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A Study on Microorganisms and Its Biodegradation Capability Biosurfactant

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Abstract The present study describes the potential applications of biosurfactants in the oil industry and the remediation of environmental pollution caused by oil spills where they occurred. The work was focused on the isolation of biosurfactant producing microorganisms from oil contaminated soil. Isolated microorganisms were identified and their biosurfactant work was detected through GC-MS of *Bacillus subtilis*. The results showed that a biosurfactant produced by a novel strain of *Bacillus subtilis* to enhance the biodegradation and bioavailability of organic contaminants.

Keywords Biosurfactant, Microorganisms, Oil spills.

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Introduction

Surface active agents produced by different groups of microorganisms are known as biosurfactants. Biosurfactants reduce surface tension in both aqueous and hydrocarbon mixtures. El-Sheshtawy et al. (2015) biosurfactants can be categorized into four main groups: Lipopeptides and lipoproteins, glycolipids, phospholipids, and polymeric surfactants (Mulligan et al. 2001). Removal of hydrocarbon oil by natural process such as photo-oxidation, evaporation and microbial degradation may take several years. Biosurfactants are surface-active compound produced by many microorganisms. They are amphiphilic molecules produced on microbial cell surfaces that stimulating release of oil entrapped within the capillaries, wetting of solid surfaces, reduction of oil viscosity and oil pour point, lowering of interfacial tension, and dissolution of oil (Kapadia and Yagnik 2013). Biosurfactant is very interesting because their advantage such as biodegradability, low toxicity, ecological acceptability and ability to be produced from renewable and cheaper substrates (Nitschke and Pastore 2006). Biosurfactant or bioemulsifier-producers occurring in soils and sediments mainly belong to the genera Pseudomonas, Bacillus, Sphingomonas and Actinobacteria (Awashti et al. 1999, Banat et al. 2000). The objective of this study was to isolate and screening biosurfactant producing bacteria from different oil contaminated terrestrial samples collected in Thanjavur, Tamil Nadu.

Table 1. Sample sources.

Sl. No.	Sources	Collected From
1.	Petrol bunk soil	Thanjavur
2.	Mechanic workshop soil	Thanjavur
3.	Oil mill soil	Thanjavur
4.	Engine oil soil	Thanjavur

Materials and Methods

Sample collection

The samples were collected from oil contaminates areas such as, petrol bunk, automobile workshop (tractor workshop), oil mill, and engine pump set around Thanjavur District. The sample was collected in a sterile polyethylene bag with the help of sterile tea spoon and it was taken to the laboratory immediately and analyzed for the isolation of biosurfactant producing bacteria (Table 1).

Isolation of bacterial species from soil sample

Bacterial species were isolated from the collected soil samples by serial dilution and agar plating method. The soil sample was diluted from 10^{-1} to 10^{-6} dilutions, and the diluted soil samples were spread on sterile nutrient agar plates. The inoculated plates were incubated at 37 ^{0}C for 24 hours. A mixed culture was obtained after incubation and it was purified by quadrant streaking on sterile Nutrient agar plates and Cetrimide agar plates.

GC-MS analysis

Biosurfactant oil analysis was estimated by gas chromatography method. A computer related Nucon series gas chromatograph equipped with flame ionization detector (FID) was employed for the separation and quantification of fatty acid profile.

GC Program

Column : Elite-5MS (5% Diphenyl / 95% Dimethyl poly siloxane), 30 × 0.25 mm × 0.25 µm df
Equipment : GC Clarus 500 Perkin Elmer
Carrier gas : Iml per min, Split : 10:1
Detector : Mass detector Turbo mass gold- : Perkin Elmer
Software : Turbomass 5.2
Sample injected : 2µl

Oven temperature program

110^0 C-2 min hold
UP to 200^0 C at the rate of 10 ^{0}C/min-No hold
Up to 280^0 C at the rate of 5^0 C / min-9 min hold
Injector temperature 250^0 C
Total GC running time 36 min

MS program

Library used NIST Version-Year 2005
Inlet line temperature 200^0 C
Source temperature 200^0 C
Electron energy : 70 eV
Mass scan (m/z) : 45-450
Solvent Delay : 0-2 min
Total MS running time : 36 min

Results and Discussion

The present study was conducted to determine the oil degrading activity of biosurfactant producing organism and all the isolated strains were screened for the confirmation of bio-surfactant producing bacteria is *Bacillus subtilis* and the strains showed good result finally that strains taken for the further study.

Isolation of *Bacillus subtilis*

Morphological identification

The strain was identified as *Bacillus subtilis* on the basis of their morphological and biochemical characteristics. The bacterial isolate was Gram negative, rod shaped, spore forming and motile organisms (Tables 2 and 3).

Characterization of isolated bacteria

(a) Colony morphology : The shape, size, elevation, margin and cooler of the colony were observed in the

Table 2. Colony morphology of nutrient agar and cetrimide agar plate.

Sl. No.	Sample	Colony morphology on nutrient agar	Colony morphology on cetrimide agar
1	Petrol bunk soil	Smooth whitish pin pointed colony	Greenish colony
2	Tractor work shop soil	Smooth whitish pin pointed colony	Greenish colony
3	Oil mill soil	Smooth whitish pin pointed colony	Greenish colony
4	Engine oil soil	Smooth whitish pin pointed colony	Greenish colony

culture plates with nutrient agar used as the nutrient medium. The observations were noted down.
(b) Gram's staining : Morphological characterizations of isolate strains were done by Gram's staining and it was found that both strains were Gram negative Bacillus (Figs. 1—4).

The results of GC/MS analysis confirmed the capability of the bacterial consortium to degrade crude

Table 3. Identification test: Gram's staining.

Sl. No.	Sources	Gram's staining
1	Petrol bunk soil	Gram negative, rod shaped bacteria
2	Tractor work shop soil	Gram negative,rod shaped bacteria
3	Oil mill soil	Gram negative, rod shaped bacteria
4	Engine oil soil	Gram negative, rod shaped bacteria

oil. (Figs. 5-8). During processing and form some of the fatty acid components and FFA in biosurfactant oil produced from *Bacillus subtilis* in this study, 8 fatty acid components were identified by GC-MS. The major fatty acid components identified in the petrol bunk soil is Phenol, 2, 4-bis (1, l-dimethylethyl) Tridecane, 4-methyl-Hexadecane, l-Pentanone, l-{1-[hydroxyl (phenyl) methyl] -2- methylcyclopropyl}, 6-Chloro-2,2,9,9-tetramethyl-3,7-decadiyn-5-ol 4-Tetradecene, (E)- Dodecyl acrylate, 1,2-Benzenedicarboxylic acid, diisooctyl ester and the major fatty acid components identified in the tractor workshop soil is Decanoic acid, methyl ester, Phenol, 2, 4-bis (l,l-dimethyleth-

FIG:1 SOIL SAMPLES

FIG: 2 NUTRIENT AGAR PLATE

FIG: 3 CETRIMIDE AGAR SLANT

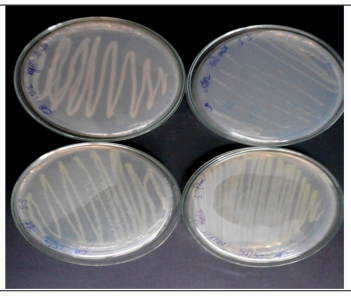

FIG:4 CETRIMIDE AGAR PLATE

Fig. 1. Soil samples. **Fig. 2.** Nutrient agar plate. **Fig. 3.** Cetrimide agar slant. **Fig. 4.** Cetrimide agar plate.

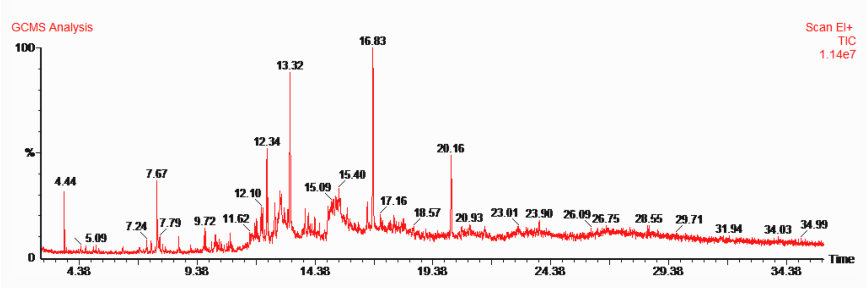

Fig. 5. Gas chromatography-mass spectrometry analysis of petrol bunk soil.

yl), Tridecane, 4-methyl-Hexadecane, 1-Pentanone, 1-{1-[hydroxyl (phenyl) methyl]-2-methylcyclopropyl}, 6-Chloro-2,2,9,9-tetramethyl-3,7-decadiyn-5-ol, Dodecyl acrylate, 1,2-Benzenedicarboxylic acid, diisooctyl ester and the major fatty acid components identified in the oil mill soil is Decanoic acid, methyl ester, Phenol, 2, 4-bis (l, l-dimethylethyl), Tridecane, 4-methyl- Hexadecane, l-Pentanone, l-{l-{hydroxyl (phenyl) methyl]-2-methylcyclopropyl}, 6-Chloro-2,2,9,9-tetramethyl-3, 7-decadiyn-5-ol, Octadecanoic acid, methyl ester, Dodecyl acrylate, 1, 2- Benzenedicarboxylic acid, diisooctyl ester and the major fatty acid components identified in the engine oil soil is Decanoic acid, methyl ester, Phenol, 2, 4-bis (l, l-dimethylethyl), Tridecane, 4-methyl-Hexadecane, l-Pentanone, 1-{1-[hydroxyl (phenyl) methyl]-2-methylcyclopropyl}, 6-Chloro-2,2,9,9-tetramethyl-3,7-decadiyn-5-ol, Octadecanoic acid, methyl ester, Dodecyl acrylate Dodecanoic acid, isooctyl ester the results are shown in (Tables 4–7) respectively.

Molecular techniques for the identification of hydrocarbon-degrading bacteria have been rarely used in environmental studies (Chang et al. 2001).

In this present study, initially the growth pattern by the isolates on relatively simple hydrocarbon source (engine oil) was studied. Zobell (1973) has reported that growth can be taken as a parameter for microbial utilization of substrate. Eventually screening that about Arabinose, Salicin, Trebulose, Cellobiose, Maltose, Lactose, Sucrose, Fructose and Glucose carbon content of hydrocarbons present as bacterial protoplasm.

Table 4. GC-MS analysis of petrol bunk soil.

Sl. No.	RT	Name of the compound	Molecular formula	MW	Peak area %
1	7.67	Phenol, 2,4-bis (1, l-dimethylethyl)-	$C_{14}H_{22}O$	206	10.1
2	7.79	Tridecane, 4-methyl-	$C_{14}H_{30}$	198	1.4
3	9.72	Hexadecane	$C_{16}H_{34}$	226	2.2
4	12.34	1-Pentanone, 1-{1-[hydroxy (phenyl) methyl]-2-methyley clopropyl}	$C_{16}H_{22}O_2$	246	8.7
5	13.32	6-Chloro-2,2,9,9-tetramethyl-3,7-decadiyn-5-ol	$C_{14}H_{21}ClO$	240	21.7
6	15.40	4-Tetradecene, (E)-	$C_{14}H_{28}$	196	13.0
7	16.83	Dodecyl acrylate	$C_{15}H_{28}O_2$	240	29.0
8	20.16	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390	13.8

Table 5. GC-MS analysis of tractor workshop soil.

Sl. No.	RT	Name of the compound	Molecular formula	MW	Peak area %
1	4.44	Decanoic acid, methyl ester	$C_{11}H_{22}O_2$	186	4.51
2	7.67	Phenol, 2,4-bis (1, l-dimethylethyl)-	$C_{14}H_{22}O$	206	10.1
3	7.79	Tridecane, 4-methyl-	$C_{14}H_{30}$	198	1.4
4	9.72	Hexadecane	$C_{16}H_{34}$	226	2.2
5	12.34	1-Pentanone, l-{1-[hydroxy (phenyl) methyl]-2-methyley clopropyl}	$C_{16}H_{22}O_2$	246	8.7
6	13.32	6-Chloro-2,2,9,9-tetramethyl-3, 7-decadiyn-5-ol	$C_{14}H_{21}ClO$	240	21.7
7	16.83	Dodecyl acrylate	$C_{15}H_{28}O_2$	240	29.0
8	20.16	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390	13.8

Table 6. GC-MS analysis of oil mill soil.

Sl. No.	RT	Name of the compound	Molecular formula	MW	Peak area %
1	7.67	Phenol, 2, 4-bis (1,1-dimethy-lethyl)-	$C_{14}H_{22}O$	206	10.1
2	7.79	Tridecane, 4-methyl-	$C_{14}H_{30}$	198	1.4
3	9.72	Hexadecane	$C_{16}H_{34}$	226	2.2
4	12.34	l-Pentanone, l-{l-[hydroxy (phenyl) methyl]-2-methyl-cyclopropyl}	$C_{16}H_{22}O_2$	246	8.7
5	13.32	6-Chloro-2,2,9,9-tetramethyl-3, 7-decadiyn-5-ol	$C_{14}H_{21}ClO$	240	21.7
6	14.25	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	298	3.34
7	16.83	Dodecyl acrylate	$C_{15}H_{28}O_2$	240	29.0
8	20.16	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390	13.8

Table 7. GC-MS analysis of engine oil soil.

Sl. No.	RT	Name of the compound	Molecular formula	MW	Peak area%
1	7.67	Phenol, 2,4-bis (l,l-dimethy-lethyl)-	$C_{14}H_{22}O$	206	10.1
2	7.79	Tridecane, 4-methyl-	$C_{14}H_{30}$	198	1.4
3	9.72	Hexadecane	$C_{16}H_{34}$	226	2.2
4	12.34	I-Pentanone, l-{l-[hydroxy (phenyl) methyl]-2-methyl-cyclopropyl}	$C_{16}H_{22}O_2$	246	8.7
5	13.32	6-Chloro-2,2,9,9-tetramethyl-3,7-decadiyn-5-ol	$C_{14}H_{21}ClO$	240	21.7
6	16.83	Dodecyl acrylate	$C_{15}H_{28}O_2$	240	29.0
7	17.67	Dodecanoic acid, isooctyl ester	$C_{20}H_{40}O_2$	312	4.51
8	20.16	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390	13.8

Meckenstock et al. (2000) have also observed incorporation of [^{13}C] bicarbonate into the carboxylic group of 2-naphthoic acid, indicating that NAP was activated through addition of a Cl compound. In another study, naphthalenol was detected as an intermediate consistently by GC/MS analysis in anaerobic NAP degradation by a sulfidogenic culture (Bedessem et al. 1997). This study proposed hydroxylation as the

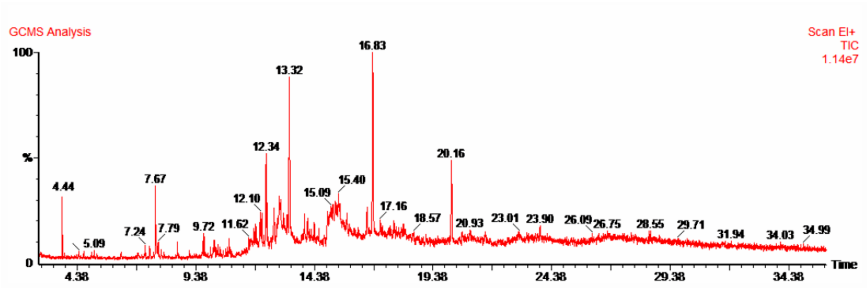

Fig 6. Gas chromatography- mass spectrometry analysis of tractor work shop soil.

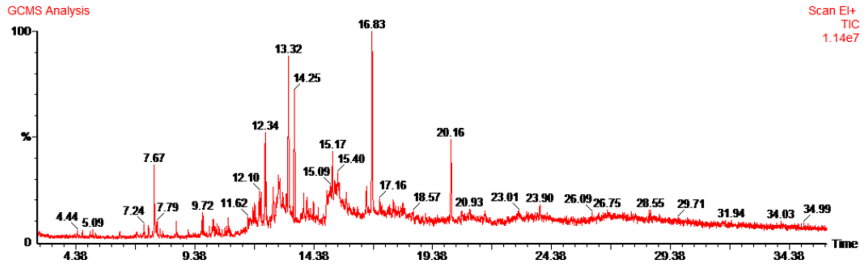

Fig. 7. Gas chromatography- mass spectrometry analysis of oil mill soil.

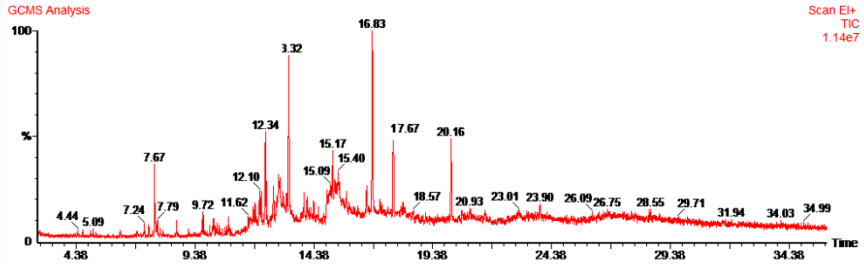

Fig. 8. Gas chromatography-mass spectrometry analysis of engine oil soil.

initial step in NAP degradation under sulfate-reducing conditions.

Conclusion

The results suggest that *Bacillus subtilis* is a good producer of biosurfactant and against hydrocarbon contaminated places. Thereby the oil contaminated soils can be degraded by introducing the biosurfactant producing organisms such as *Bacillus subtilis*, which is able to degrade the oil contaminated soil from the environment.

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