation, serial dilutions were made for the medium from 10^{-1} to 10^{-9} in sterile saline. From the dilutions 10^{-3} to 10^{-5}, 0.1 mL was spread on the nutrient agar plates and from dilutions 10^{-6} to 10^{-8}, 0.1 mL was spread on the Sabouraud agar plates. The plates were kept for incubation at 37 °C for 48 hrs (bacterial) and at 25 °C for two weeks (fungal). Post incubation time, distinct colonies which were different morphologically, were selected for all the further protocol [12].

Maintenance of isolates

The selected bacterial isolates were streaked on nutrient agar slants and fungal isolates in Sabouraud's dextrose agar slants for obtaining pure culture isolates. Post incubation, these slants were then refrigerated for further use [12].

Identification of biosurfactant producing organism

The morphological identification of bacterial isolates was done by performing Gram staining and fungal isolates were stained using Lacto phenol cotton blue stain and observed under light microscope (Olympus, Germany).

Biochemical tests

For the identification process of bacterial isolates, different biochemical tests such as Indole Test, Methyl Red Test, Voges-Proskauer Test, Citrate Test, Oxidase Test, Catalase Test, Urease test, Triple sugar iron test, carbohydrate fermentation tests with glucose, sucrose and lactose, nitrate reduction and motility test were performed.

Indole test

This test is useful in determining the bacteria's ability to split tryptophan to indole. Broth containing tryptophan peptone was prepared, sterilized and inoculated with the isolates (MB1 and MB2), and incubated at 37 °C for 24 hrs. Also, a sterile broth medium was maintained as control. Post incubation time of 24 hrs, Kovac's reagent was added to both test and control tubes. Red coloration confirms the indole test [13].

Methyl red- Voges Proskauer (MR-VP)

The isolates MB1 and MB2 were inoculated in MR-VP broth and incubated for 48 hrs at 37 °C. Post the incubation time, methyl red reagent (methyl red as indicator) was added drop-wise to the tube, mixed well and observed for the color change of the broth from yellow to red, red indicating a positive test. Similarly, for Voges Proskauer test, 15 drops of Baritt's reagent A and B each were added, mixed well and observed for color change from yellow to red. Red coloration confirms VP positive [14].

Citrate test

In this test, bacteria are determined for its ability to utilize citrate as carbon and energy source, wherein ultimately alkaline carbonates and bicarbonates are the end products. Isolates MB1 and MB2 were streaked onto Simon citrate agar slants containing Bromophenol blue as the indicator. Post inoculation, slants were observed for color change from green to blue. A color change to blue demonstrates the isolate to be citrate positive [15].

Catalase test

This test demonstrates the presence of catalase enzyme which functions to carry out the breakdown of hydrogen peroxide molecule into oxygen and water moieties. Hence, catalase producers can be differentiated from catalase non-producers. To perform this test, a drop of culture broth was placed onto grease free slide containing a drop of hydrogen peroxide (3 %). If effervescence was produced it was considered as a positive test [16].

Oxidase test

This test is performed to identify microbes which produce intracellular oxidase enzyme that functions to carry out the oxidation of Cytochrome C, part of bacterial electron transport chain. Test was performed by picking the bacterial colony through the loop and touching the oxidase disc or by adding a loopful of broth culture to the disc. It was then observed for blue coloration which indicates the organism to be positive [17].

Nitrate reduction test

This test is performed to check the ability of the bacterial isolate to produce nitrate reductase enzyme which basically functions to hydrolyze the nitrates to nitrites. These can be further broken down into various other nitrogenous products. Bacterial isolates were inoculated into nitrate broth and incubated for 24 hrs at 37 °C. Post incubation, one drop each of sulphanilic acid and α-naphthylamine were added to observe the color change from yellow to red. Red coloration indicates positive result [18].

Carbohydrate fermentation test

This test was performed to check utilization ability of the isolates for specific carbohydrates like glucose, sucrose and lactose. Carbohydrate fermentation broth with phenol red as indicator was added along with the inoculation with loopful of bacterial isolate and incubated for 24 hrs at 37 °C. The color change from red to yellow indicates sugar utilization. Inverted Durham's tube was added to each tube to check for the gas formation [19].

Urease test

This test helps determining the organisms' ability to split urea, through the production of urease enzyme. To perform this test, bacterial culture was inoculated into urease broth with 40 % sterile urea solution added to it for 24 hrs at 37 ºC and observed for color change. A color change from light orange to bright pink is considered to be positive test [20].

Taxonomic identification of efficient fungal strain producing biosurfactant

Fungal isolate which exhibited good biosurfactant production was identified by 28S rRNA sequence analysis [6]. The fungal isolate was isolated using ABI PRISM® BigDyeTM Terminator Cycle Sequencing Kits with AmpliTaq® DNA polymerase (FS enzyme) (Applied Biosystems). The kit consists of cell wall lysing chemicals which break open the cellulosic cell wall and plasma membrane thereby extracting DNA from fungal cells. Polymerase chain reaction was used to amplify specific genomic DNA sequence by adding 25 µL of PCR reaction solutions (1.5 µL of forward primer 5' TAC TAC CAC CAA GAT CT3' and reverse primer 5'ACC CGCTGA ACT TAA GC 3', 5 µL of deionized water and 12 µL of Taq Master Mix). The sequenced result was submitted to the GenBank National Center for Biotechnology information (NCBI) database. The phylogenetic tree analysis was also performed by neighbor joining method using MEGA software (Pennsylvania State University, United States).

Screening of biosurfactant producing bacteria

Obtaining the cell free extract

Before starting the screening process for biosurfactants, 10 mL of MSM broth medium was prepared and the isolates were inoculated. The flasks were incubated at 37 °C for 72 hrs. Post incubation, the broth was centrifuged at 2500 - 3000 rpm for 30 min. Discarding the pellet with the cells, only the supernatant was collected. This supernatant was used further for various kinds of screening methods [20].

Oil displacement test

In this particular test, a Petri dish (diameter 9 cm) was filled up with 50 mL of distilled water. Onto this exact 1 mL of the oil was added and the oil was allowed to spread all over the water surface forming a uniform layer. After attaining the equilibrium, 1 mL of supernatant of culture broth was added to the center. The extent to which the oil film undergoes clearance, forms the basis of this test [21].

Drop Collapse test

The liquid droplet dispersion ability of the biosurfactant was performed by drop collapse test. 2 µL of oil was added on a glass coverslip and was left to settle for about an hour. Followed by this, 5 µl of the supernatant from centrifuged culture broth was added and was allowed to stand for a minute. It was then observed for the dispersion of the supernatant on the oil coating. If the drop collapses from its shape and disperses, indicates biosurfactant activity, hence positive. However, if the drop remains intact without any change in the geometrical shape, it is concluded as negative result. For a proper assurance of the test, it was simultaneously tested using distilled water as a control [22].

Hemolytic activity test

Hemolytic activity test is also one of the important methods which is often performed to test for the presence of biosurfactants. The principle underlying the test is that the biosurfactant acting species have the ability to hemolyze the blood's red blood cells. The isolates were streaked on blood agar plate and were incubated at 37 ºC for about 24 hrs. Post incubation time, plates were checked for the type of zone of hemolysis. Beta - hemolytic zone, indicates the presence of biosurfactants [23].

Emulsification Capacity

The test was conducted to detect the presence of any stable emulsified layer present in the tubes. Equal volume of oil was added to equal volume of the supernatant of broth culture (approx. 2 mL) and vortexed at very high speed for couple of min. The vortexed mixture was then allowed to standby of 24 hrs. The emulsification index was calculated for both bacterial strains.

Foaming activity

The culture isolates were inoculated in 100 mL of MSM broth each and incubated for 96 hrs under shaker conditions. Post incubation, the flasks were shaken vigorously for 2 min and observed for any production of foam [24].

Blue agar plate method (BAP)

The test is used to screen for the biological surfactants that are anionic in nature. Mineral Salt agar medium containing glucose (2 %), cetyltrimethylammonium bromide (CTAB: 0.5 $mg \cdot mL^{-1}$) and methylene blue (MB: 0.2 $mg \cdot mL^{-1}$) was prepared for well diffusion agar plate 100 µl of supernatant from the centrifuged broth culture were inoculated on MSM media plate. The plate was then left for incubation at 37 °C for 48 - 72 hrs. A distinct dark blue colored halo zone around the culture was considered as positive for presence of anionic biosurfactant [25].

Confirmatory test

Phenol: H_2SO_4 method

The supernatant of bacterial isolates (MB1 and MB2) and fungal isolates (MF1 and MF2) were inoculated into 100 mL of MSM broth and incubated for a week's time on orbital shaker conditions. Post incubation, centrifugation of the broth was carried out at 3000 rpm for about 30 min and the pellet were discarded. 1 mL of 5 % phenol was

added to 1 mL of culture supernatant vortexed for 1 min. To this mixture, 5 mL of conc. H_2SO_4 was added drop wise. Color change from yellow to orange confirms the presence of biosurfactant in supernatant [26].

Extracting the biosurfactants

50 mL of MSM broth containing 1 mL of kerosene oil was prepared with 500 µL of culture inoculum was added. The flask was then incubated at 37 °C for 14 days under orbital shaker condition. Post incubation period, the broth was centrifuged at 3000 rpm for about 30 min to completely separate the cells. The fresh supernatant was taken carefully and its *p*H was adjusted exactly to 2, by adding conc. H_2SO_4 drop by drop. Followed by this, chloroform and methanol were added in a ratio of 2 : 1. Their total amount was equal to the volume of the supernatant, approximately 3 mL. The mixture was mixed well to ensure proper mixing and allowed to stand over an entire night for evaporation in the hot air oven (SS - HAO – 005, Krishna Scientific supplier, Chennai, India). It was then detected for the presence of a solid white colored sediment residue which was successfully extracted [15].

Antimicrobial activity

Antimicrobial screening of the biosurfactant producing isolates was carried out using well diffusion method. Isolates were studied against Gram positive clinical pathogens such as *Staphylococcus aureus* and *Enetrococcus* sp*,* Gram negative clinical pathogens such as *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilus, Shigella sp.* Muller Hinton agar plates were prepared swabbed with the clinical pathogen and wells were bored using sterile cork borer. Cell free supernatant of two biosurfactant isolates with varying concentration of 40, 80, 120, 140 and 160 µL were added into the well, and distilled water was used as negative control. The plates were kept for incubation for 24 hrs and checked for the zone of clearance around the well.

Molecular weight analysis using analytical method GC MS

For the preparation of biosurfactant extract for GC-MS analysis, 4 g of extracted white sediment of biosurfactant produced bacterial isolate was mixed in 1 mL chloroform, 0.85 mL methanol and 0.15 mL of sulphuric acid and heated at 100 ºC for 140 min. To the above mixture 1 mL of distilled water was added and vortexed for 1 minute. The mixture was allowed to stand for phase separation to occur [15]. Bottom layer of the mixture that is chloroform layer containing the fatty acid mixture was analyzed by GC-MS (Perkin Elmer, USA) analysis (Elite-5MS capillary GC column, length 30 m, ID 0.25 mm film thickness 250 µm) [27]. Helium was used as the carrier gas at a flow rate of 1 $mL \cdot min^{-1}$. The injector temperature was 280 ºC. The oven temperature was initially at 68 ºC with a hold time of 5 min followed by an increase of 180 ºC at 4 $ºC \cdot min^{-1}$, which lastly increased to 280 ºC for 37 min. The scans were initiated over 50 - 1000 m/z range and positive ion mode was used for the analyses.

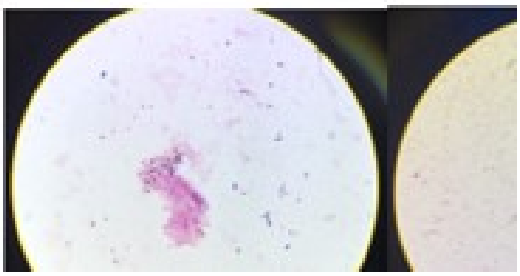
RESULTS AND DISCUSSION

Isolation of microbial species that produce the biosurfactant

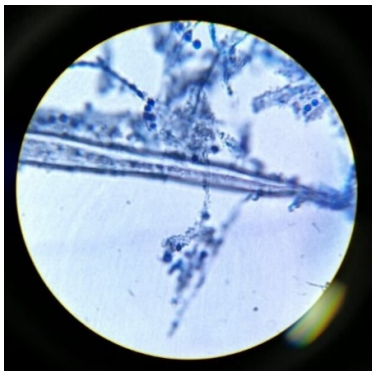
After the enrichment of the soil using the minimal media supplemented with kerosene, five bacterial strains and two fungal strains were isolated on the MSM media plates. Out of which two bacterial (MB1 and MB2) and one fungal strain (JAD1) gave good result for biosurfactant production, therefore used for further screening tests.

Identification of biosurfactant producing organism

Gram staining of the two bacterial strains were carried out and MB1 was observed to be Gram positive cocci in clusters and MB2 as Gram negative coccobacilli in pairs or chains as shown in Figure 1(a). Lactophenol cotton blue staining was carried out for fungal strains (JAD1) and observed hyphae and spores under 100X magnification as shown in Figure 1 (b).



a



b

Figure 1. *Morphological identification of isolates,*
a) Gram staining of MB1 isolate (above) and MB2 isolate (below)
b) Lactophenol blue staining of fungal strain JAD1

Biochemical tests result of both MB1 and MB2 isolates after 48 hrs of incubation have been tabulated in Table 1.
Based on the biochemical tests the bacteria MB1 was *Staphylococcus aureus* and MB2 was *Acinetobacter sp.* It can be confirmed using Bergy's manual.

Table 1. *Biochemical test results of MB1 and MB2*

Tests	MB1	MB2
MR-VP	-	-
Indole	-	-
Citrate	+	+
TSI	-	-
Urease	-	-
Nitrate	-	-
Glucose	-	-
Lactose	-	-
Sucrose	-	-
Catalase	+	+
Oxidase	-	-
Starch	-	-
Motility	-	-

'-' = negative, '+' = positive

Taxonomic identification of biosurfactant producing fungal strain

Aspergillus niger JAD1 was identified based on 28S rRNA gene sequence analysis. The nucleotide obtained were compared with the reference sequences by using BLAST online similarity check software and closely related sequences were obtained from GenBank. JAD1 sequence strain was deposited to GenBank and received accession number as MK114162. The phylogenetic analysis (Figure 2) indicates that strain JAD1 showed
99 % similarity with *Aspergillus flavus* V5F-13 and *Aspergillus oryzae* RIB40.

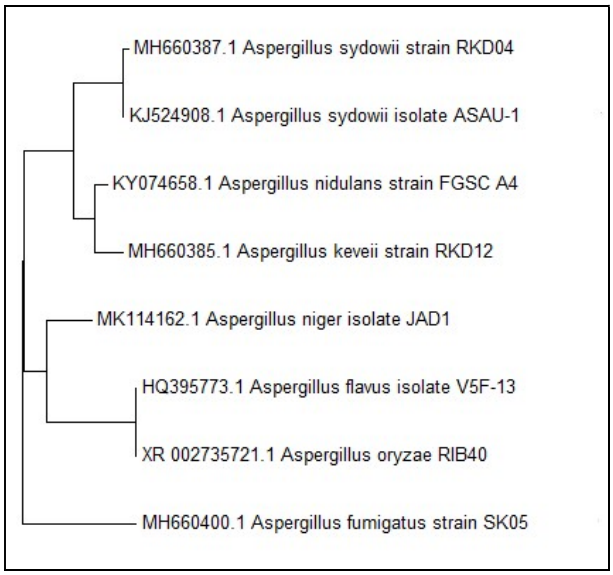


Figure 2. *Phylogenetic analysis of 28S rRNA gene sequence of Aspergillus nigerJAD1 and reference sequences retrieved from NCBI GenBank and phylogenetic tree constructed using neighbor joining method*

Earlier works have been reported to used *Aspergillus flavus* and *Aspergillus niger* to produce new glycosophorolipid-surfactant [7]. However, this is the first report where *Aspergillus niger* JAD1 produce two of the compounds (Trindene,2,3,4,5,6,7,8,9-octahydro-1,1,4,4,9,9-hexamethyl- and Cyclotetrasiloxane, octamethyl) obtained after the GC-MS analysis, biosurfactant found in the environment [9, 10].

Screening of biosurfactant producing bacteria

Oil displacement test

Cell free supernatant was added onto the petri plate containing kerosene and water. Isolates MB1, MB2 and strain JAD1. displaced showing a clear zone on the water. The diameter of the displaced zone is tabulated in Table 2. This confirms the production of biosurfactant by the two isolates and used for the further study. The displaced zone is shown in Figure 3.

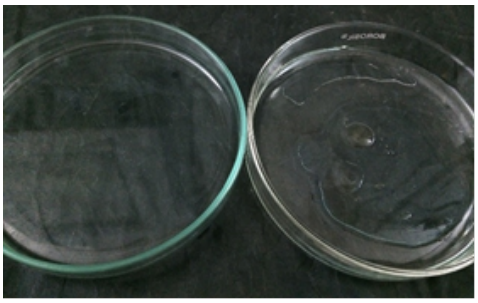


Figure 3. *Drop displacement test (left) control and (right) test*

Table 2. *Result of diameter of displaced zone of oil displacement test*

Isolate	Diameter of displaced zone (in cm)	Inference
MB1	3.2	Positive
MB2	5.0	Positive
JAD1	6.5	Positive

Drop Collapse test

Cell free supernatant of MB1, MB2 and JAD1 were placed on the glass slide covered with thin layer of kerosene oil and it was observed that they collapsed and spread. Above test can be used for confirming the isolates are biosurfactant producers and have the ability to attach to the oil drop as shown in Figure 4. This test relies on the destabilization of liquid droplet by the surfactant produced all the three isolates.

Figure 4. *Drop collapse test for bacterial (a) and fungal (b) isolates*

Foaming activity result

Biosurfactant producing isolates MB1 and strain JAD1 were cultured into MSM and M1 broth respectively for 96 hrs and then the flasks were shaken vigorously for about 2 mins. Foaming activity was observed for both the isolates as shown in Figure 5.

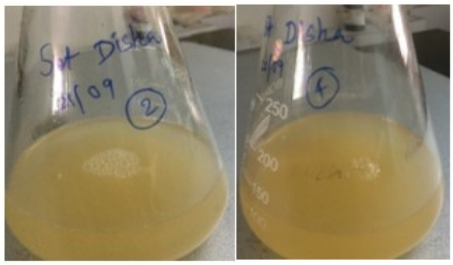


Figure 5. *Foaming activity of MB1 (left) and strain JAD1 (right)*

Hemolytic activity test

Biosurfactant producing isolates MB1 and MB2 were isolated onto the blood agar plates. Post incubation, MB1 showed β hemolysis and MB2 showed γ hemolysis as shown in Figure 6.

Figure 6. *Beta hemolysis activity of MB1 strain and γ hemolysis of MB2 strain*

Emulsification Assay

Fungal isolate JAD1 showed good emulsifying activity as shown in Figure 7 whereas bacterial isolates (MB1 and MB2) showed low E_{24} index. The crude oil used in this test was kerosene and post incubation results are tabulated in Table 3. Formula for calculating E_{24} index is given in (Equation 1):

$$E_{24} = \frac{Emulsification\ layer's\ total\ height}{Aqueous\ layers'stotal\ height} \times 100 \quad (1)$$

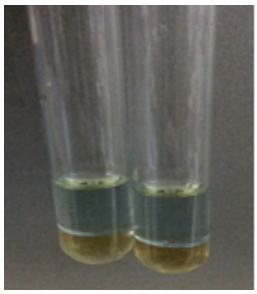


Figure 7. *Emulsification activity of MB1 strain (left) and MB2 strain (right)*

Table 3. *Emulsification index of both bacterial strains*

Isolate	Emulsification layer (cm)	Liquid layer (cm)	E_{24} (%)
MB1	1.0	1.1	41.66
MB2	1.1	1.7	64.70
JAD1	0.5	1.2	90.90

It can be concluded that *Aspergillus niger* JAD1 showed good E_{24} value and a potent biosurfactant producer compared to the bacterial isolate.

Blue agar plate method (BAP)

Post incubation of 72 hours, isolates MB1, MB2 and JAD1 showed no blue colored halo zones around the colonies on the CTAB-methylene blue agar medium therefore denoting negative for anionic biosurfactant production.

Confirmatory test

Both fungal and bacterial isolates showed positive result for the confirmatory tests. The yield of biosurfactant produced by *Aspergillus niger* JAD1 was more than that of the bacterial isolates. There was change in color in the medium from colorless to orange brown as shown in Figure 8.

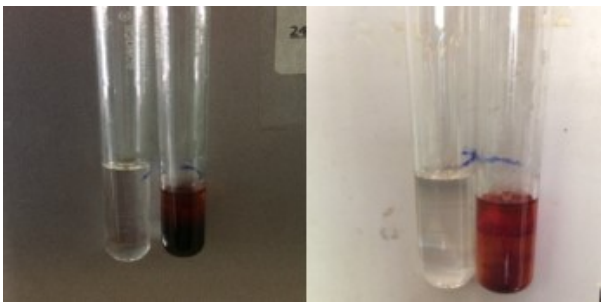


Figure 8. *Phenol sulphuric confirmatory test for (above) bacterial and (below) fungal isolate*

Antimicrobial activity

Isolates (MB1, MB2 and JAD1) extracts have been tested against clinical pathogens such as *Staphylococcus aureus, Escherichia coli, Proteus mirabilis, Klebsiella pneumoniae, Enteroccus* sp. and *Shigella* sp. The diameter of zone of inhibition was tabulated in Table 4 below.

It can be concluded that bacterial isolates producing biosurfactant have narrow spectrum activity against Gram positive clinical pathogens whereas fungal culture biosurfactant extract showed no activity against clinical pathogens.

Table 4. *Antimicrobial activity result for bacterial isolate*

Concentration of isolate suspension (µL)	***Staphylococcus aureus***	***Enterococcus* sp.**	***Escherichia coli***	***Klebsiella pneumoniae***	***Proteus mirabilis***	***Shigella* sp.**
MB1						
40	-	-	-	-	-	-
80	-	-	-	-	-	-
120	12 mm	-	-	-	-	-
160	22 mm	-	-	-	-	-
200	25 mm	-	-	-	-	-
MB2						
40	-	-	-	-	-	-
80	-	-	-	-	-	-
120	15 mm	-	-	-	-	-
160	18 mm	-	-	-	-	-
200	20.5 mm	-	-	-	-	-

'-' Negative

Detection of compound using GC-MS

Molecular mass spectra of the biosurfactant produced by *Aspergillus niger* JAD1 was obtained after GC-MS analyses as shown in Figure 9. The chromatogram displayed anticipated signals at molecular masses of 267.01 and 281.0. From the mass spectral library using NIST (National institute of standards and technology) search the compounds were identified as 1H-Trindene,2,3,4,5,6,7,8,9-octahydro-1,1,4,4,9,9-hexamethyl- and Cyclotetrasiloxane, octamethyl- as tabulated in table 5. There were many other compounds of saturated and unsaturated bonds visible in the chromatogram (Figure 9), compounds having biosurfactant property with high molecular weight are shown in Figure 10.

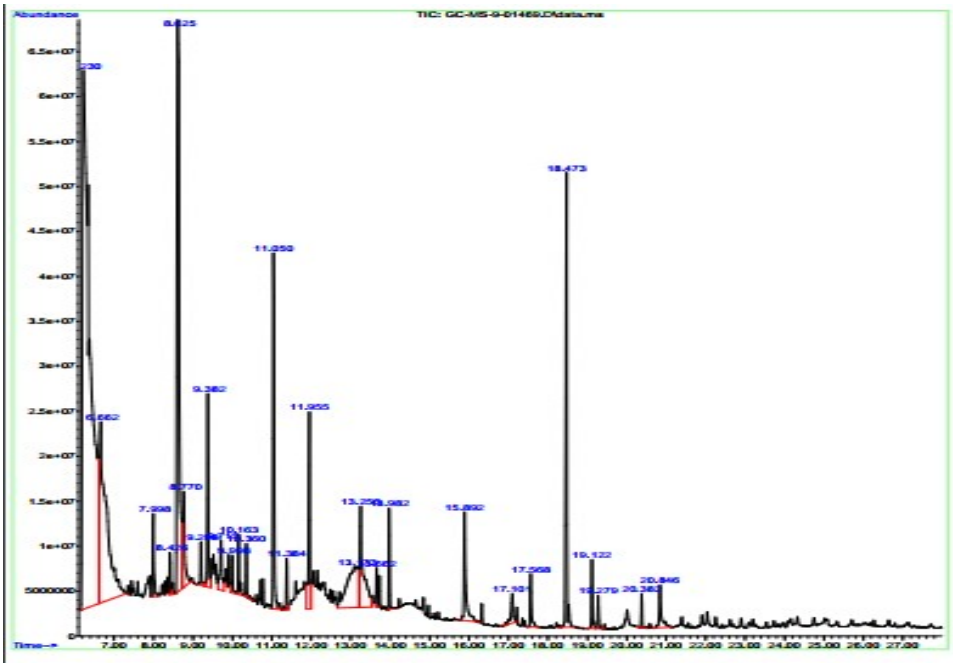


Figure 9. *GC-MS analysis chromatogram for Aspergillus niger JAD1*

Table 5*. NIST library search results of Aspergillus niger JAD1 by GC-MS analysis.*

S.no.	RT (min)	Name of Compound	Molecular formula	Molecular weight	Quality (%)
1.	6.231	Cyclotetrasiloxane, octamethyl	$C_8H_{24}O_4Si_4$	281.0	70
2.	7.998	Trindene,2,3,4,5,6,7,8,9-octahydro-1,1,4,4,9,9-hexamethyl-	$C_{21}H_{30}$	267.0	68

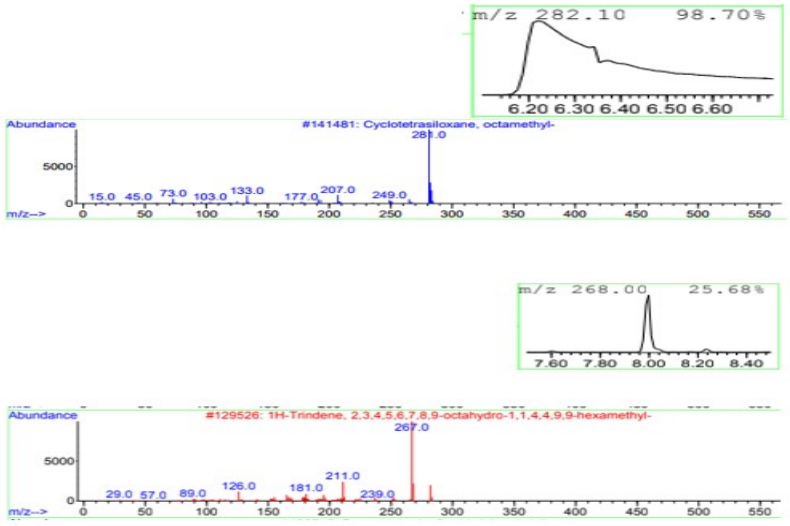


Figure 10. *Two biosurfactant compounds found in the chromatogram*

CONCLUSION

In this study, bacterial and fungal isolates were isolated from soil sample collected from Vellore in Tamil Nadu having the ability to produce biosurfactant. *Aspergillus niger* JAD1 showed good production of biosurfactant activity compared to bacterial isolates. Biochemical tests were performed and were identified using Bergy's manual as *Staphylococcus* sp. The bacterial isolate (MB1) showed a good narrow spectrum antimicrobial activity against the clinical gram-positive pathogens. *Aspergillus niger* JAD1 showed good biosurfactant production and GC-MS analysis was performed to identify the biosurfactant compounds present. Two of the compounds (Trindene,2,3,4,5,6,7,8,9-octahydro-1,1,4,4,9,9-hexamethyl- and Cyclotetrasiloxane, octamethyl) obtained after the analysis was considered to be biosurfactant found in the environment. Biosurfactant producing organism can be used for many other applications like in agricultural, petrochemical such as oil, pharmaceutical industry, etc. They can be used to enhance the solubility of bio hazardous chemicals usually found in waste water. Thus, playing a vital role in wastewater treatment process. They can be also used to increase the bioavailability of hydrophobic substrates using methods like desorption and solubilization.

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