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Research Article

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# Identification and Isolation of Biosurfactant Producing Bacteria from Oil Spill Contaminated Soil of Ennore

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**Abstract:** The bacteria were isolated from the soil samples that were collected in oil spill-contaminated sites located at Kamarajar port in Ennore, Chennai, Tamil Nadu, India. The main aim of this study was to isolate the bacteria that produce biosurfactants from the oil spill contaminated soil in Ennore, Chennai and to produce a growth medium for the bacteria to enrich the growth of the bacteria. For the growth of bacteria, Minimal Medium (MM) and Mineral Salt Medium (MSM) were the two types of growth enrichment medium used and serial dilutions of the bacterial cultures were done for the production of colonies. The objective of the study was to perform tests to identify whether the bacteria can be able to produce the bio-surfactant or not. Tests like Drop collapse method and oil displacement method were done to check for the production of biosurfactant. The bacteria which produce the surface active substances were the one which produces the bio-surfactant. As a result, gram staining was done for identifying the nature of bacteria. Most of the gram positive bacteria are known to have the properties of bio-surfactant. These bacteria has peptidoglycan outer layer that are easier to kill - their thick peptidoglycan layer which absorbs antibiotics and cleaning products easily. Hereby we conclude that the bacteria which produces the biosurfactant is *Bacillus* and further studies should be done on identifying the bacteria at the species level. Therefore, this study was done to eradicate the oil spill contaminated areas with the help of bacteria in a natural way without harming the environment. *Bacillus* sp. is a gram-positive bacteria which helps in, liver and renal function, and act as an inflammatory markers and in maintaining blood glucose levels. It does not have an outer cell wall and only has a membrane of peptidoglycan which makes the *Bacillus* more absorbent and eco-friendly.

**Key words:** Biosurfactant, *Bacillus*, Drop collapse method, Gram positive bacteria, Hydrocarbons, Oil displacement Assay, Oil spill contamination, Peptidoglycan.

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## INTRODUCTION

Surfactants are amphiphilic molecules with hydrophilic and hydrophobic moieties; hence they contain both the water-insoluble and water-soluble component[1]. It has the ability to orient at the interface of two immiscible compounds as it lowers the surface and interfacial tension of the fluids forming micro-emulsion and hydrocarbon and can solubilize in water or where water can be solubilized in hydrocarbon. The tails of most surfactants are fairly similar which consist of a hydrocarbon chain, and can be branched, linear, or aromatic. Bio-surfactants are surface-active compounds, heterogeneous groups of surface-active molecules produced by microorganisms, which either adhere to the cell surface or are secreted extracellularly in the growth medium[2]. Bio-surfactants contain two parts: a polar (hydrophilic) moiety and a nonpolar (hydrophobic) group. A hydrophilic group of the bio-surfactant consists of mono-, oligo- or polysaccharides, peptides, or proteins, and a hydrophobic moiety which contains saturated, unsaturated, and hydroxylated fatty acids or fatty alcohols. The characteristic feature of bio-surfactant could also be hydrophilic-lipophilic balance (HLB) which specifies the portion of hydrophilic and hydrophobic[3]. Bio-surfactants had been isolated and characterized as glycolipids, phospholipids, lipopeptides, natural lipids, fatty acids, lipopolysaccharide, and other fully characterized. The bio-surfactants are majorly synthesized by microorganisms, grown on water-immiscible hydrocarbons, but some are produced on such water-soluble substrates as glucose, glycerol, and ethanol[4]. They are eco-friendly, biodegradable, less toxic, and non-hazardous in nature. They have better foaming properties and better selectivity. They are active at extreme temperatures, pH, salinity and can also be produced from industrial wastes and from by-products. This feature makes cheap production of biosurfactants possible and allows utilizing waste products and reducing their polluting effect at the same time[5]. Therefore, the aim of the study was to isolate the bacteria that produces biosurfactants and to identify the type of bacteria that produces biosurfactants. The compounds that are derived from microbes are often easily degraded in comparison to synthetic surfactants and are suitable for environmental applications like bioremediation and biosorption. As there is an increase in environmental concern, it forces the people to seek alternative products like bio surfactants.[6] One of the promising ways to improve bioremediation in hydrocarbon-contaminated environments is the use of bio-surfactants. Hydrocarbon bioremediation can be improved through two mechanisms. The first mechanism involves an increase in microbial substrate bioavailability, and the second mechanism involves interaction with the cell surface. This increases surface hydrophobicity and makes it easier for hydrophobic substrates to bind to bacterial cells.[7] by reducing surface and interfacial tensions; bio-surfactants increases the surface area of insoluble compounds, improving hydrocarbon mobility and bioavailability[8]. As a result, bio surfactants promote biodegradation and promote hydrocarbon removal. The addition of bio surfactants is often expected to increase hydrocarbon biodegradation through recruitment, solubilisation, or emulsification[9]. As most of the research studies done until now, have been performed and identified only with rhamnolipids that were obtained from P*seudomonas* when compared to other biosurfactants. Only less information has been available when it comes to concerning the influence of sand/soil components on the remediation process that was done with the biosurfactant. Due to this, the other biosurfactants and the organisms that are producing the biosurfactants are in need to be identified and investigated as the organisms may have the possibilities of having more promising properties. So in this study, the microorganisms have been collected as whole as a soil sample and from that, the bacteria was isolated as the soil may contain many microbes. Bacteria were isolated from the soil sample collected in Ennore port, because the place has been contaminated by oil spills. Once the samples were collected and the specific growth medium was provided to enrich the growth of bacteria. The bacteria were identified by a number of assays performed.

## MATERIALS AND METHODS

100 ml of Minimal medium (MM) and Mineral salt medium (MSM) each into 250ml of conical flasks were taken. The composition of MM and MSM were prepared accordingly[10].
11 test tubes with 10 ml of sterile distilled water was used for serial dilution. MM and MSM were poured in 12 test tubes for inoculation. 6 Test tubes used for the slants. 300ml of Nutrient agar prepared in a 500ml conical flask. Six of 100ml conical flasks were used to prepare MM and MSM for inoculation of colonies . About 3.9g of Nutrient broth and 3g of Agar was mixed in the 300ml of distilled water for the preparation of Nutrient agar. 10 disposable petri plates were used to inoculate serial dilution. 9 disposable petri plates were used for streaking colonies. Micro centrifuge tubes were used for the isolation of supernatant and test for the production of biosurfactants.[10]

## METHODOLOGY

### *Isolation of Sample*

Soil samples were collected in 3 sterile bags and labelled as A, B, C from an oil spill contaminated site located at Kamarajar port in Ennore, Chennai, Tamil Nadu, India. One gram of soil was mixed in 100 ml sterile water. 1ml of soil suspension was added in two different 500ml Erlenmeyer flasks each containing 200ml of Minimal Medium (MM) and Mineral Salt Medium (MSM). [10]

### *Production Medium*

After sterilization by autoclave, solution A and solution B of Minimal medium (MM) were mixed and the trace elements were mixed with Mineral Salt Medium. 1ml of soil suspensions were added to two 250ml Erlenmeyer flask, each containing 100 ml of MM and MSM. 1 ml of filtered hexadecane was used as substrate (precursor) was added to MM and MSM to enrich biosurfactant producing microorganisms. The MM and MSM were inoculated in the laminar hood and incubated it in the shaker of having a 160 rev $min^{-1}$ for about 10 days. The temperature in the shaker should be maintained at 37° C. All the above procedures were done in sterile condition.

### *Serial Dilution Of Culture In Enrichment Broth (MSM)*

After 10 days of incubation, 1ml of the sample in enrichment culture MSM was serially diluted in the ten folds ratio of $10^{-10}$ in 10 test tubes. The sterilized nutrient agar was prepared and poured in 10 nutrient agar plates. The six active colonies A, B, C, D, E and F were selected for production of biosurfactants. The sterilized nutrient agar was poured in 10 Petri plates. 1ml of the serially diluted solutions of each test tube were micro pipetted and inoculated over the prepared nutrient agar plates.

The samples were spread over the agar plates with the spreader in a sterile condition and incubated overnight at 37°C. Numerous colonies were grown.

### *Isolation of Bacteria Producing Biosurfactant*

The colonies which were active that were marked as A, B, C, D, E and F , isolated and streaked on the different agar plates with the sterile inoculating loop and kept in the refrigerator at 4°C as stock culture The colonies from the stock culture were isolated and inoculated into a 6 test tube containing 3ml of production medium, MM and MSM each and marked respectively in a sterile condition and incubated for 3 consecutive days in shaker at 37° C and 160 rev $min^{-1}$. After each 24 hours, 1ml of MM and MSM of each colony was micro pipetted and centrifuged for 10,000 rpm at 4°C. The supernatant was collected and tested for the presence of surface active agents by the tests: [10]

### *Tests for The Bio Surfactant Production*

### *Drops Collapse Method*

A paraffin sheet was marked with the controls and colonies A, B, C, D, E and F of MM and MSM enrichment broth. Water was taken as negative control as it remains the same which does not contain any surface active substances whereas 0.1mg of SDS in 1ml water was taken as a positive control as it spreads in the paraffin sheet. 10µl of positive control, negative control and supernatant of cultured broth were micro pipetted and poured into the paraffin sheet in the marked respective places. 10µl of drop were spread over the paraffin sheet indicated the presence of bio surfactant. If the drop remains the same, then the bacterial colony does not produce the bio surfactant. The test was taken after the incubation of the cultured broth in 24 hours, 48 hours and 72 hours.[10]

### *Oil Displacement Assay*

About **20 ml** of water was taken in a petri plate. 1000µl of paraffin oil was micro pipetted and poured into the petri plate containing water which forms a layer in the surface of water. 1000µl of supernatant was added on the layer of paraffin oil. The ability of oil displaced after adding the supernatant which determines the bacterial colony produced the biosurfactant. If the oil was not displaced, then there is no biosurfactant producing bacteria in the cultured broth.[10]

### *Gram Staining*

After the tests of the bacteria for the production of biosurfactant, the biosurfactant producing bacterial colonies were collected and stained by Gram staining method for the biochemical test to identify whether the bacterial colonies are gram positive or gram negative.[10]

### *Preparation of The Glass Microscopic Slide*

Oil free slides were essential for the preparation of microbial smears. Grease or oil from the fingers on the slides was removed by washing the slides with soap and water. The slides were wiped with spirit or alcohol. After cleaning, the slides were air-dried until ready for use.[10]

### *Preparation Of The Smear*

With a sterile cooled loop, a drop of sterile water or saline solution was placed on the slide. The loop was sterilized and cooled again to pick up a very small sample of a bacterial colony and it was gently stirred into the drop of water/saline on the slide to create an emulsion. Smears were typically done with only a small amount of bacterial culture.[10]

### *Heat Fixing*

Thermal fixation is known to kill bacteria in the smear, attach the smear firmly to the slide, and allow the specimen to pick up dirt more easily. The smear was air dried. The smear was air-dried, and stained by holding one end of the slide and passing the entire slide through a Bunsen burner flame 2-3 times with the smear side up.[10]

### *Identifying The Nature of Bacteria*

Under the microscope, the gram positive bacteria observed as a violet stain by the methyl violet due to thick layer of peptidoglycan with numerous teichoic acid cross linking which resists the decolourization. Whereas the gram negative bacteria observed as a pink stain due to the safranin and due to the thin layers of peptidoglycan, one to three layers deep with a slightly different structure than the peptidoglycan of gram-positive cells. With ethanol treatment, the excess stains were washed out.[10]

## RESULTS

A numerous bacterial colonies were formed. From the plates, prominent single bacterial colonies with definite round shape were produced in the ratio of $10^{-4}$, $10^{-5}$ and $10^{-6}$ over the agar plates. The particular colonies with different characteristics were isolated with the help of an inoculation loop in a sterile condition. Those single active colonies A, B, C, D, E and F were selected and marked (Fig. 1).

### *Morphology of The Selected Colonies*

### *The morphology of the selected colonies*

***Shape of Colony*** : All the selected colonies A, B, C, D, E and F were round shaped.

***Chromogenesis:*** The colour of all the six colonies was white.
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***Opacity of Colony:*** The selected colonies were opaque, not transparent or clear.

***Elevation of Colony:*** Colonies were slightly raised from the agar.

***Surfaceo of Colony:*** Smooth and glistening.

***Consistency Or Texture*:** Butyrous, viscid which sticks to loop and is hard to get off.

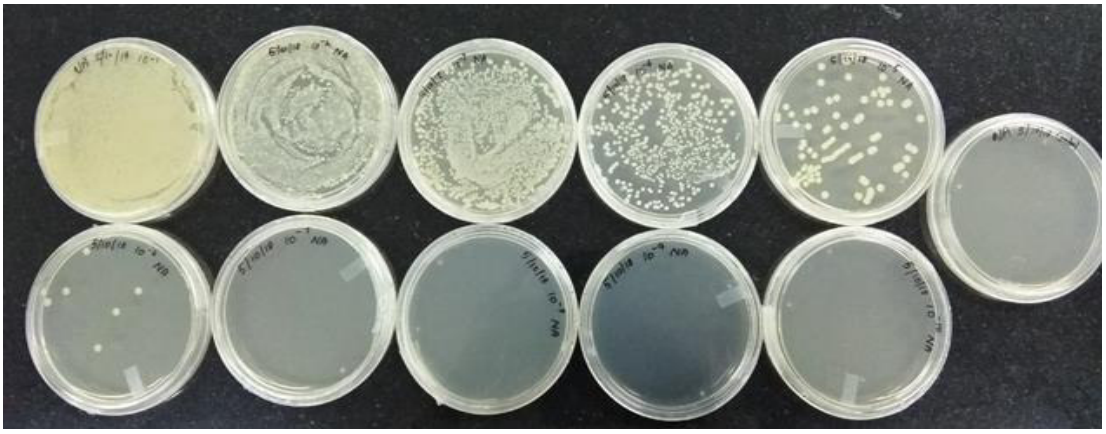

Fig.1 Bacterial colonies after incubation(24hr or 48hr)

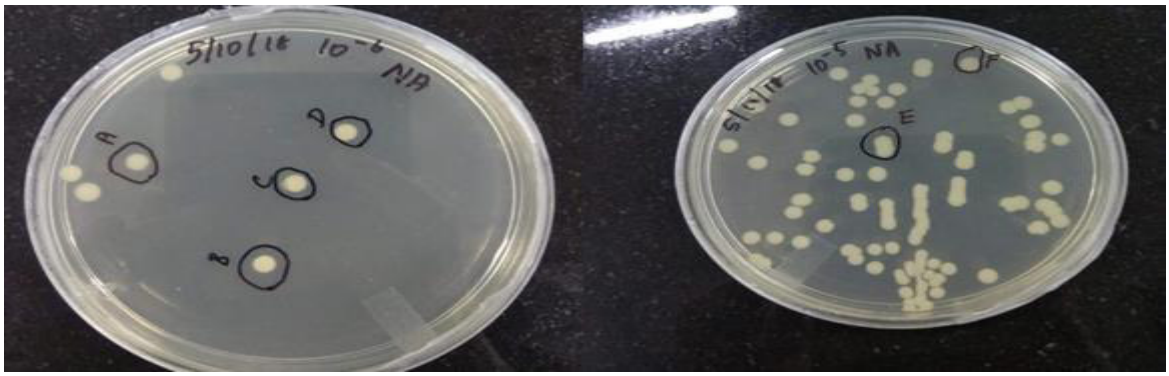

Fig.2: Bacterial colonies in ratio of $10^{-6}$ and $10^{-5}$

*From (Fig. 2) the six active colonies were selected for production of biosurfactants. A, B, C and D bacterial colonies were selected from $10^{-6}$ and E, F colonies selected from $10^{-5}$.*

**Bacterial production**

After 24 hours, 1 ml of MM and MSM culture filtrate were centrifuged. The supernatant was collected and tested for the presence of surface active agents.

**Tests for the biosurfactant production**

**DROPS COLLAPSE METHOD**

A paraffin sheet was marked with the controls and colonies A, B, C, D, E and F. Water was taken as negative control as it remains the same which do not contain any surface active substances, whereas SDS solution was taken as a positive control as it has surfactant property in the paraffin sheet. 10µl of positive control, negative control and supernatant of cultured broths were micro pipetted and poured into the paraffin sheet in the marked respective places. A 10µl of drop spread over the paraffin sheet indicated the presence of biosurfactant. The test was taken after the incubation of the cultured broth in 24 hours, 48 hours and 72 hours[1]

**Table 4: Drop collapse test for the bacterial production of biosurfactants (after 24 hours)**

| Supernatant | MM | MSM |
|---|---|---|
| A | -ve | -ve |
| B | -ve | -ve |
| C | -ve | -ve |
| D | -ve | -ve |
| E | -ve | -ve |
| F | -ve | +ve |


Fig.3: Drop collapse test for the bacterial production of biosurfactants (after 24 hours)

**F showing the presence of biosurfactants.**

After 24 hours of incubation in the shaker, table 4 shows that only the supernatant of F colony in Mineral salt medium spreaded slowly over the paraffin sheet (fig 3).

**Table 4: Drop collapse test (after 48 hours)**

| Supernatant | MM | MSM |
|---|---|---|
| A | -ve | +ve |
| B | -ve | -ve |
| C | -ve | -ve |
| D | -ve | -ve |
| E | -ve | -ve |
| F | -ve | +ve |


***The next day, precisely after incubation of 48 hours (table 4) the supernatant of A and F colonies in Mineral salt medium showed the slight movement of spreading on the paraffin paper (Fig.4).***

**Fig.4: Drop collapse test (after 48 hours) showing in supernatant A and F.**

**Table 5: Drop collapse test (after 72 hours)**

| Supernatant | MM | MSM |
|---|---|---|
| A | -ve | +ve |
| B | -ve | +ve |
| C | -ve | -ve |
| D | -ve | -ve |
| E | -ve | -ve |
| F | -ve | +ve |

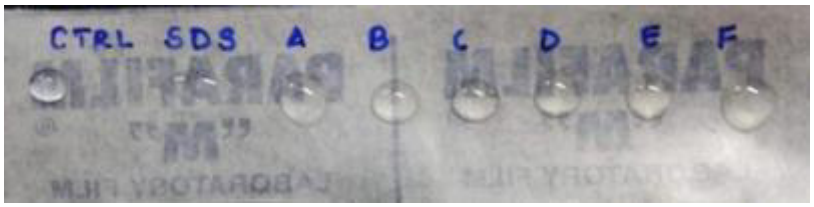


**Fig. 5: Drop collapse test (after 72 hours) showing in colonies of A, B and F.**

Then the next day after 72 hours (table 5), the supernatant of the colonies A, B and F of Mineral salt medium were spread in the paraffin sheet (fig 5). By this drop collapse test, the bacterial colonies of A, B and F in the Mineral salt medium (MSM) produces the bio-surfactant. As the colonies in the Minimal medium (MM) did not show any spreading movement, it was concluded that the bacteria in the MM do not produce the bio-surfactant. since, it consists of less minerals below than the required amount for the production of biosurfactants.

## OIL DISPLACEMENT ASSAY

20 ml of water is taken in a petri plate.1000 µl of paraffin oil is micro pipetted and poured into the petri plate containing water to form a layer on the surface of water. 1000µl of supernatant which collected after 24 hours of incubation was added on the layer of paraffin oil. F colony from the Mineral salt medium has slightly displaced by forming a small bubble on the layer of the paraffin oil in water[10]. After 72 hours, the supernatant of the colony A, B and F in MSM was collected and tested for the oil displacement. The supernatant slightly displaced the paraffin oil layer in the petri plate of water forming a small bubble on the layer of the paraffin oil in water which was shown in (fig 6).

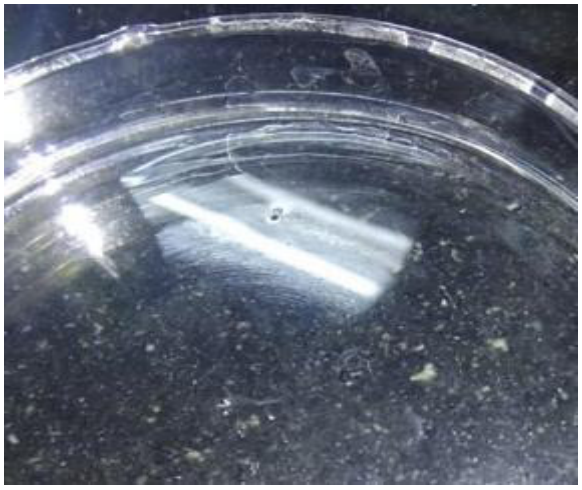

**Fig.6: Oil displacement assay (after 24 hours) of supernatant F in petri plate of water and paraffin oil**

**Biochemical Test of Biosurfactant Producing Bacteria**

***Gram Staining***

The staining was done for the bacterial colonies A, B and F which showed the production of bacteria by the above tests were stained by gram staining method and observed under the microscope. Thus only the three colonies were isolated from the stock plate and stained by the gram staining technique.

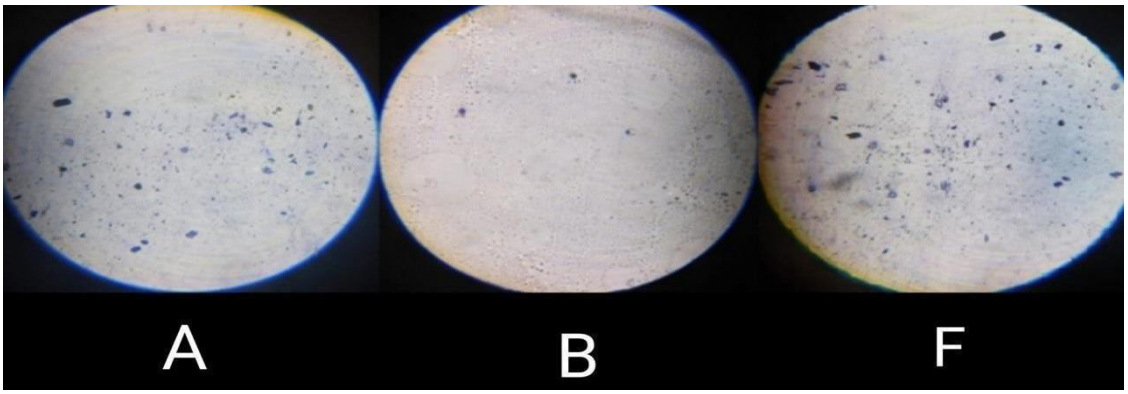


**Fig.7: Gram stained bacterial colony A, B, F**

By observing (fig 7) the slide with bacterial colonies A, B and F under microscope were with the **blue colour** stained and **rod shaped bacteria**. The gram positive bacteria observed the blue stain from the methyl violet due to thick layer of peptidoglycan with numerous teichoic acid cross linking which resists the decolorization. Hence all the bio-surfactant producing bacterial colonies A, B and F were Gram positive and it may be *Bacillus sp.* which produces the lipoprotein bio-surfactant known as SURFACTIN.

## DISCUSSION

Because of the increased need for environmentally acceptable products, biosurfactants are being used more frequently in numerous industrial sectors. Biosurfactants that are produced by microorganisms are very much helpful in the process of bioremediation. A study has analyzed biosurfactant-producing bacteria from the floodplain and highland Amazon soils in Brazil in this study. Drop collapse, oil dispersion, emulsification, and surface tension tests were used to analyze the isolated extracts which are used to identify the selected bacteria[11]. The strains that produce biosurfactants are known to be of terrestrial origin. However, there are few findings on marine surfactant molecules. Although E.Coli did not produce extracellular biosurfactants, it act as a biosurfactant. Surfactin, a lipo heptapeptide produced by *Bacillus subtilis,* is one of the most effective biosurfactants known; it can lower water's surface tension by up to 27 mN/m, has critical micelle concentrations as low and has high emulsifying activity; it also has antimicrobial, antiviral, and anti-tumor properties. S. aureus was also discovered as a possible producer. Bacillus had the lowest surface tension, showing that it has a strong surface tension-reducing ability. The molecular structure of the biosurfactant produced has a big impact on its ability to lower surface ten sion[12]. The good qualities of biosurfactant produced by sponge-associated bacteria suggest that it could be used in marine bioremediation efforts, and they adds to already impressive biotechnological capabilities of these bacterial populations[13]. A study demonstrated the isolation and identification of novel indigenous biosurfactant-producing bacterial strains from the formation water of Assam oil reservoir. The experiments aimed to optimize the carbon source percentage, pH and temperature to maximize the surface production in terms of surface tension reduction which reduced to 29.85 mN/m. Like our study, an early research has concluded that the isolated strain Bacillus subtilis MG495086 was found suitable for the both ex-situ and in-situ applications [14]. *B. licheniformis* was isolated from soil as a

powerful bacteria. In carbohydrate-based minimum media, the isolated bacterial strain was capable of producing biosurfactant within 24 hours. The biosurfactant was identified as a lipopeptide (lichenysin-A) and demonstrated outstanding surface and interfacial tension activity, as well as changing the wettability to be more water-wet[15]. A study has been discovered to be extremely stable in the face of harsh environmental conditions. This study also provides a detailed explanation of the sand-packed column's design and operation as a quick, accurate, and cost-effective method for determining the potential of biosurfactants. The bacterial isolate was shown to recover 32.10 percent of the entrapped crude oil from the sand-packed column[16]. As a result, the separated bacteria and biosurfactants developed could be extremely useful in environmental applications such as microbial enhanced oil recovery and hydrocarbon removal from a polluted environment. In the presence of crude oil as a substrate, Bacillus subtilis A1 produced large amounts of biosurfactant and degradative enzymes. pH 7.0, temperature of 40°C, sucrose and yeast extract as the optimum carbon and nitrogen sources, respectively, were validated as optimal growing conditions for maximum biosurfactant synthesis. The synthesised biosurfactant was lipopeptide in origin and displayed high emulsification activity under optimal circumstances. The crude oil biodegradation efficiency was 87 percent, which was linked to significant biosurfactant, alkane hydroxylase, and alcohol dehydrogenase enzyme production. These findings show that *B. subtilis* A1 is a highly efficient crude oil degrader. The crude oil hydrocarbon's bio-availability can be attributed to their ability to synthesise biosurfactants and emulsify them, as well as their biosurfactant synthesis and emulsification capacities[17]. From 50 terrestrial samples taken in a study which was done in regions contaminated with petroleum in several Bangkok districts, biosurfactant-producing bacteria were identified. The potential of bacteria to produce biosurfactants was examined using an oil displacement agar plate and the emulsification index method. The samples yielded a total of 42 biosurfactant-producing microorganisms. *Serratia marcescens* was found as the top biosurfactant generating isolate with the maximum oil displacement and emulsification activity based on microscopic and biochemical research[18]. In a study, *Bacillus subtilis* and *Bacillus cereus* are bacteria that create a lot of biosurfactants. Because *B. cereus* was collected from a contaminated environment, it was expected to create biosurfactants. B. subtilis, on the other hand, was isolated from a non-contaminated water sample and demonstrated biosurfactant activity[19]. The fact that the biosurfactant activity of B. subtilis differed significantly between the experiment and the control suggested that the biosurfactant generating activity of *B. subtilis* may be induced in the presence of a pollutant. The isolation of the relevant gene and subsequent PCR amplification could be used to create environmentally friendly surfactants artificially. These two strains may be useful in future studies involving biosurfactants and bioremediation technologies. It is possible to deduce that B. subtilis and B. cereus can produce biosurfactants, that aid in the degradation of oil and other hydrocarbon pollutants in the environment[20]. The ability of a biosurfactant from *Bacillus sp*. to release oil from oily sand at a concentration of 0.04 mg/mL was also reported[21]. The ability of biosurfactants to solubilize and remove hydrocarbon pollutants from polluted soil has also been established. Rhamnolipid biosurfactants from *Pseudomonas aeruginosa* were studied for their ability to extract hydrocarbons from sandy-loam soil and silt-loam soil[22]. It centred on isolating biosurfactant-producing bacteria from environmental samples (contaminated soil and uncontaminated water) and evaluating their potential using several conventional methods. The potential of isolates to produce biosurfactant was tested using oil displacement and emulsification index methods. Diesel and engine oil were also used as hydrocarbon sources in studies[23]. In another study, 22 bacterial isolates were obtained from 8 distinct sites using a culture-dependent approach. *Bacillus* and *Pseudomonas* were the most common bacteria, accounting for 22.73 percent of all cases. The isolates were subjected to traditional biosurfactant screening assays, both subjectively and quantitatively (drop collapse and microplate assay) (oil speading and emulsification activity). *Bacillus* and *Pseudomonas sp.* were found to be positive in all tests, with a clearing zone of 4mm and emulsification capacities of 51.61 percent and 53.13 percent, respectively. This demonstrated their ability to create biosurfactants that lowered interfacial and surface tension, resulting in an increase in oil solubility and emulsification[24]. *Bacillus subtilis* and *Bacillus cereus*, which were isolated from water and soil, respectively, were discovered to have the properties of biosurfactants as mentioned in this study[25].

## CONCLUSION

In the present study, the presence of biosurfactant producing bacteria in the oil spill contaminated soil was successfully identified by the drop collapse test and oil displacement assay. By Gram staining, the bacteria that produce biosurfactants were found to be gram positive and the bacteria *Bacillus sp*. which produces surfactin. This study will be used to know the basic procedure to isolate and identify the bacteria that produces biosurfactant. And the further studies should be done on identifying the biosurfactant bacteria at species level and molecular level more specifically and aided by proteomics genetics, and metabolic studies, will undoubtedly provide a more thorough understanding of their structure and function, enhancing the knowledge required for broad applicability of biosurfactant.

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## AUTHORS CONTRIBUTION STATEMENT

This study was initially designed and developed by Ms. Madhumitha Vijayanand, inspired from the work done by Dr.Mukesh Doble, and collaborated with Sree Balaji Medical College and hospital. The methodology and tests were performed in the laboratory by Ms. Madhumitha Vijayanand and by Ms. Arthi Arul. Dr. Archana and Dr.Aishwarya J Ramalingam helped us in framing the article.

## CONFLICTS OF INTEREST

Conflicts of interest declared none.

## REFERENCE


1. Abu-Ruwaida, A., Banat, I., Haditirto, S., Salem, A. and Kadri, M., 1991. Isolation of biosurfactant-producing bacteria, product characterization, and evaluation. *Acta Biotechnologica*, 11(4), pp.315-324.
2. Agarwal, P. and Sharma, D., 2009. Studies on the Production of Biosurfactant for the Microbial Enhanced Oil Recovery by Using Bacteria Isolated from Oil Contaminated Wet Soil. *Petroleum Science and Technology*, 27(16), pp.1880-1893.
3. Banat, I., 1995. Biosurfactants production and possible uses in microbial enhanced oil recovery and oil pollution remediation: A review. *Bioresource Technology*, 51(1), pp.1-12.
4. Cameotra, S. and Makkar, R., 2004. Recent applications of biosurfactants as biological and immunological molecules. *Current Opinion in Microbiology*, 7(3), pp.262-266.
5. Desai, J. and Banat, I., 1997. Microbial production of surfactants and their commercial potential. *Microbiology and Molecular Biology Reviews*, 61(1), pp.47-64.
6. Md, F., 2012. Biosurfactant: Production and Application. *Journal of Petroleum & Environmental Biotechnology*, 03(04).
7. Fiechter, A., 1992. Biosurfactants: moving towards industrial application. *Trends in Biotechnology*, 10, pp.208-217.
8. Gharaei-Fa, E., 2010. Biosurfactants in Pharmaceutical Industry (A Mini-Review). *American Journal of Drug Discovery and Development*, 1(1), pp.58-69.
9. Kosaric, N., Gray, N.C.C.& Cairns, W.L.(1987). Biotechnology and the surfactant industry. In *Biosurfactants and Biotechnology,* Surfactant Science Series, Vol. 25, eds Kosaric, N., Cairns, W.L. and Gray, N.C.C. pp. 1–19
10. Arutchelvi, J. and Doble, M., 2010. Characterization of glycolipid biosurfactant from Pseudomonas aeruginosa CPCL isolated from petroleum-contaminated soil. *Letters in Applied Microbiology*, p.no-no.
11. Oliveira, E., Sales, V., Andrade, M., Zilli, J., Borges, W. and Souza, T., 2021. Isolation and Characterization of Biosurfactant-Producing Bacteria from Amapaense Amazon Soils. *International Journal of Microbiology*, 2021, pp.1-11.
12. Nayarisseri, A., Singh, P. and Singh, S., 2018. Screening, isolation and characterization of biosurfactant producing Bacillus subtilis strain ANSKLAB03. *Bioinformation*, 14(06), pp.304-314.
13. Rizzo, C., Syldatk, C., Hausmann, R., Gerçe, B., Longo, C., Papale, M., Conte, A., De Domenico, E., Michaud, L. and Lo Giudice, A., 2018. The demospongeHalichondria (Halichondria) panicea(Pallas, 1766) as a novel source of biosurfactant-producing bacteria. *Journal of Basic Microbiology*, 58(6), pp.532-542.
14. Datta, P., Tiwari, P. and Pandey, L., 2018. Isolation and characterization of biosurfactant producing and oil degrading Bacillus subtilis MG495086 from formation water of Assam oil reservoir and its suitability for enhanced oil recovery. *Bioresource Technology*, 270, pp.439-448.
15. Ali, N., Wang, F., Xu, B., Safdar, B., Ullah, A., Naveed, M., Wang, C. and Rashid, M., 2019. Production and Application of Biosurfactant Produced by Bacillus licheniformis Ali5 in Enhanced Oil Recovery and Motor Oil Removal from Contaminated Sand. *Molecules*, 24(24), p.4448.
16. Sakthipriya, N., Doble, M. and Sangwai, J., 2015. Action of biosurfactant producing thermophilic Bacillus subtilis on waxy crude oil and long chain paraffins. *International Biodeterioration & Biodegradation*, 105, pp.168-177.
17. Sharma, S. and Pandey, L., 2020. Production of biosurfactant by Bacillus subtilis RSL-2 isolated from sludge and biosurfactant mediated degradation of oil. *Bioresource Technology*, 307, p.123261.
18. Rajesh, M., Samundeeswari, M. and Archana, B., 2017. Isolation of Biosurfactant Producing Bacteria from Garbage Soil. *Journal of Applied & Environmental Microbiology*, 5(2), pp.74-78.
19. Batool, R., Ayub, S. and Akbar, I., 2017. Isolation of biosurfactant producing bacteria from petroleum contaminated sites and their characterization. *Soil & Environment*, 36(01), pp.35-44.
20. Parthipan P, Preetham E, Machuca L, Rahman P, Murugan K, Rajasekar A. Biosurfactant and Degradative Enzymes Mediated Crude Oil Degradation by Bacterium Bacillus subtilis A1. Frontiers in Microbiology. 2017;8.
21. Ebadi A, Olamaee M, Khoshkholgh Sima N, Ghorbani Nasrabadi R, Hashemi M. Isolation and Characterization of Biosurfactant Producing and Crude Oil Degrading Bacteria from Oil Contaminated Soils. Iranian Journal of Science and Technology, Transactions A: Science. 2017;42(3):1149-1156.
22. Ibrahim Ewida A, Salah El-din Mohamed W. Isolation and Characterization of Biosurfactant Producing Bacteria from Oil-Contaminated Water. Biosciences, Biotechnology Research Asia. 2019;16(04):833-841.
23. 4. Saikia R, Deka S, Deka M, Banat I. Isolation of biosurfactant-producing Pseudomonas aeruginosa RS29 from oil-contaminated soil and evaluation of different nitrogen sources in biosurfactant production. Annals of Microbiology. 2011;62(2):753-763.
24. Zhang X, Xu D, Zhu C, Lundaa T, Scherr K. Isolation and identification of biosurfactant producing and crude oil degrading Pseudomonas aeruginosa strains. Chemical Engineering Journal. 2012;209:138-146.
25. 25.Janaki S, Thenmozhi S, Muthumari R. A study on Hydrocarbon Degradation by Biosurfactant Producing Bacillus cereus in Oil Contaminated Soil Samples. International Journal of Life-Sciences Scientific Research. 2016;2(4).