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IN-VITRO STUDIES ON OIL DEGRADING BACTERIA ISOLATED FROM OIL CONTAMINATED SOIL OF VANIYAMBADI AND AMBUR AREAS OF VELLORE DISTRICT

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Keywords:

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ABSTRACT: The oil polluted soil has many hydrocarbons which can be degraded by microbes. These oil degrading microbes were isolated from the oil contaminated soil through plate and dilution technique. The oil contaminated soils were collected from the oil spilled places of Vaniyambadi and Ambur areas of Vellore District, Tamil nadu, India. Through the biochemical tests, the isolated bacterial strains were identified as *Pseudomonas aeruginosa* and *Bacillus subtilis*, the strains were further confirmed by starch hydrolysis test and by growing on cetrimide agar. It is screened for the biosurfactant production through drop collapsing test and Emulsification Index (E_{24}) using diesel, petrol, kerosene and tween 20. Biosurfactants are amphiphilic compounds which reduce surface and interfacial tension. Here, *Pseudomonas aeruginosa* had shown higher biosurfactant activity, when compared to *Bacillus subtilis*.

INTRODUCTION: Pollution of the soil system with the excess amount of chemicals or other substances resulted in the turndown of its fertility and which resulted in the poor yield of crops. The soil pollution is mainly caused by the accidental oil spills, agricultural activities, waste disposal and industrial outlets which mix with the soil and spoil the nature of the soil. This problem may be overcome by bioremediation. Bioremediation is the biological process to degrade or transform contaminants or impairments from the soil and water.

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This process totally depends on microbes, which carry out their metabolic process and use this chemical contaminants as an energy source and converts contaminants into harmless or less toxic products [1].

Biosurfactants are amphiphilic compounds produced on living surfaces, mostly microbial cell surfaces or excreted extracellularly and contain hydrophobic and hydrophilic moieties that reduce surface tension and interfacial tension between individual molecules at the surface and interface respectively [2]. Biosurfactants are grouped into six major classes based on the producing microorganism. These classes are glycolipids, phospholipids, polysaccharide lipid complexes, lipoprotein-lipopeptides, hydroxylated and cross linked fatty acids and the complete cell surface [3]. Microorganisms exhibit emulsifying activity by producing biosurfactants and utilize contaminants as substrate and mineralize them into harmless

products. The present study was focused on the isolation of oil degrading bacteria and screening the biosurfactant activity of the isolated bacterial strains.

MATERIALS AND METHODS:

Collection of soil sample:

The oil contaminated soil samples were collected from the oil spilled places of Vaniyambadi and Ambur areas of Vellore District, Tamil nadu, India.

Isolation of bacteria from the soil:

The bacterial strains were isolated from the soil through serial dilution technique and nutrient agar as a media. Staining techniques and biochemical tests were performed to identify the isolated bacterial strains[4]. These two strains were further confirmed through starch hydrolysis test and growth of the streaked culture on cetrimide agar.

Screening for Biosurfactant Activity:

Biosurfactant activity of the isolated bacterial strains were detected by using emulsification stability testand Drop collapsing test using four different oil sources namely Petrol, Diesel, Kerosene and Tween 20.

Emulsification Test:

The emulsifying capacity was evaluated by an emulsification index E_{24}[5]. The emulsifying activity of the culture supernatant was estimated by adding 3ml of the supernatant and adding equal volume of oil of interest to the same tube. The tube was vortexed for 10 seconds to 1 minute. Then held stationary for 1 minute and then visually examined for turbidity of stable emulsion. Emulsifying power was measured by vortexing equal volumes of the centrifuged culture with the oil for 1 minute and determining the percentage of volume to settle for 24 hours and the height of the emulsion was measured[6].

E_{24} (%) = (Height of emulsion formed / Total height of solution) X 100

Drop collapsing test:

2µl of oil was added to each well of plate lid. The lid was equilibrated for 1 hour at room temperature and then 5 microliter of the culture supernatant was added to surface of oil. The shape of the drop on the oil surface was inspected after 1 minute. Biosurfactant producing cultures giving flat drops were scored as 'positive'. Those cultures that have rounded drop were scored as 'negative' indicative of the lack of biosurfactant production[7].

RESULTS AND DISCUSSION: Totally eight oil contaminated soil samples were collected from the oil spilled places like Petrol bunk, Diesel bunk, Mechanical shop and Coconut oil spills. From these samples, the bacterial strains were isolated by using nutrient agar plate and serial dilution techniques. **Table 1** shows the presence (+) and Absence (-) of the growth of bacterial colonies **(Fig. 1**) from the different soil samples at various dilution conditions. Here, most of the all eight samples have shown better bacterial growth which can be further isolated and subcultured.

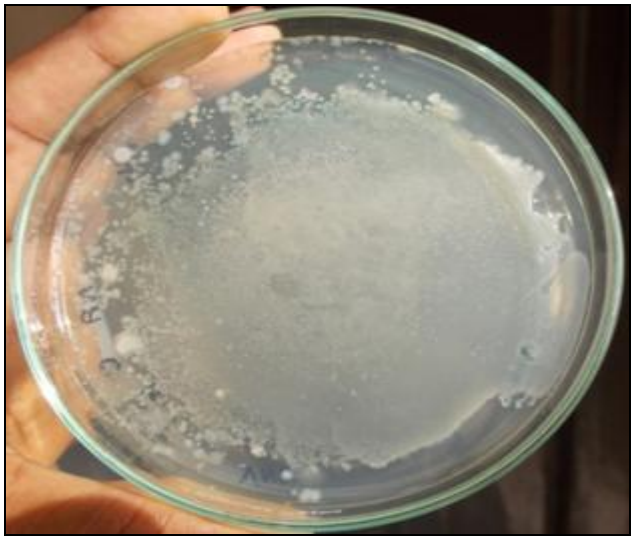

FIG. 1: BACTERIAL COLONIES

TABLE 1: ISOLATION OF OIL DEGRADING BACTERIA FROM THE OIL CONTAMINATED SOILS

S.No.	SAMPLE	Concentrations (µg/ml)				
		1000	500	250	125	62.5
1.	P_{VNB}	+	+	+	+	+
2.	D_{VNB}	+	+	+	+	+
3.	M_{VNB}	+	+	+	+	+
4.	C_{VNB}	+	+	+	+	-
5.	P_{AMB}	+	+	+	+	+
6.	D_{AMB}	+	+	+	+	+
7.	M_{AMB}	+	+	+	+	+
8.	C_{AMB}	+	+	+	+	+

(+) – Presence, (-) – Absence, P - Petrol, D - Diesel, M - Mechanical Shop,
C - Coconut oil, VNB – Vaniyambadi, AMB – Ambur.

Table 2 shows the biochemical test results for the identification of bacterial isolates. Out of eight different soil samples, totally 15 bacterial strains

were isolated using nutrient agar medium and these strains were undergone for gram staining and biochemical tests like Voges-proscauer test (**Fig.2**), Urease test (**Fig. 3**), Indole production test (**Fig. 4**), Simmons citrate agar test (**Fig.5**), Triple sugar iron test (**Fig.6**), Methyl red test (**Fig.7**),Catalase and Oxidase test etc. From these test results the strains 9, 10, 11 and 12 were identified as a gram negative bacteria *Pseudomonas aeruginosa* and strains 13, 14 and 15 are identified as a gram positive bacteria *Bacillus subtilis.*

TABLE 2: BIOCHEMICAL TEST FOR THE IDENTIFICATION OF BACTERIAL ISOLATES

Bacterial Strains	VP	MR	INDOLE	SCA	UREA	TSI Slant/Butt	CATALASE	OXIDASE
Strain 1	-	-	-	+	-	K/A	-	+
Strain 2	-	-	-	-	-	A/A	-	+
Strain 3	+	-	-	+	-	A/A, G	+	-
Strain 4	+	-	-	+	-	A/A, G	+	-
Strain 5	+	-	+	+	+	A/A	+	-
Strain 6	+	+	-	-	-	K/K	+	+
Strain 7	+	-	-	+	-	A/A	-	-
Strain 8	-	+	+	+	+	A/A	+	+
Strain 9	-	-	-	+	+	K/K	+	+
Strain 10	-	-	-	+	+	K/K	+	+
Strain 11	-	-	-	+	+	K/K	+	+
Strain 12	-	-	-	+	+	K/K	+	+
Strain 13	+	+	-	+	-	K/K	+	-
Strain 14	+	+	-	+	-	K/K	+	-
Strain 15	+	+	-	+	-	K/K	+	-

VP - Voges-Proscauer, MR – Methylred test, SCA – Simmon Citrate Agar test, TSI – Triple sugar iron test

Biochemical test for the identification of bacterial isolates:

FIG. 2: VOGES-PROSCAUER (VP) TEST

FIG. 3: UREA PRODUCTION TEST

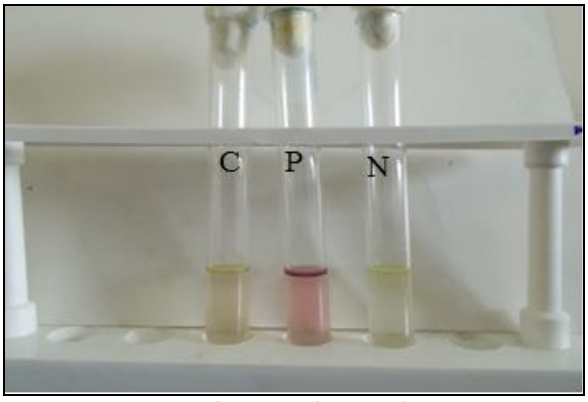

FIG. 4: INDOLE TEST

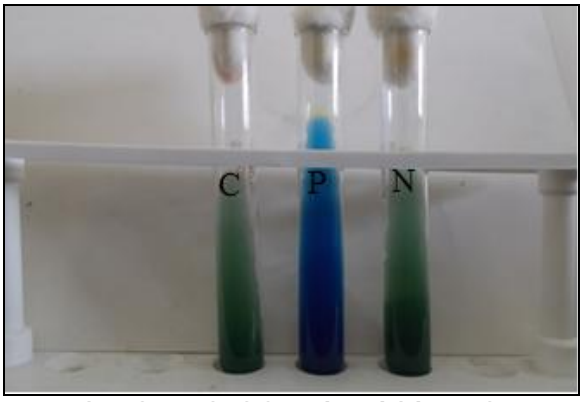

FIG. 5: SIMMONS CITRATE AGAR TEST

FIG. 6: TRIPLE SUGAR IRON (TSI) TEST

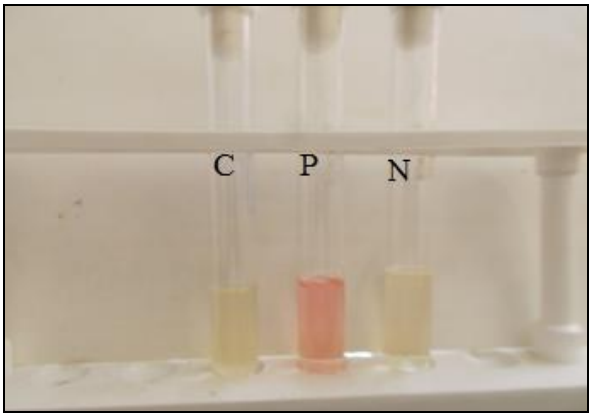

FIG.7: METHYL RED (MR) TEST

C- Control, P- Positive, N- Negative

For further confirmation of *Pseudomonas aeruginosa,* it was streaked on a Cetrimide Agar plate. Cetrimide Agar was used as a selective medium for the isolation of *Pseudomonas aeruginosa* (**Fig. 9**). This medium inhibits bacteria other than *Pseudomonas aeruginosa*. Same way *Bacillus subtilis* was streaked on starch agar medium. There is no color change found in the medium when organisms hydrolyze the starch, then the plate turns black after the addition of iodine (depending on the concentration of iodine). A clearing zone around the bacterial growth confirms that the organism streaked was *Bacillus subtilis* (**Fig.8**).

Confirmation test for the Bacterial isolates:

FIG.8: ***BACILLUS SUBTILIS***

FIG.9: ***PSEUDOMONAS AERUGINOSA***

TABLE 3: SCREENING OF EMULSIFICATION INDEX (E_{24}%) OF BACTERIAL ISOLATES

S.No:	Sample Sources	Control	S9	S10	S12	S14	S15
1.	Petrol	57.14	57.14	62.85	57.14	60.00	57.14
2.	Diesel	57.14	60.00	57.14	57.14	62.86	60.00
3.	Kerosene	57.14	62.85	57.14	57.14	60.00	57.14
4.	Tween 20	57.14	68.57	57.14	57.14	57.14	57.14

(+) – Presence, (-) – Absence.

Table 3 shows the Emulsification index (E24%) of the bacterial isolates. The confirmed bacterial strains *Pseudomonas aeruginosa* and *Bacillus subtilis* were sceened for the biosurfactant activity. Biosurfactant play a role in emulsifying hydrocarbons. Out of seven isolated bacterial

strains, based on the growth five bacterial strains were selected for the screening of biosurfactant production. These 5 bacterial isolates have shown better results against different oils (**Fig.10, 11, 12** and **13**). In this study S9, S10, S12 were *Pseudomonas aeruginosa* and S14, S15 were *Bacillus subtilis*. These two bacterial strains have the ability of emulsifying oils. S9 have shown the best of 62.85% , 68.57% against Kerosene and Tween 20 respectively. Same way S12 shown best of 62.86% against Diesel[8].

Emulsification activity of bacterial isolates on different oils:

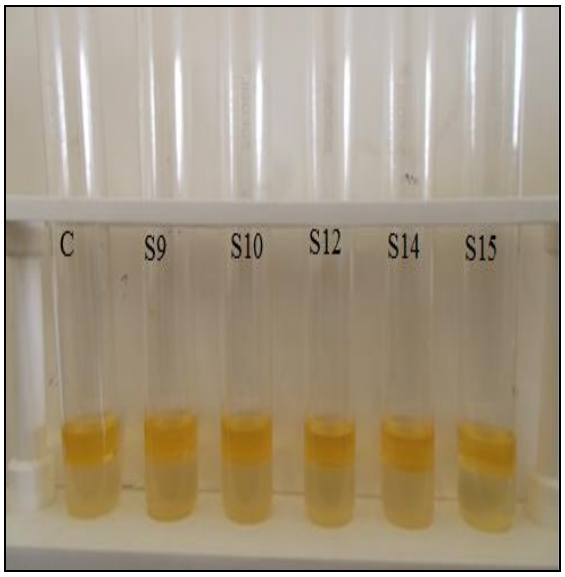

FIG.10: PETROL

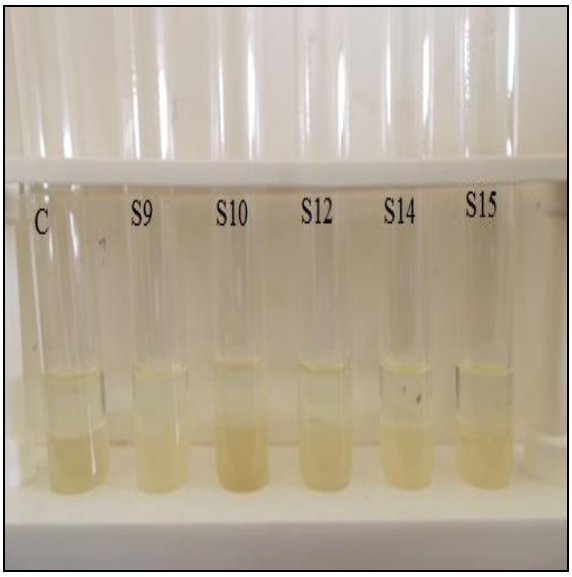

FIG.11: DIESEL

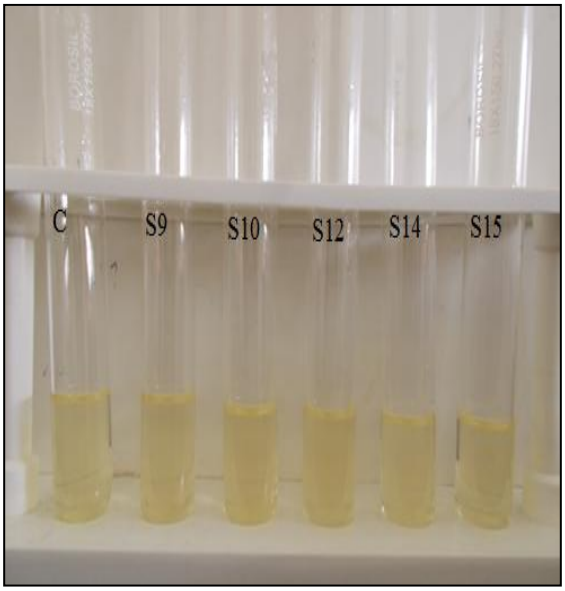

FIG. 12: TWEEN 20

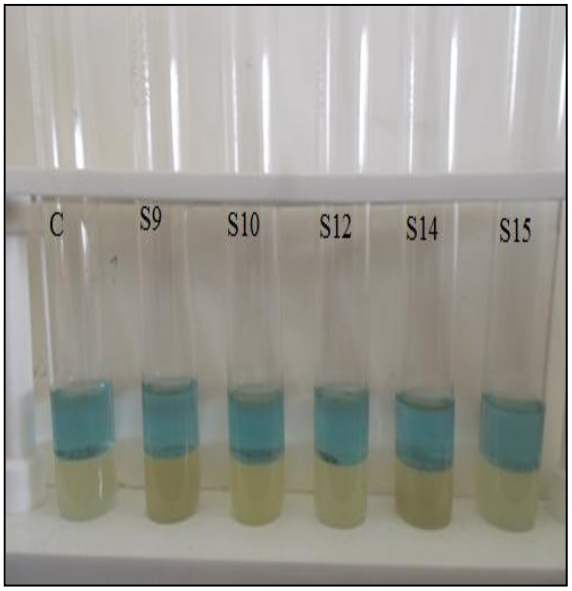

FIG. 13: KEROSENE

TABLE 4: DROP COLLAPSING TEST FOR THE BACTERIAL ISOLATES

S.no:	Sample Sources	Control	S9	S10	S12	S14	S15
1.	Petrol	-	-	-	-	+	-
2.	Diesel	-	+	-	-	+	+
3.	Kerosene	-	+	+	-	+	-
4.	Tween 20	-	+	-	-	-	-

(+) – Presence, (-) – Absence.

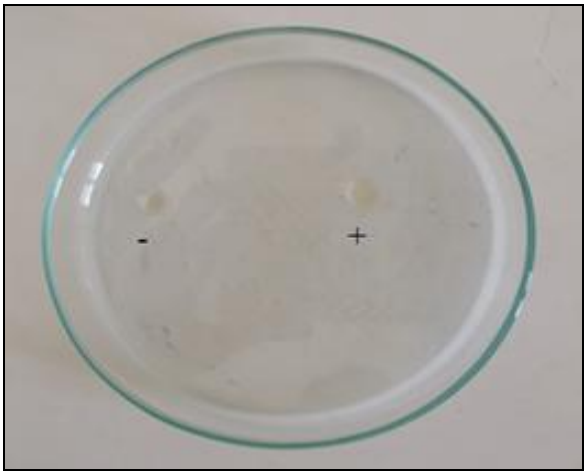

FIG.14: DROP COLLAPSING TEST FOR BACTERIAL STRAINS

Table 4 shows thedrop collapsing test for the biosurfactant production from bacterial isolates. Biosurfactant production of bacterial strains were further screened by using the qualitative drop collapsing test against different oils. Here, based on the flat drop appearance(+) and round drop appearance (-), the biosurfactant production was confirmed (**Fig.14**). Same way S9 and S14 have given the flat drop appearance against different used oils.This two test results confirms that *Pseudomonas aeruginosa* and *Bacillus subtilis* have biosurfactant acrivity[9,10].

CONCLUSION: This study concludes that the oil degrading bacteria has the biosurfactant activity. The microorganisms utilize hydrocarbons present in the oils as a source and convert that as a usable energy. It is very clear that *Pseudomonas aeruginosa* and *Bacillus subtilis* has biosurfactant activity. The biosurfactants isolated from the microbes can be used in the bioremediation process to treat the contaminated soil, waste water and gases.

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