Cite This: J.of. Toxicol. and Environ. Sci., 2021, 1, 36-47

Sciforce

Journal of Toxicology and Environmental Sciences

Journal homepage: www.sciforce.org

Isolation And Characterization Of Biosurfactant Producing Bacteria From Different Environmental Soil Samples

Abrar Hussain Mian[1*]

[1]*Department of Microbiology, Hazara University Mansehra, 21300,KPK, Pakistan.*

ARTICLE INFO

Article history:
Received: 20211011
Received in revised form: 20211020
Accepted : 20211020
Available online: 20211102

Keywords:
Biosurfactants;
Oil-contaminatedsites;
biochemical techniques;
Pseudomonas aeruginosa;
Chemical Removal.

ABSTRACT

Biosurfactants are natural substances produced by several bacterial and fungal organisms that are amphiphilic and are extracellular (a part of the cell membrane). Biosurfactants can reduce the stress between solids and liquids on the surface and at the end. Biosurfactants have several properties, i.e. they are stable, less harmful, as well as readily degradable, and extremely eco-friendly. Biosurfactants also have a wide range of industrial uses because they are a versatile category of chemical substances. The principal justification for conducting such research was the isolation of possible biosurfactants containing bacteria. Sampling was performed for the isolation of bacteria producing biosurfactants from different oil-polluted sites That is to say, experiment for emulsification, test for oil spreading, test for drop collapse, and measure for hemolysis. The capability to produce biosurfactants was seen in 22 different isolates from polluted sites B1, B2, and B3. Through different biochemical tests and Gram staining, it was identified that isolated bacterial strains are Pseudomonas spp and that is Pseudomonas aeruginosa. The procedure used as characterizing biosurfactants was the TLC plate's procedure, by using TLC plates process yellow dots emerged after spraying on silica gel plates with an throne and ninhydrin reagents. These yellow spots confirmed the presence and production of rhamnolipid in the biosurfactant. Hence, it was concluded that identified strains in the study can be helpful in the heavy metals, pesticides, and hydrocarbons bio-degradation and bioremediation. These may also be used as biological control agents to protect plants from various pathogens, resulting in improved crop yields.

Keywords; Biosurfactants., Oil-contaminatedsites, biochemical techniques, Pseudomonas aeruginosa., Chemical Removal.

*Corresponding author. e-mail: *abrar.microbio@hu.edu.pk

Introduction

Biosurfactants are natural substances produced by several bacterial and fungal organisms that are amphiphilic and are extracellular (a part of the cell membrane) (Chen et al., 2007; Ghayyomiet al., 2012). Main purpose of the bio-surfactantsgeneration or production is a consequence of financial availability (Van Dyke et al., 1993 It is reported that almost 50 percent of the world's surfactants are used because of the need for cleaning agents as well as the rate of growth grows every day (Deleu and Paquot, 2004). Appropriate use of bio-surfactants will control environmental emissions what these are the most dangerous, constantly rising gradually and disrupting the routine maintenance of life every day. Awareness campaign initiatives have been introduced and also increase for environmental laws, various innovative approaches need to be implemented and even the issue of pollution focused entirely. Developing appropriate advanced technologies to help clear up chemicals and toxins from the ecosystem, like hydrocarbons (both inorganic and organic). Studies on biosurfactants are being launched by scholars and researchers with significant health issues like adverse environmental effects, air contamination, environmental change, and waste management (Makkar and Cameotra, 2002 Biosurfactants contribute to expanded demand for such microbial products as alternatives to chemical surfactants (Benatet al., 2000). Microbes seem to have the capability to degrade contaminants, but their biodegradation is limited leading to hydrophobicity, low solubility in water, and inadequate bioavailability, of such pollutants (Patil, et al., 2012). GhayyomiJazeh, Mishraet. al (2001) those bacteria that produce

biosurfactants were isolated from the site of petroleum spills and afterward, 160 strains and as well as 59 strains were able to produce biosurfactants have shown better performance in a test for hemolysis of blood, and 45 strains with positive findings within oil spread experiment were applied in the laboratory to isolate and segregate the media cultured Banat process (Rahman et al., 2002) These were observed and researched that biosurfactants of Pseudomonas aeruginosa spp are most likely to disrupt the bonding of hydrocarbons like nonadecane, octa, Hexa, and hepta, in marine Water contaminated with oil spills up To approximately 47%, 53%, 73% and 60%(Abrar et al., 2020). Current study concluded that the isolated strain having the ability to degrade hydrocarbon as well as the ability to degrade the heavy metal. The strain also can protect the plant from various diseases. The present research found that the isolated strain is capable of degrading hydrocarbon while also being capable of degrading the heavy metal. As well as the strain does have the capability to defend plants from different diseases.

Material And Methods

Area of Study

The investigation was conducted at HazaraUniversity(HU) Microbiology Laboratory, MansehraPakistan.

Assemblage of Samples

Thehomestay area of the city Mansehra Pakistan which is named as a township, where oil spills arose, oil spills soil samples were obtained as well as sampling from various Mansehra automobile workshops were also done. Sterilized bags of polythene were being used to collect samples of the soil, after thatthe sample was taken towards the Hazara University (HU) Mansehra Microbiology Laboratory to examine and extract bacterial strains that could develop biosurfactants. The soil temperature at the time of sample selection was around 30 ° C. The pH was also verified by Galvano science companies at the time of selection by pH meter, and the pH being reported was 7.

Preparation of Media

15 x 100 mm Petri dishes were being used to prepare the media. Agar plates were thoroughly cleaned with water from the tap and then carefully covered in aluminum foil following cleaning then placed within autoclave at 121°C for about 15 min at 15 psi for sterilization. The nutrient agar which contains 0.5% NaCl, 0.3% beef extract, 0.5% peptone, and 1.5% agar, in 500 ml of distilled water, 14 g of the nutrient agar media (Merck) were dissolved. The nutrient level used mainly for the production of non-fastidious species. Nutrient agar is widely known as it's capable of growing a variety of bacteria types and provides nutrients required for the growth of bacteria. Upon sufficient dissolution of such nutrient agar in distilled water, these were then sterilized by autoclaving for 15 min at 15 psi in the autoclave and held at 121 °C Upon autoclaving, pouring of the media was done in laminar flow hood, and then packed and placed for yet more use in a fridge at 4°C. **2.4 Preparation of serial dilution**

The bacteria are isolated using the serial dilution process. During this process, 10 test tubes were taken and distilled water (9ml) was added in each tube. After that tubes were put for 15 minutes in the autoclave machine at 121°C. After that 1gm of a crude oil sample from the soil was added in a test tube containing distilled water. Further, 1 ml of the solution was taken from the first test tube and poured to the adjoining tubes for the preparation dilution as under $1/10, 1/102, 1/103, 1/104, 1/105, 1/106, 1/107$. Afterward, 10µl of the solution was pipetted from both the dilution of $1/104$ *and* $1/106$ *tubes*, and shifted for spread culture techniques, then incubated the plates at 37°C for 48hrs.

Biosurfactants extraction

Firstly, in nutrient broth solution theculture of bacteria was added and inoculated with oil, the bacterial colony was then incubated at the temperature of 25°C in a shaking incubator just for 7 days. Incubation after seven days of trembling. Thebacterial Crop was then taken and centrifuged at 5000rpm at temperature 4°C for 20minutes. Following centrifugation, the supernatant was collected and then mixed in the equivalent amount in Methanol: Chloroform. White sediment was then retained and collected for further use

.

Bacterial Colonies Isolation

1 g of the soil polluted with oil was diluted serially up to 106 dilutions.10 µl of 104 and 106 dilutions for spread culture were transferred to the MSM agar plates and nutrient agar. The plates were then incubated at 37°C for 48hrs. Twenty-two morphologically separate colonies were separated for further specific examination just after the incubation and processed by using the technique of streak plate.

Screening of Isolates' Biosurfactants Behavior

To check the activity of biosurfactants produced by the bacterial species the following methods of screening were done.

Hemolytic Activity of Biosurfactants for Erythrocytes

Blood agar containing 5% of blood was prepared as after the fresh isolates were added and inoculated on blood agar plates, then the plates were taken and placed in the incubator at temperature 37°C for 48hrs (Rashedi*et al*., 2005). Thereafter the observation of clear zone in the colonies indicated the existence of bacterial species that produce biosurfactants. This experiment was undertaken to control the ability of isolated bacteria to induce blood agar hemolysis. Three forms of hemolysis usually involve; alpha, beta, and hemolysis of the gamma. The agar underneath the species is dark greenish, then it is Alpha, the yellowish color produced in beta hemolysis and gamma hemolysis does not affect the bacterial *spp*whichadded on the plates (Anandaraj and Thivakaran, 2010).

Bio-surfactant identification with process of CTAB

MSM (Mineral salt agar medium) with (2%) of glucose serving both as carbon source, (0.5 mg / ml) acetyl-tri-methyl-ammonium-bromide (CTAB), and methylene blue (MB: 0.2 mg/ml) are used to detect anionic bio-surfactants (Satpute *et al*., 2008). For this method, thirty microliters (30µl) of cell-free

supernatant were added to each of the wells of the methylene blue agar plate that comprises of borer (4 mm in diameter). after that, the incubation of the plates was done for 48-72 hrs at 37°C. Just after incubation in each of the wells, a dark blue halo zone was being used to show the successful anionic bio-surfactant production.

Table 1: Composition of MSM Media

S. No	Ingredients	Amount (gm/L)
I	Potassium dihydrogen phosphate (KH_2PO_4)	1 $01gm$
II	Magnesium Sulfate ($MgSO_4$)	0.55 gm
III	Iron Sulfate ($FeSO_4$)	0.001 gm
IV	Sodium Nitrate ($NaNO_3$)	1.51 gm
V	Calcium Chloride ($CaCl_2$)	0.0002 gm
VI	Ammonium Sulfate $(NH_4)_2SO_4$	1.4 gm

Technique for Spreading of Oil

A sufficient number of isolated bacteria were inoculated into a solution of 100ml nutrient broth. Over 3 days, the culture was incubated at 37 ° C in a rotating shaker incubator (150 rpm). After that biosurfactants synthesis was checked in culture suspensions (Priya and Usharani, 2009; Anandaraj and Thivakaran, 2010). For this process, thirty milliliters (30ml) of distilled water was added in a Petri dish. In the middle of the distilled water, 1 milliliter (1ml) of diesel oil was added, and then a centrifuged twenty microliter (20µl) culture was introduced to the middle of a plate, which was isolated from oil spilled soil or local oily groundwater. The species producing the bio-surfactant displace the hydrocarbons and disperse it even in the water. Then it was calculated and analyzed within 1 mint (Ali *et al*., 2013).

Technique for Drop collapse

In this process, 96-wellsformed in each of the plates of nutrient agar. Afterwards, all the 96-wells of microliter plates was then filled withmineral oil of about 2ml. Then stabilized the plate at 37°C for 1 hour, after which the oil surface was filled with 5µl of supernatant culture. Therefore, the drop shape was taken to be observed on the oil surface after 1min. The drop which was collapsed, generated by the supernatant culture which is used to signify positive(+ive) outcome and the drops which stayed the same and displayed no changeindicates negative(-ive) outcome. And was taking distilled water as a control(Plaza et al., 2006).

Emulsification index

The emulsification index was calculated, as stated by the process followed by Cooper and Goldenberg (1981) In this process, 2 ml of kerosene oil was taken and inserted in each of the test tubes to the same amount of cell-free supernatant, and then homogenized for 2 min in a vortex at high speed and allowed for 24 hours to stand. The emulsification steadiness was then determined after the 24 hours, and the emulsification value was estimated by measuring the emulsified layer height by the total liquid layer height, then multiplied by 100.

$$E24 = Total\ emulsified\ layer\ height \times 100/\ Total\ liquid\ layer\ heigh$$

Quantification for the Dry weight of Biosurfactants

The bacterial colony was inserted and inoculated in the nutrient broth medium, followed by oil and centrifuged at 5000rpm and after that, the supernatant was clutched and treated with chloroform and methanol and mixed. The white colored deposits were taken and used for the furtherprocess of dry weight. Afterwards, took the clean Petri plate and determined the empty plate weight. Next, the sediment was poured onto Petri plates. Now, for the drying process the hot air oven was used and set the 100ºC of temperature for 30minutes and the plates were put in the oven. After the drying process, the plates were weighted again. The dry weight was calculated for the biosurfactants using the formula which described below:

$$Bio - surfactant\ dry\ weight = Plate\ weight\ after\ drying - Empty\ plate\ weight$$

Selected strains Identification and their characterization

Instead, various basic biochemical methods were used to identify the isolated bacterial strains. Various biochemical tests, such as Gram staining, Oxidase test, Urease test.Catalase test, Methyl red test, Motility test, Indole test, Starch hydrolysis, Citrate test, Spore staining, Gelatin hydrolysis. Then afterwards, for the preliminary characterization of the biosurfactant, the thin layer chromatography process was used.

Physical characterization of the strains selected

Gram staining

First, on the slide, using the wire loop the bacterial pure culture was taken, and smear was prepared on the slide, and then a drop of purified water was applied. Then, the sterile loop or needle was correctly mixed the bacterial colony and purified

water, then mixed up until it is somewhat turbid. Then, spirit lamp was used to fixed the bacterial smear on slide and cooled to room temperature. With this glass slide was loaded with solution of crystal violet and stood for 1minute anddistilled water was applied on slide. Meanwhile the slide was submerged for 1 minute with the iodine solution, and then flushed and rinsed with water. Therefore, decolorizer of about 1 to 2 drops(5 percent acetone and 95 percent alcohol) were added to the slide's smear and stand for 30seconds, and then treated with water. After then slide was rinsed with safranin for 60seconds, and then treated with water anddry in air. Microscopic analysis was done with 100x objective lenses using emersion oil on smear.

Cell morphology

The isolates of the bacterial cell were gram stained on slides and then the slides were observed under the light microscope, showing the shape and color of the cells.

Biochemical characterization of the selected strains

Catalase test

Aim of this study is to identify, evaluate and examine that, whether or not the microbes are capable of producing catalase enzymes, while catalase is a protective enzyme, i.e. catalase has the potential to protect against the lethal chemicals known as (H_2O_2). In this study a bacterial culture that was clarified overnight was used. This culture has been smeared on a glass slide, and 3 percent hydrogen peroxide (H_2O_2) has been applied and observed on smear. Effects have been observed for bubble formation.

Citrate test

This study was performed to check the amount or ingest the citrate as the carbon and energy supply for growth and metabolism. Medium containing bromothymol blue and sodium citrate as pH indicator, bacterial was introduced. Ammonium chloride is also present in this medium used as a nitrogen source. Results were noted with variations of color from green to blue.

Urease test

The capability of urease enzyme for degrading urea was calculated in this bacterial capacity test. Bacterial culture was taken and inoculated for 48 hours at 37 ° C in urease broth, and then color was observed.

Methyl red test

Through using the process known as mixed acid fermentation which is used to evaluate the bacteria's acid production. The bacterial culture was taken and introduced in the broth of MR-VP and then incubated for 3days at a temperature of 37°C. Two (2) to three (3) drops of Methyl red were added in the broth medium after the incubation period. The change in broth color was observed for final results after a few seconds.

Indole test

Through using the process to assess the bacteria 's capability to crash indole from tryptophane molecules. After the 24 hours of incubated, taken the fresh inoculum of bacteria and then inserted into the tryptone medium, 24 hours of incubation of about 30°C, 2ml of the tryptone broth medium was added into a sterile test tube. Kovac's reagent was taken to be added (few drops) in sterile test tube and stimulated for a few minutes, and variations of color were detected.

Gelatin test

It is the approach assess to figure out the use of enzymes known as gelatins from bacterial organisms that precipitate the gelatin. Fresh inoculum of bacteria was taken after 24 hours, and inserted into the media of gelatin agar. This was incubated for around 24 hours, so the temperature did not exceed 30 ° C. Media was observed after incubation time.

Starch hydrolysis

Several of the micro-organisms that use the starch as a carbon energysource. Therefore, this method has been used to assess whether or not bacteria may use starch as a source of carbon. The bacterial fresh inoculum was spread on the petri starch agar plates, and after that the plate was incubated for 24 hours andmaintained the temperature at 30 to 35 ° C, then gradually applying the supplements of iodine to the plates to flow the change, and then examining the plates.

Preliminary characterization of the strains selected

Experimental characterization of the bio-surfactant was performed by using the process of TLC (Anandaraj *et al.,* 2010). On a silica gel plate, crude portion of the rudimentary bio-surfactant was separated using Methanol: Chloroform: water (CH3OH: CHCl3: H2O) in the ratio of$10:70:0.5, v/v/v$ as an eluent with a different color producing reagents. Ninhydrin reagent (0.5 g ninhydrin in 100ml anhydrous acetone) was used to find bio-surfactant lipopeptide as red spots and anthrone reagent (1 g anthrone in 5ml sulfuric acid combined with 95ml ethanol) as yellow spots to identify rhamnolipid bio-surfactant (Yin *et al.,* 2008).

Results and Discussion

Isolation of bacteria

At first, twenty-two (22) strains from a polluted soil sample were isolated from nutrient agar media.Mixed culture provided by these colonies, so they were taken and smeared on the plates of nutrient agar and then fresh inoculum was collected and stored at temperature of 4°C for the further analysis. Bio-surfactants (surface-active compounds)are formed by a variety of amphiphilic bacterial and fungal organisms that are extracellular (a part of the cellular membrane) (Chen *et al.,* 2007).

Screening of Isolated strains for biosurfactant producing colonies

Different experiments were carried out to identify, isolate and screen bacteria that are capable of generating bio-surfactants and that is Oil spreading technique(OST), blood hemolysis test(BHT), CTAB test, Emulsification operation. There were twenty-two distinct isolates observed in the current research. And the B1, B2 and B3culture were taken and selected from the twenty-two (22) strains isolated from the polluted spot, which

were found to produce biosurfactant. And the oil spreading technique showed promising results for these strains. And strain B2 showed a greater displacement of oil and this is 4 mm. Oil spreading method is quick and often easy to handle, and this technique requires no particular equipment, only a very small amount of sample is used. This approach can be applied when the production and quantity of biosurfactant is small (Plaza *et al.,* 2006) and (Youssef et al., 2004) Only bacterial cultures have been allocated and screened for bacterial species that can generate or use biosurfactants. Just three (3) strainsamong them presented the best results.Those 3 strain,s (B1, B2 and B3) were selected as an additional analysis.

Blood hemolysis test

On the petri plates of blood agar, the . Isolated bacteriaof B1, B2 and B3 were taken andstreak at the temperature about 37°C for 48 hours. Strain B1 demonstrated β (Beta) hemolysis after the incubation cycle and B2 and B3strains demonstrated γ (Gamma) hemolysis. The B1 strain had an emulsification index of about 74 percent and that was very high as compared to 70 percent for B2 and about 53 percent for B3 respectively. Around the same time, B1 strain showed β (Beta) hemolysis and γ (Gamma) hemolysis was shown bystrains B2 and B3 on the platesof bloodagar. The β hemolysisshowed by the strain B1 in the blood agar test, and the strain B2 and B3 showed γ (Gamma) hemolysis. It is determined that 20 percent strains that are the bestproducer of rhamnolipid have not fully lysed the blood, because the ability of the producer strains capacity not be responsible for the hemolytic activity. According to many researchers, who have shown that this is not such an effective tool for biosurfactant detection due to many bioproducts that may also induce red blood cell lysis, that is not so sufficient to be the surface-active molecule (Youssef et al., 2004). (Rashedi and others, 2005).

Table2 Blood Hemolysis Test

Bacterial culture	Hemolysis results
B, 1	β (Beta)
B, 2	γ (Gamma)
B, 3	γ (Gamma)

CTAB agar plate test

This test confirms the anionic biosurfactants development. After plate incubation at a temperature of 37 ° C for 72 hours, dark blue hollow zone was existedaround each of the B1 strains wells, which clearly indicated the positive (+ive) development of anionic Biofactant. In addition, the B1 and B2 strains showed positive (+ I ve) results and, in the CTAB analysis, the B3 strain was found to be negative (-ive). The growing microorganisms when secreted the anionic biosurfactants on the plates of CTAB (cetyl-tri-methyl-ammonium-bromide) and methylene blue, then as a result the dark blue-purple insoluble ion pairs formed on the plates. The halo zone around each of the colonies was developed that can recognize rhamnolipid production and that was dark blue in colour, and could correlate with production of rhamnolipid (Siegmund *et al.,* 1991). As indicated in (Fig1)

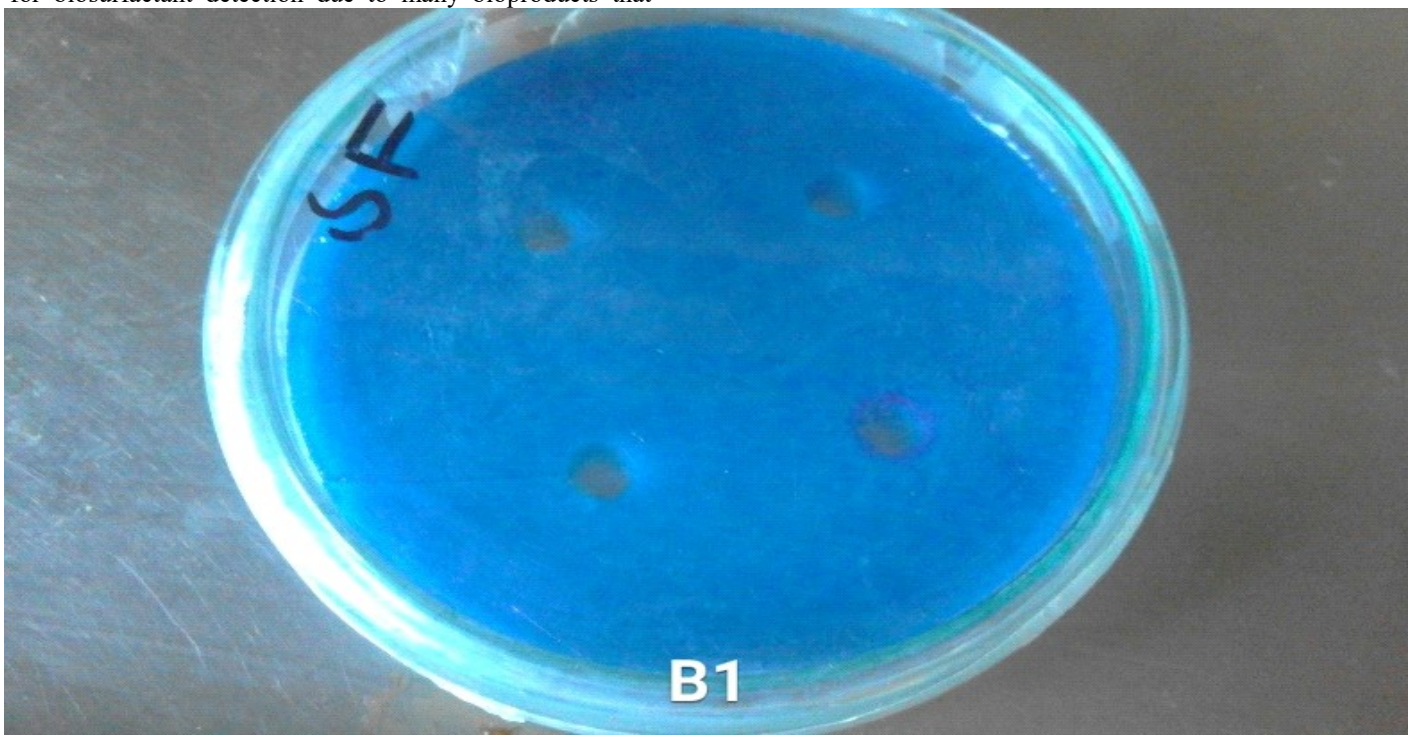

Fig1: B1 positive on CTAB agar plate

Oil Spreading Technique

The oil was displaced by B1, B2and B3 strains in this test strain and showed a zone that was so clear. The bacterial strains capable of developing biosurfactant were tested and separated from the sample of soil which was oil spilled and brought from the District of Mansehra, Pakistan and from automobile workshops of Mansehra. As shown in (Fig.2).

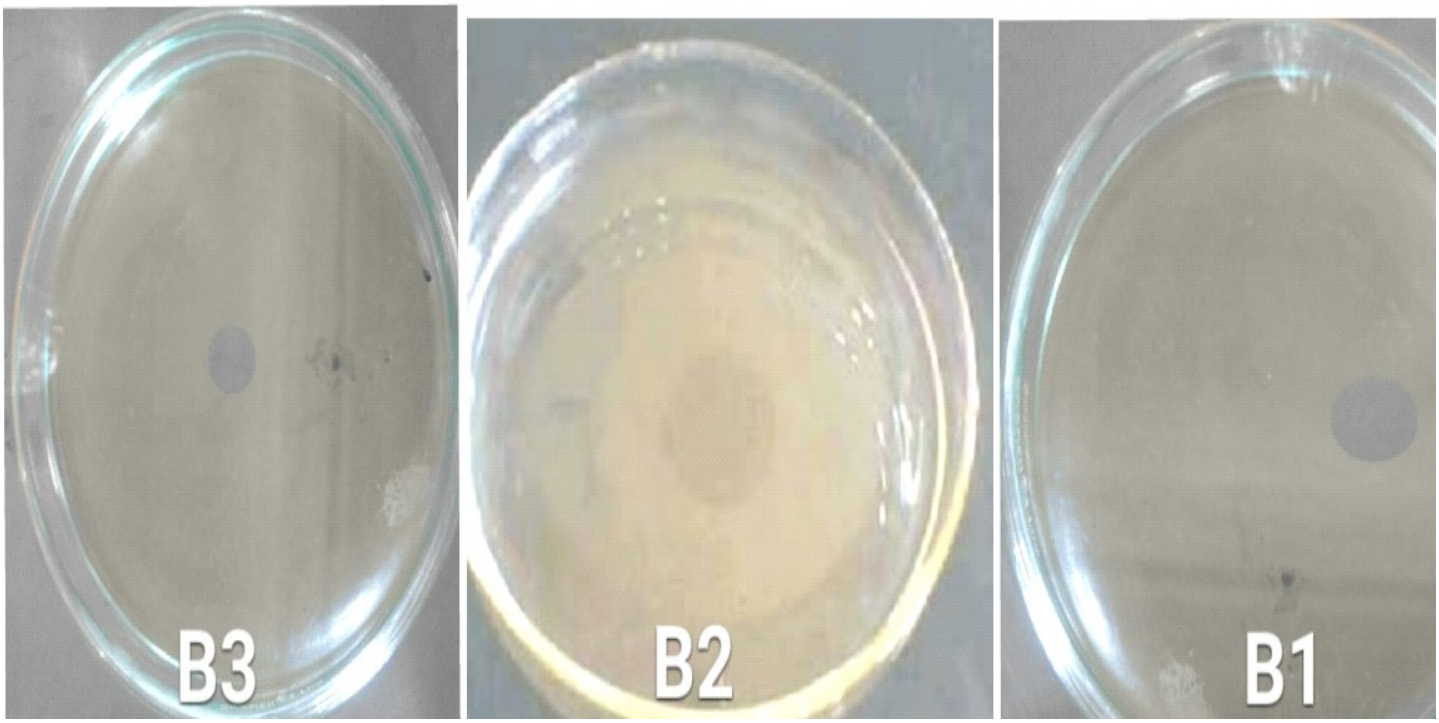

Fig.2: Results of Oil Spreading by B1, B2 and B3

Table 3;.Test for oil spreads

Bacterial culture	Formation of zone (mm)	Readings
B,1	3.03	$+ive$
B,2	4.1	$+ive$
B,3	1.05	$+ive$

Drop-collapse technique

During this process the drop shape was observed at the oil surface. As seen in Fig 3, the collapsed drop was provided by the supernatant culture B1 , B2 and B3..

Emulsification index

Emulsification stability was measured with the use of kerosene oilin this test, and then observed the results. Since this emulsification index was calculated by dividing the height of the emulsion layer by the total height of the liquid layer and then multiplying by 100, as shown in the formulation below.

$$E24 = total\ emulsified\ sheet\ height = 100/\ total\ liquid\ layer\ height$$

Emulsification index

Emulsification stability was measured with the use of kerosene oilin this test, and then observed the results. Since this emulsification index was calculated by dividing the height of the emulsion layer by the total height of the liquid layer and then multiplying by 100, as shown in the formulation below.

$$E24 = total\ emulsified\ sheet\ height = 100/\ total\ liquid\ layer\ height$$

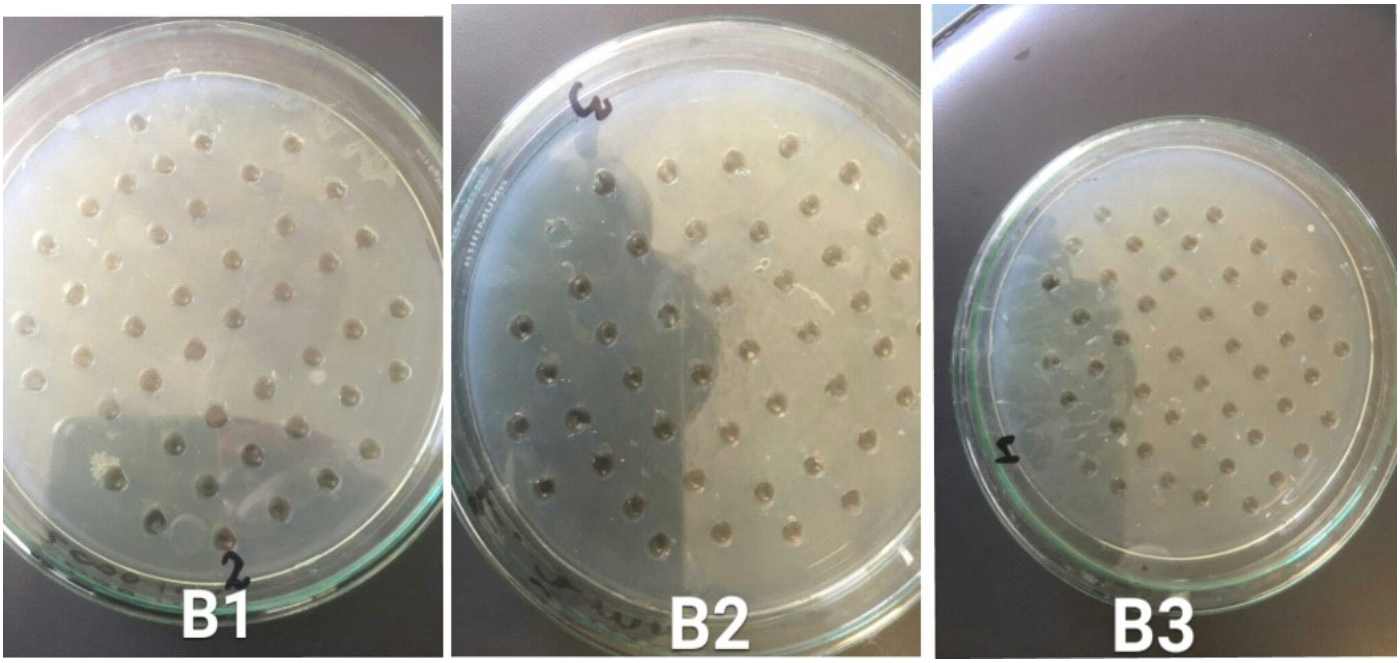

Fig 3: Result of Drop-collapse test

Table 4: The activity of Biosurfactant emulsification

Bacterial culture	***Emulsified layer*** (cm)	***Total layer of liquid*** (cm)	***E24*** (%)
$B.1$	2.01	2.077	73.11
$B.2$	1.08	2.077	69.21
$B.3$	1.00	1.088	53.51

Dry weight of bio-surfactants

In this examination, white-colored sediment was collected. Then measured the weight of the sterile Petri plate which was empty in the first step. Then, the sediment was poured into plates. The plates were taken and weighted after 30 minutes of drying on a hot air oven, following the process of drying. The weight of biosurfactants (dry weight) was measured using the following formulations:

$$Biosurfactant\ dry\ weigh = Plates\ after\ drying\ weight - Empty\ plate\ weight.$$

Fig 4: Dry weight of biosurfactants

Table: 5: Dry weight of the biosurfactants

Bacterial Culture	**Weight of the plate (g)**	**biosurfactant in The plate after drying (g)**	**Dry weight of Biosurfactant (g)**
B,1	48.0120	48.2451	0.124
B,2	48.0120	48.441	0.320
B,3	48.0120	48.511	0.390

Identification of selected strains and their characterization

Gram staining

For structural applications, and stroke analysis gram staining method was used.(Fig.5) shows findings from the process of gram staining.

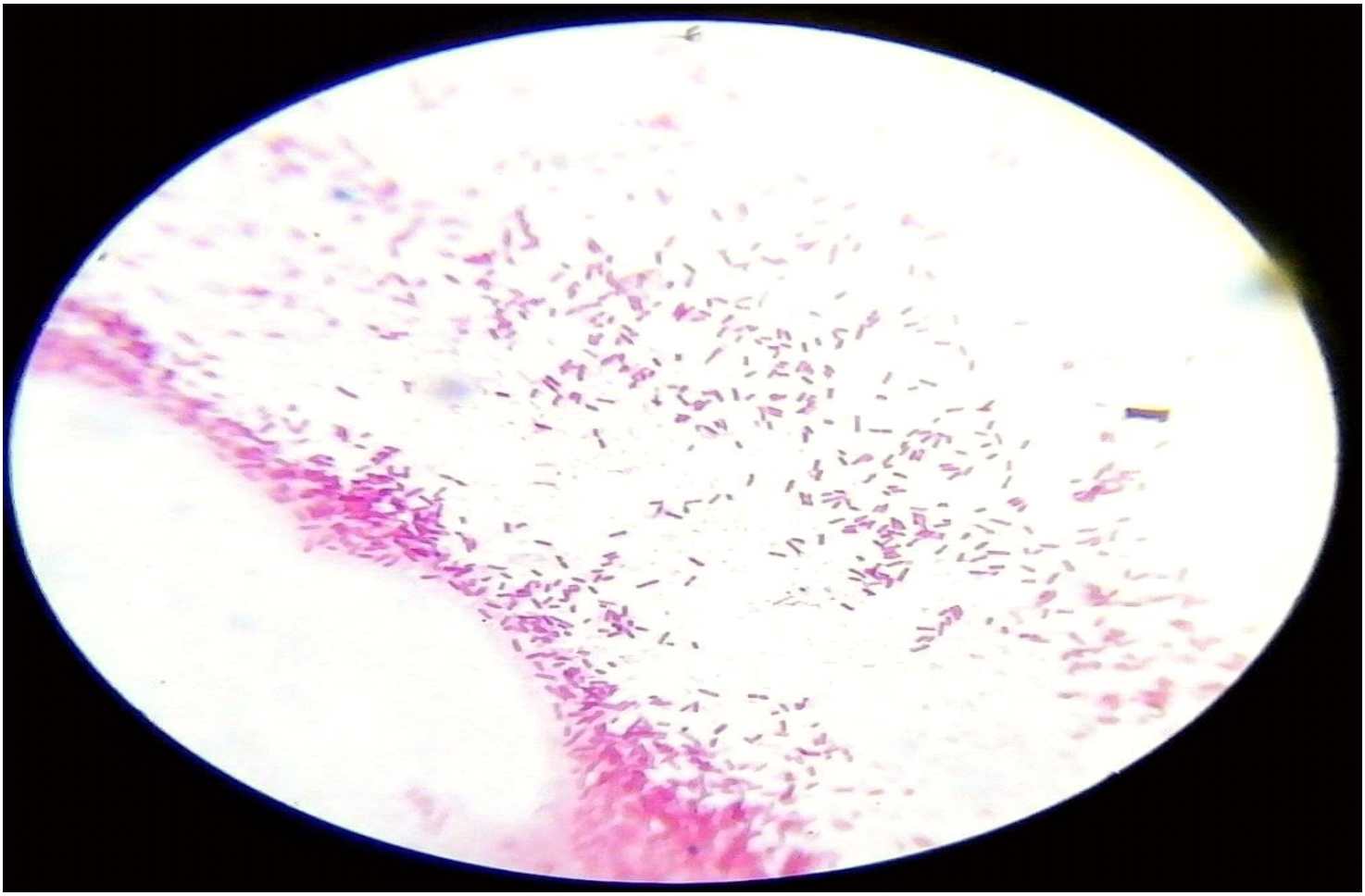

Fig 5: Microscopic view of Gram staining

Biochemical identification of bacterial strains and their characterization

Specific biochemical studies were performed to identify the species for further recognition and characterization. The bio-surfactant producing microorganism was found to be *Pseudomonas aeruginosa* after conducting various characterizations and the biochemical tests(Eric Deziel *et al.,* 1996), Which can be used to further analyze and study the industrial development of the biosurfactant. Rhamnolipid is also isolated and produced from the Pseudomonas aeruginosa species on the silica gel plate (Rashedi *et al.,* 2005), a form of biosurfactants highly recommended for processes of bioremediation. All the findings collected from biochemical testing were labeled as Berge 's Manual and it revealed that the protected microorganism was (*Pseudomonas aeruginosa)*. Results of biochemical test were tabulated in (Table.5)

Table 6: Bacterial strain identification

Tests	**B1**	**B2**	**B3**
Gram staining	Negative	Negative	Negative
Oxidases	Positive e	Positive	Positive
Catalase	Positive	Positive	Positive
Indole	Positive	Negative	Negative
Citrate	Positive	Negative	Negative
Urease	Negative	Positive	Negative

Nitrate	Positive	Positive	Positive
Motility	Positive	Positive	Positive
Gelatin hydrolysis	Positive	Negative	Negative
Lactose	Negative	Positive	Positive
Methyl red	Negative	Positive	Positive
Voges Proskauer	Negative	Negative	Negative

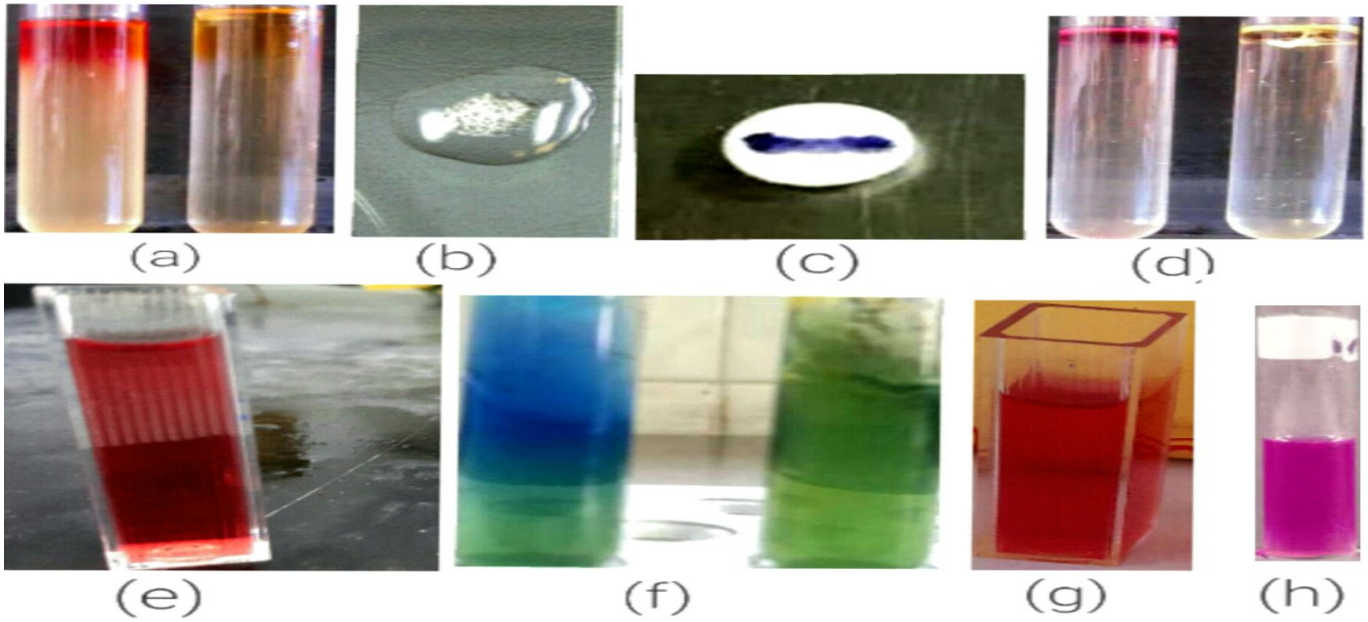

Fig 6: Results of biochemical tests

(A) Methyl red and Voges Proskauer tests (b) catalase tests (c) oxidase tests (d) indole tests (e) citrate tests (g) lactose tests (h) urease tests

Preliminary bacterial strain's characterization

The plates showed yellow dots, when sprayed with anthrone reagent. It indicated the existence of biosurfactants of rhamnolipid in the organism on the plate of TLC as seen in theFig.7

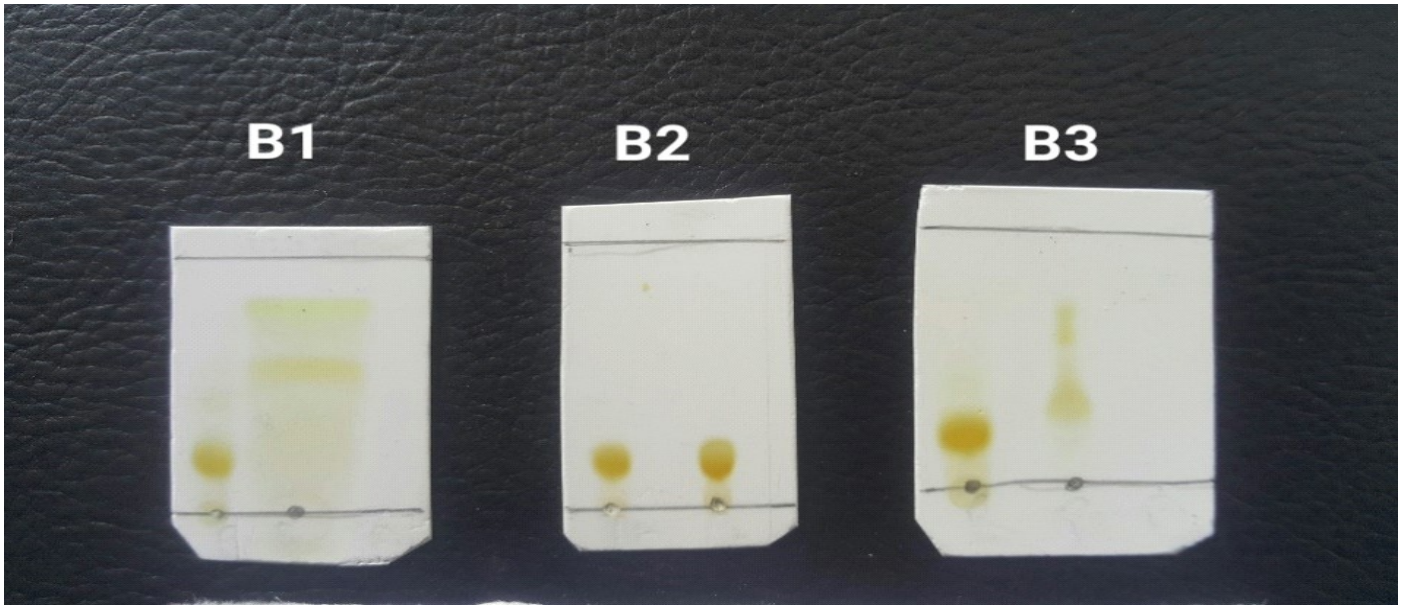

Fig 7: Biosurfactant characterization by TLC

Conclusion

Biosurfactant development is exciting and perceptible across industries to clean up oil waste and pollutants, particularly in the ecosystem.Compared with chemical surfactants, the biosurfactants are less harmful. It plays an important role in defining the advantages and the importance of industrial applications. Therefore, it is not possible to disregard the growing role and importance of biosurfactants in environmental sustainability.Biosurfactant formulations which can be used for bacterial, fungal, and viral organisms as growth inhibitors. Such biosurfactant inhibition properties can make them components that are applicable to Numerous illnesses that are used as medicinal agents. Therefore it was decided that the described strain could be used as a potential source for heavy metal bioremediation pesticide and hydrocarbon polluted sites. And also used as shielding the plant from different pathogens, contributing to improved crop yields. There is no doubt that the biosurfactants are a multifunctional, advanced, versatile, long-lasting and updated type not only for the twenty-first century but beyond.

Conflict of interest

The authors declared that they have no conflict of interest and the paper presents their own work which does not been infringe any third-party rights, especially authorship of any part of the article is an original contribution, not published before and not being under consideration for publication elsewhere.

References

1. Ali, S.R.; Chowdhury, B.R.; Mondal, P. and Rajak, S. "Screening and characterization of biosurfactants producing microorganism from natural environment (Whey spilled soil)". *J. Nat. Sci. Res.* **2013**, 3(13), 34–64.
2. Anandaraj, B. and Thivakaran, P. "Isolation and production of biosurfactants producing organism from oil spilled soil". *J. Biosci. Tech.* **2010**, 1(3), 120–126.
3. Banat, I.M.; Makkar, R.S. AND Cameotra, S.S. "Potential commercial Application of Microbial Surfactants". *Applied MicrobialBioethanol.* **2000**, 53, 495-508.
4. Cooper, D. G, Zajic, J. E. and Denis, C. J. Am. Oil Chem. Soc. **1981**, 58, 7780.
5. Deleu, M. and Paquot, M. "From Renewable Vegetables Resources to Microorganisms: New Trends in Surfactants"C.R. *Chimie.* **2004**, 7, 641-646.
6. Eric, Deziel.; Gilles,Pauette.; Richars, Villemur.; Francois,Lepine.; and Jean-Guy, Bisaillon. "Biosurfactants Production by a Soil Pseudomonas Strain Growing on Polycyclic Aromatic Hydrocarbons". Applied and Environmental Microbiology. **1996**, 62(6), 1908-1912.
7. Ghayyomi, J.M.; Forghani, F.; Deog, Hwan, Oh. "Biosurfactant production by Bacillus sp. Isolated from petroleum contaminated soil of Sirri Island". Ame. J. Appl. Sci, **2012**, 9(1), 1-6.
8. Makkar, R.; & Cameotra, S. An update on the use of unconventional substrates for biosurfactant production and their new applications. *Applied microbiology and biotechnology.* **2002**, 58(4), 428-434.
9. Mishra, S.; Jyot, J.; Kuhad, R. C.; & Lal, B. Evaluation of inoculum addition to stimulate in situ bioremediation of oily-sludge-contaminated soil. *Appl. Environ. Microbiol.* **2001**, 67(4), 1675-1681.
10. Patil, T. D.; Pawar, S.; Kamble P. N. & Thakare, S. V. "Bioremediation of complex hydrocarbons using microbial consortium isolated from diesel oil polluted soil". *Der*

ChemicaSinica Journal of Biotechnology. **2012**, 3(4), 953-958.

11. Plaza, G.; Zjawiony, I.; and Banat, I. "Use of different methods for detection of thermophilic biosurfactants producing bacteria from hydrocarbon contaminated bioremediation soils". *J. Petro. Sci. Eng*. **2006**, 50(1), 71–77.
12. Priya, T.; Usharani, G. "Comparative study for bio-surfactant production by using Bacilus subtilis and Pseudomonas aeruginosa". *Bot. Res. Int*. **2009**, 2(4), 284–287.
13. Rahman, K.S.M.; T.J. Rahman.; S, McClean.; R, Marchant.; and I, M. Banat. "Rhamnolipid biosurfactants production by strains of pseudomonas aeruginosa using low-cost raw materials". **2002**, 18, 1277-1281.
14. Rashedi. H.; Jamshidi, E.;Mazaheri, Assadi. M.; and Bonakdarpour, B. "Isolation and production of bio-surfactant from Pseudomonas aeruginosa isolated from Iranian southers wells oils". *Int. Environ. Sci. Tech*. **2005**, 2(2), 121–127
15. Satpute, S.K.; Bhawsar, B.D.; Dhakephalkar, P.K.; and Chopade, B.A. "Assessment of different screening methods for selecting bio-surfactant producing marine bacteria". *Indian J. Marine Sci*. **2008**, 37, 243–250.
16. Shafeeq, M.; Kokub, D.; Khalid, Z. M.; Khan, A. M.; Malik, K. A. (1989). MIRCEN J. Appl. Microbiol. Biotech. **1989**, 5, 505–510.
17. Siegmund, I. and Wagner, F. "New method for detecting rhamnolipids excreted by Pseudomonas species during growth on mineral agar". *Biotech. Tech.* **1991**, 5, 265–268.
18. Van Dyke, M. I.; Couture, P.; Brauer, M.; Lee, H. and Trevors, J. T. "Pseudomonas aeruginosa UG2 rhamnolipid biosurfactants structural characterization and their use in removing hydrophobic compounds from soil". *Can. J. Microbiol.* **1993**, 39, 1071-1078.
19. Yin, H.; J, Qiang.; Y, Jia.; J, Ye.; H,Peng.; H, Qin.; N, Zhang. B. "Characteristics of bio-surfactant produced by Pseudomonas aeruginosa S6 isolated from oil containing water". *Process Biochemistry*. **2008**, 44: 302–308.
20. Youssef, H.; Duncan, El.; Nagle, P.; Savage, N.; Knapp, M.; McInerney, J. "Comparison of methods to detect biosurfactant production by diverse microorganisms". *Microbiol Methods.* **2004**, 56, 339-347.