Petroleum Hydrocarbon Degradation Potential of Biosurfactant extracted from Bacteria isolated from Oil Contaminated Sites

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Abstract

Petroleum hydrocarbons are widely used in day-to-day life and pollution caused by them affects the environmental health, as it can readily alter/ disturb the fragile nature of the terrestrial and marine ecosystem and human health. Bacterial biosurfactants enhance the bioavailability of hydrophobic substances and thereby increase the rate of biodegradation of crude oil. Petroleum crude oil degrading bacteria (VITGK5) were isolated from soil samples collected from oil contaminated sites. A biosurfactant was extracted from the isolate VITGK5 and studied for biodegradation of crude oil. Spectrophotometric analysis of crude oil biodegradation by the bacterial isolate VITGK5 was found to be 61.05%. The isolate showed an emulsification index of 69.7%, a zone of 5 cm in the oil dispersion test and a triple positive for the drop collapse test. The isolate was characterized by 16S rDNA sequencing and identified to be belonging to the genus Klebsiella and designated as Klebsiella penumoniae sp. *VITGK5.*

The partially purified biosurfactant was characterized by FTIR and GC-MS and were found to be lipid in nature. This study explores the use of bacterial biosurfactants for bioremediation of oil spills as an eco-friendly approach to protect the environmental health.

Keywords: Environmental pollution, Petroleum hydrocarbons, Crude oil, Bioremediation, Biosurfactants.

Introduction

Petroleum hydrocarbons (PHCs) are complex substances as the major contaminant of the environment and cause severe pollution worldwide. It is of great economic importance due to their use as fuels, chemicals and plastics[31]. Majority of the PHC pollution is due to oil mining and accidental oil spills. Natural leakage including spilling pipelines, aged discharges and overflow from land-based sources also contribute to pollution. A few months after the Gulf War oil spill of 1991, it was reported that within the first four months, the spilled oil degraded significantly. But recent investigations have revealed that the concentration of toxic polycyclic aromatic hydrocarbons (PAHs) is still high in tar mats released before 27 years, even after years of natural weathering indicating the magnitude of the problem[7]. The majority of marine oil spills are due to accidents during transportation (74%) and coastal offshore production[10]. Marine oil spill leads to many physical and chemical changes including the formation of film slick on the surface and water-in-oil emulsion, which persist along the shore for a prolonged period of time affecting various marine life forms[2]. Polycyclic aromatic hydrocarbons (PAHs), aliphatic hydrocarbons, benzene, toluene, ethyl benzene and xylene (BTEX) are considered as persistent organic pollutants. These toxic substances have prolonged half-lives, remaining in the marine environment and cause eco-toxicity to marine animals[16].

The impact of the marine oil spill and their effect on marine species, including crustaceans and mollusks is reported to be associated with ecotoxicity on other marine life forms[11]. Oil spill affects the dynamics of planktonic communities in the sea by disrupting the energy transmission between the trophic levels. It also affects the reproductive system of coral reefs and decreases their colony viability, lowering the number of ovaries per polyp on reef corals and resulting in premature planulae shedding. Mangrove vegetation, fisheries and mariculture resources are also affected by oil spills[3]. PAH exposure up regulates certain genes including CYP450s, GST, SOD, GPx, CAT and HSPs which results in lack of control over antioxidant defence and detoxification mechanisms on marine benthic macro invertebrates[21].

Oil along with microplastics affects the bio-indicators of sea animals including marine fish, Asian sea bass and *Lates calcalifer*[28]. Bioremediation is defined as the utilization of living organisms to eliminate the contaminants and pollutants from the environment. Bacteria are described as the most active agents that can cause biodegradation. Bacterial isolates can degrade as well as utilize crude oil as a source of carbon and energy. *Bacillus cereus* LY-1 isolated from Indonesian oil sand has been shown to be very effective in degrading complex PHCs[15]. Fungal cultures were also shown to be very effective in degrading polycyclic aromatic hydrocarbons (PAHs). *Fusarium equiseti* showed 97.8% and NIOSN-M126 (*Penicillium citrinum*) showed 100% PAHs degradation[10].

The hydrocarbon degrading organisms produce chemically diverse biosurfactants, which increase the surface area of water-insoluble substrates, thereby increasing their bioavailability and the rate of bioremediation[12]. Microorganisms of the genera *Bacillus, Pseudomonas, Rhodococcus, Halomonas and Stenotrophomonas* have been reported to increase the degradation rate by releasing biosurfactants[30]. Even under extreme conditions like high

salinity or temperature, these organisms maintain their activity and can also be produced from organic and other cheap sources[14]. Biosurfactants have higher biodegradability, lesser toxicity, higher foaming ability and environmental tolerance when compared to chemical surfactants[30].

Biosurfactants have been successfully used in remediating crude oil contamination. In the EXXON Valdez oil spill, 1% of biosurfactant obtained from *Pseudomonas aeruginosa* SB30 was applied and it was able to remove twice the oil above water[19]. It was reported that lipopeptide produced by *Bacillus subtilis* enhanced motor oil degradation twofold and exhibited stability under saline conditions in the range of 5 to 20% w/v[5]. Bacterial biosurfactant-mediated crude oil degradation and oil recovery are eco-friendly, cost-effective techniques for marine oil spill remediation. In this study, crude oil biodegrading efficiency of bacteria and their biosurfactant production was studied.

Material and Methods

Chemicals: Hexane was purchased from Finar, Ahmedabad, India and ethyl acetate was procured from Hi Media, Mumbai, India and both were of HPLC grade. Bushnell Haas broth, nutrient agar, glucose, sodium chloride and sodium-sulfate anhydrous were purchased from Hi Media, Mumbai, India. The detergent Triton X 100 was bought from Sisco Research Laboratories, Taloja, India. Analytical grade HCl acid was purchased from SDFCL, Mumbai, India.

Sample collection: Crude oil degrading organism was isolated from soil samples collected from motor waste oil contaminated sites in Katpadi, Vellore, Tamil Nadu, India. Soil samples were collected from a depth of 10 to 12 cm after removing the surface debris with the help of a sterile spatula. After collection, the soil samples were stored in sterile autoclave bags and transported to the laboratory. All the soil samples collected were maintained at 4°C in a refrigerator until further use. The crude oil used as control in this study was sourced from Regional Geoscience Lab, ONGC, Chennai, Tamil Nadu, India.

Isolation of bacteria with crude oil degrading potential: A crude oil-degrading bacterium was isolated by enrichment method as reported earlier[29]. 5 g of soil contaminated with used engine oil was added to 100 ml of Bushnell- Haas (BH) broth with 1% (V/V) of crude oil as the sole carbon source in five 250 ml flasks. BH medium is made up of 1g K_2HPO_4, 1g KH_2PO_4, 0.2 g $MgSO_4$, 0.02 g $CaCl_2$, 1g NH_4NO_3 and 0.05 g $FeCl_3$ and made up to 1000 ml using sterile distilled water. All the flasks were incubated at 37°C at 150 rpm in an orbital shaker for 7 days. At the end of the incubation period, the culture was serially diluted up to 10^{-6}. The dilutions from 10^{-4} - 10^{-6} were spread plated onto Bushnell Haas agar plates supplemented with 100 µl of crude oil and incubated at 37°C at 150 rpm in an orbital shaker for 7 days. Morphologically distinct colonies were sub-cultured on nutrient agar plates to isolate single colonies for pure culture. The pure cultures obtained were stored in 50% glycerol (V/V) stock at -20°C for further use.

Biodegradation of crude oil by VITGK5: The crude oil biodegradation potential of the isolate (VITGK5) was determined by measuring the absorbance at 420 nm with the help of a UV/Vis spectrophotometer from day 1 to day 5 for both control and VITGK5 treated[24]. Overnight culture of VITGK5 was inoculated in 50 ml of BH broth supplemented with 1% (V/V) crude oil and 0.5 % glucose and then incubated at 37°C for 5 days at 150 rpm. The crude oil remaining in the broth after degradation was extracted by liquid: liquid extraction from cell-free supernatant (CFS) with hexane.

Gravimetric analysis of crude oil degradation: Gravimetric analysis was done to evaluate the degradation percentage of crude oil by VITGK5[16]. VITGK5 was inoculated in 50 ml of BH broth supplemented with 1% crude oil (V/V) and 0.5 % glucose and incubated at 37°C for 5 days at 150 rpm. Liquid: liquid extraction was performed with 1:1 volume of hexane and broth in a separating funnel. 10 g of sodium sulphate anhydrous was used to remove moisture from the organic phase. The organic phase was then collected in a pre-weighed vial and the hexane was allowed to evaporate overnight. The combined weight of the vial and the oil was measured. Pre-weighed weight of the vial was subtracted from the final weight to determine the oil weight.

Degradation % =
(Weight of the control sample - Weight of test sample / Weight of control sample) × 100

Determining salt tolerance: Gravimetric analysis was used to determine the salt tolerance of VITGK5. The isolate was inoculated in 50 ml of BH broth supplemented with 1% (V/V) crude oil and 0.5 % glucose. 1 %, 2 % and 3 % of NaCl were added to the broth and incubated at 37°C for 5 days at 150 rpm. The degradation percentage at different concentrations of NaCl was determined.

Gas chromatography-Mass spectrometry (GC-MS) analysis of degraded crude oil: The biodegraded crude oil extracted with hexane was analysed by GC-MS (Agilent model GC-7890A coupled with mass spectrometer 5975C) and crude oil as a control. Dimensions of the column are 30 m x 250 µm x 0.25 µm. Helium was used as a carrier gas with a flow rate of 1 ml/min and 1 µl of the sample was injected at an injection temperature of 250°C. The transfer line temperature was maintained at 280°C and 230°C as the ion source temperature. The compounds present in the biodegraded crude oil were detected and identified by NIST library matching.

Biosurfactant screening: Screening of biosurfactant production by the isolate VITGK5 was done by inoculating in 50 millilitres of BH broth augmented with 1% crude oil

(V/V) and 0.5 % glucose. It was incubated at 37°C for 5 days at 150 rpm. After the incubation period, the broth was centrifuged at 10,000 rpm for 15 min at 4°C. The culture supernatant collected was used for further tests.

Emulsification index measurement: Emulsification index measurement was carried out by mixing equal volumes of CFS and crude oil (2ml each) in a test tube and mixed vigorously in a vortex mixer for 2 min and left undisturbed. After 24 hours, the emulsification index was measured as per the formula:

Emulsification index (%) =
(Height of the emulsified layer /
Total height of the liquid column) × 100

Oil displacement test: Oil displacement test was done in a Petri plate containing 50 ml of distilled water and 1 ml of crude oil. 50 µl of CFS was added and the resulting clearing zone was measured immediately. The detergent triton X100 was used as the positive control while distilled water was used as the negative control[17].

Drop collapse assay: A clean glass slide was taken and a drop of crude oil was placed on the surface. A droplet of CFS was added to the oil droplet. The collapse of the oil drop was considered as a positive result. Triton X 100 and distilled water served as the positive control and negative control respectively.

Stability of biosurfactant in saline conditions: The CFS obtained was used to assess the stability of the biosurfactant under different NaCl salt concentrations 1 %, 2 % and 3 % as described earlier. The emulsification index, drop collapse and oil displacement assays were used to evaluate the stability of the biosurfactant.

Phylogenetic identification: The isolate VITGK5 was subjected to 16S rRNA partial-gene sequencing for identification by molecular taxonomy and phylogeny. DNA was isolated using a highly pure PCR template preparation kit of Roche. Isolated DNA was amplified using PCR primer sets 27F and 1492R and re-amplified with sequencing primer sets 518F and 800R. The 16S rDNA sequence obtained was BLAST searched with the sequences available in the GenBank database. The 16S rDNA sequence was used for phylogenetic tree construction and distance matrix calculation using MEGA 11 software.

Biosurfactant extraction: The acid precipitation protocol was followed for the extraction and partial purification of the biosurfactant[18]. The isolate VITGK5 was inoculated in BH broth and incubated at 37°C for 5 days at 150 rpm. After the incubation period, the broth was centrifuged for 15 minutes at 10,000 rpm at 4°C. The pH of the CFS was adjusted to 2.0 using 6 N hydrochloric acid and left at 4°C overnight for biosurfactant precipitation. Solvent extraction method was used for the extraction of the biosurfactant using equal volumes of ethyl acetate and CFS. Subsequently, a rotary vacuum evaporator was used for evaporating the ethyl acetate to obtain a partially purified biosurfactant.

Characterization of biosurfactant: FTIR analysis: Partially purified crude biosurfactant was subjected to FTIR analysis for the identification of functional groups. FTIR spectra (4000–600 cm^{-1}) were measured using IRA Shimadzu (AVATAR 330), Japan. The FTIR spectrum obtained was analysed by OMINIC software.

GC-MS analysis: Partially purified biosurfactant was subjected to GC-MS analysis to determine its constituents. Shimadzu GC-2030 coupled with MS-QP2020 was used for analysis. Dimensions of the column are 30 m x 0.25mm x 0.25 µm. Helium gas with a flow rate of 1ml/min was used as a carrier gas. 1µl of partially purified biosurfactant was injected at 260°C (injection temperature). Interface temperature was 280°C and ion source temperature was retained at 250°C. The GC-MS spectra obtained were compared with NIST library for the identification of compounds present.

Results

Isolation and selection of strain: Culture enrichment of motor oil contaminated soil sample resulted in isolation of six morphologically different crude oil-degrading bacteria. All six isolates were subjected to biodegradation tests and screened for biosurfactant production. Based on the biodegradation potential of crude oil and biosurfactant production, the bacterial isolate VITGK5 was chosen for further studies.

Biodegradation of crude oil by bacteria: For biodegradation of the crude oil by VITGK5 OD was 1.67 on day 1 for control and 1.05 for VITGK5. After an incubation period of 5 days, OD was measured to be 0.65 and 1.66 for degraded crude oil and control crude oil respectively, indicating a considerable degradation of the crude oil in the test compared to the control.

Extraction of degraded crude oil and salt tolerance: Crude oil degradation by VITGK5 was indicated by the decrease in the weight of the test sample compared to the weight of the control sample. Gravimetric analysis revealed that VITGK5 was able to degrade 61.05% of 1% (V/V) crude oil after an incubation period of 5 days. VITGK5 showed the degradation percentage of 49.30 %, 40.12 % and 11.30 % at 1 %, 2 % and 3 % NaCl concentrations respectively.

GC-MS analysis of biodegraded crude oil: The chemical composition of crude oil and VITGK5 degraded crude oil was analyzed by GC-MS. Chromatogram of control crude oil and biodegraded crude oil is shown in figure 1 (a and b). Degraded compounds found in GC-MS analysis are tabulated in table 1. Majority of the compounds found in control crude oil are alkanes and PAHs namely

naphthalenedecahydro-2-methyl-, decahydro-4,4,8,9,10-pentamethylnaphthalene, naphthalene 2,3- dimethyl-, naphthalene, 1,6-dimethyl-, naphthalene, 1,4,6-trimethyl- and retene.

Compounds such as naphthalene, decahydro-2-methyl-, heptadecane 2,6 - dimethyl-, dodecane, 2,6,10-trimethyl-, heptadecane2,6,10,14-tetramethyl-, decahydro -4,4,8,9,10-pentamethylnaphthalene, naphthalene 2,3-dimethyl-, naphthalene, 1,6-dimethyl-, decane, 2,6,8-trimethyl-, naphthalene 1,4,6-trimethyl-, retene and nonacosane have completely degraded after biodegradation with VITGK5. Newly detected compounds after biodegradation by VITGK5 include pulegone, dodecane, naphthalene, 2-methyl-, naphthalene, 2,7-dimethyl-, 4,6,8-Trimethylazulene, hexadecane, 5-butyl-, cyclohexane, 1-(1-tetradecylpentadecyl)- and heneicosane, 11-decyl-.

Phylogenetic identification: The 16S rRNA sequence (1498 bp) alignment of VITGK5 (GenBank accession number OQ380677) with the GenBank database sequences revealed that VITGK5 showed (99.60%) sequence similarity with *Klebsiella pneumoniae* strain KPN1705. Hence, it was identified to be belonging to the genus *Klebsiella* and was named as *Klebsiella pneumoniae* sp. VITGK5. The phylogenetic tree of VITGK5 showed that it was related to *Klebsiella pneumoniae* with bootstrap value of 45% (figure 2).

Characterization of biosurfactant: VITGK5 showed emulsification index of 69.7 %, the oil dispersion test showed a zone of 5 cm and the drop collapse test showed triple positive results (figure 3). Triton X 100 and distilled water were as positive and negative control respectively.

FTIR Analysis: The partially purified biosurfactant of VITGK5 was analysed by FTIR to identify the functional groups. The broad peak at 2921.84 cm $^{-1}$ indicating O-H stretching vibrations and a peak at 1743.41 cm^{-1} indicate strong C=O stretching vibrations and a peak at 1366.37 cm^{-1} implied the presence of C-H bending vibrations, a characteristic of alkane. The peaks at 1243.43 cm^{-1} and 1035 cm^{-1} imply a strong C-O stretching vibrations and strong S=O stretching vibrations respectively. The peak at 847.35 cm^{-1} indicates a C=C bending vibrations of alkene and a peak at 784.78 cm^{-1} indicates the presence of strong C-H bending vibrations. The FTIR spectrum of the partially purified biosurfactant is shown in figure 4.

GC-MS Analysis of the Biosurfactant: The partially purified biosurfactant was subjected to GC-MS analysis to identify the compounds present. The existence of fatty acids and esters indicates the lipid nature of the biosurfactant produced by VITGK5. PHC compounds were also detected. GC-MS spectrum obtained for the crude biosurfactant is given in figure 5. The major biosurfactant compounds detected in GC-MS spectrum are given in table 2.

Table 1
Compounds detected in crude oil and VITGK5 degraded crude oil

Compounds	Retention time (Crude oil)	Retention time (Biodegraded crude oil)	Area % (Crude oil)	Area % (Biodegraded crude oil)	Biodegradation efficiency
Decane	5.288	5.263	0.75	0.89	Increased
Undecane	6.798	6.782	1.75	1.57	10.28%
Dodecane	8.316	8.291	2.57	2.14	16.73%
Tridecane	9.768	9.743	4	2.45	38.75%
Tetradecane	11.152	11.127	3.82	2.65	30.62%
Naphthalene, 1-methyl-	12.049, 12.385	12.377	1.99	0.84	57.78%
Pentadecane	12.46	12.435	4.18	2.53	39.47%
Hexadecane	13.702	13.677	4.82	2.73	43.36%
Pentadecane, 2,6,10,14-tetramethyl-	14.683	14.658	4.86	2.45	49.58%
Heptadecane	14.885	14.851	4.65	2.81	39.56%
Hexadecane, 2,6,10,14-tetramethyl-	15.875	15.85	1.82	1.01	44.50%
Octadecane	16.00, 24.461	15.975, 26.461	8.55	3.43	59.88%
Nonadecane	17.074	17.041	4.16	2.46	40.86%
Eicosane	18.089	18.056	4.75	2.41	49.26%
Heneicosane	19.062	19.037, 24.129	4.95	7.02	Increased
Docosane	20.002	28.969	4.53	3.05	32.67%
Tricosane	20.899	20.866, 26.294	4.5	7.86	Increased
Tetracosane	21.755	21.730, 23.375	4.35	6.74	Increased
Pentacosane	22.585	22.561	4.26	3.54	16.90%
Hexacosane	23.382, 26.302	11.748	5.17	0.84	83.75%
Octacosane	24.892	24.867, 27.946	2.37	8.1	Increased
Heptacosane, 1-chloro-	27.073	27.065	1.07	5.39	Increased

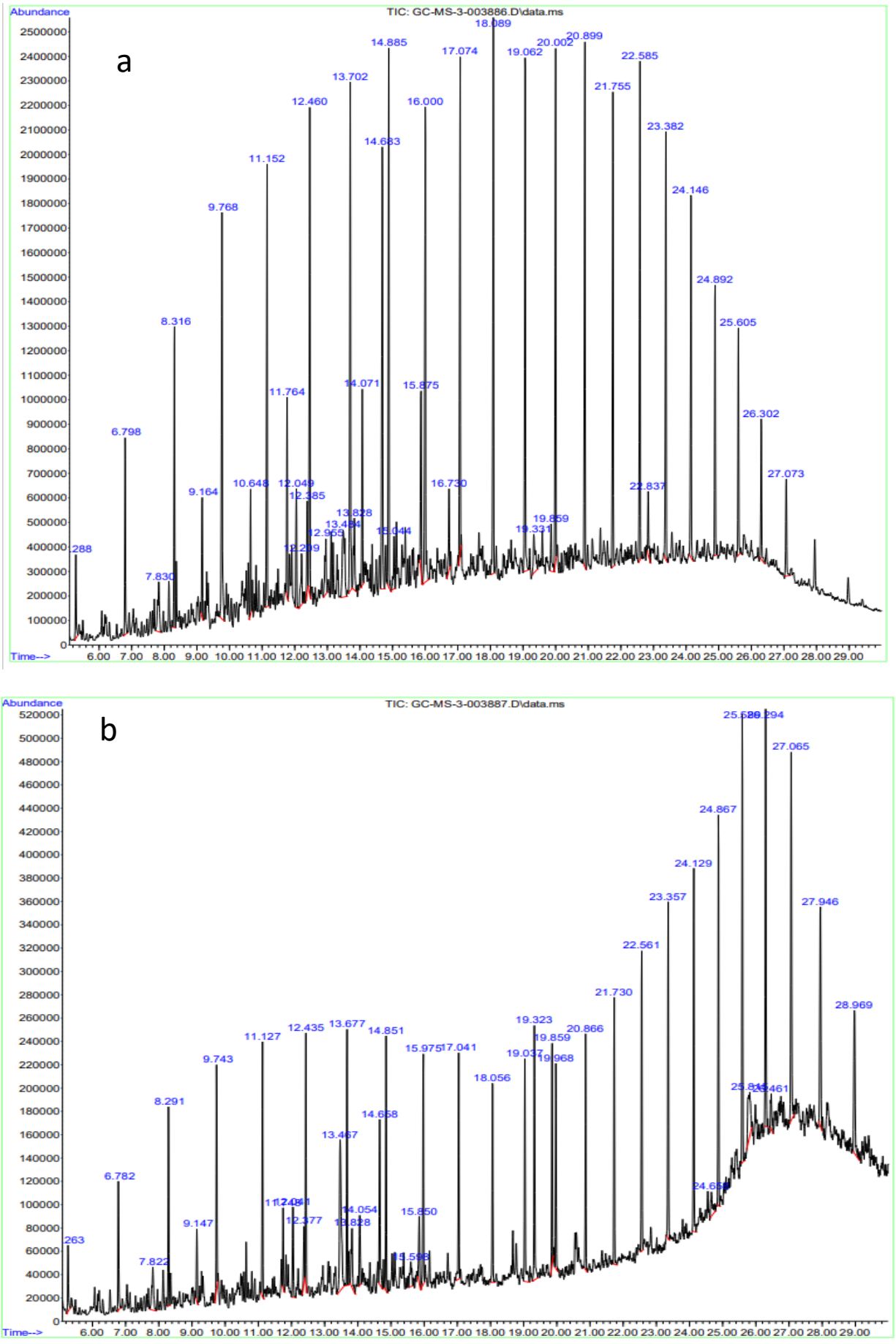

Figure 1: GC-MS spectrum of a) control and b) VITGK5 degraded crude oil.

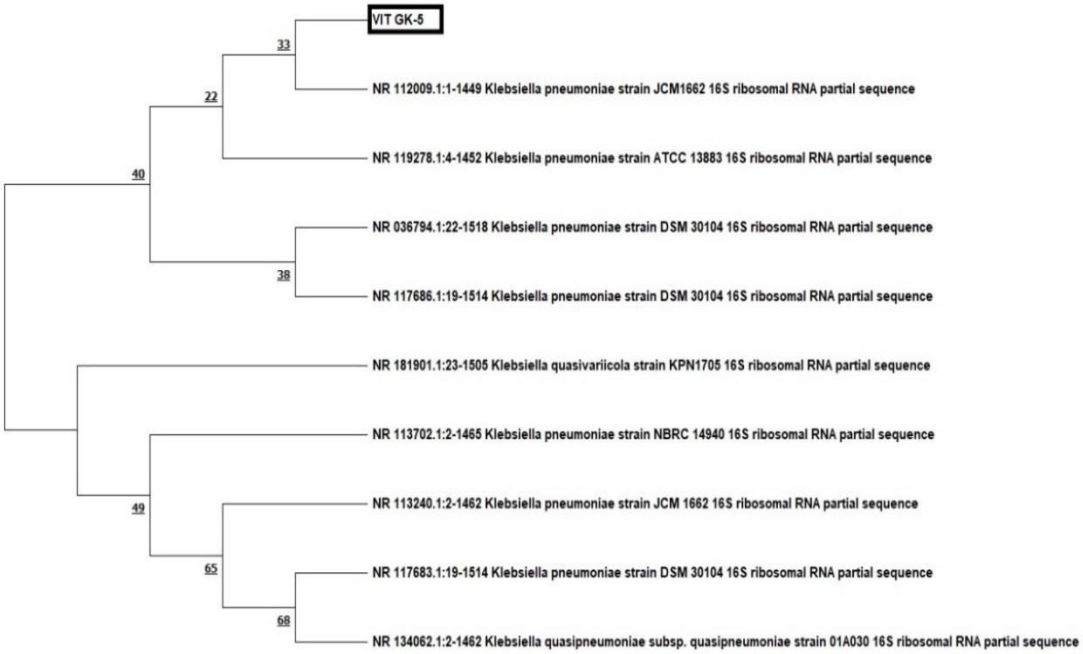

Figure 2: Phylogenetic tree of the bacterial isolate VITGK5

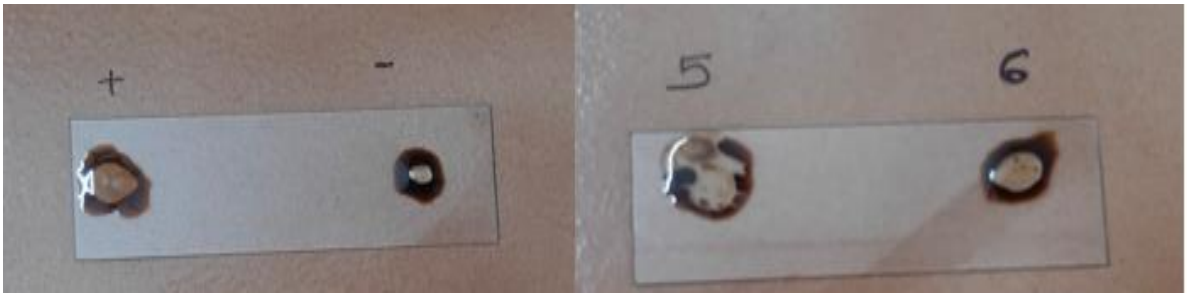

Figure 3: Drop collapse assay- (+) Positive control [Triton X 100], (-) Negative control [Distilled water], (5) VITGK5, (6) VITGK6

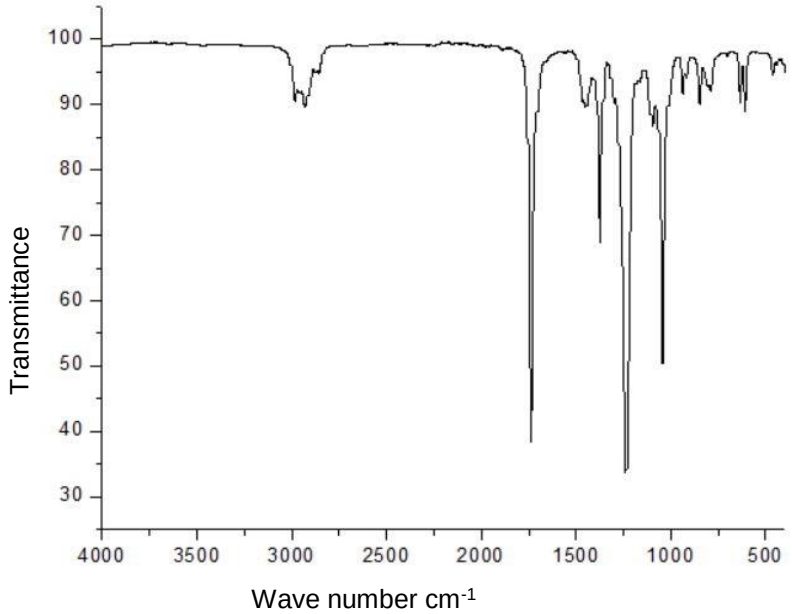

Figure 4: FTIR spectrum of partially purified biosurfactant isolated from VITGK5.

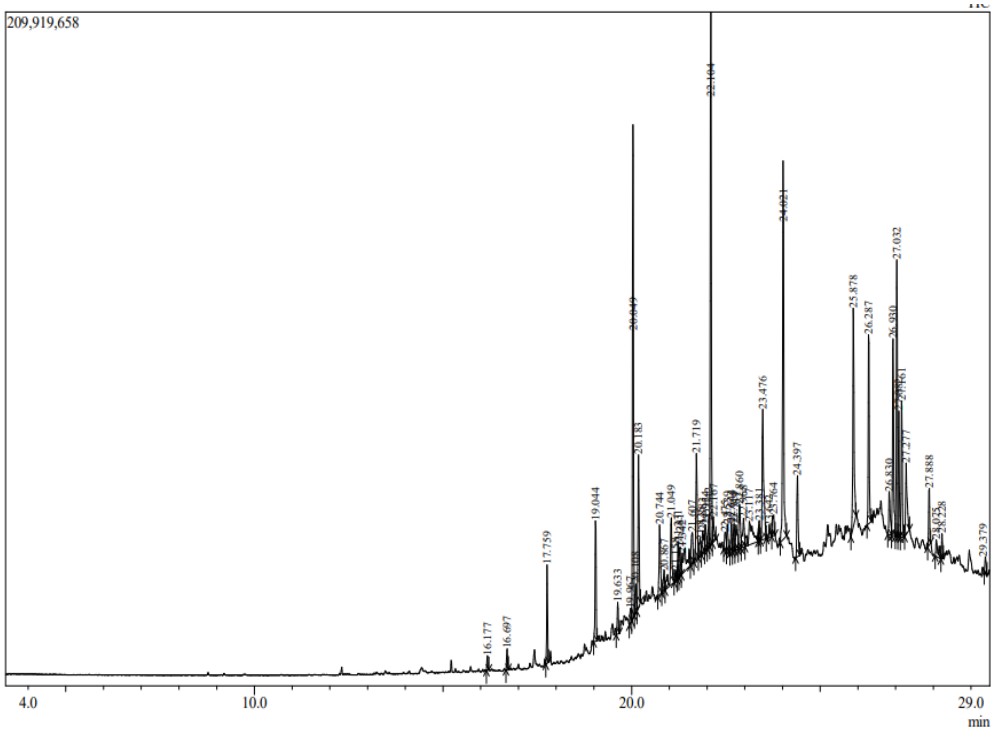

Figure 5: GC-MS spectrum of biosurfactant produced by VITGK5.

Table 2
Major compounds detected in GC-MS spectrum of biosurfactant from VITGK5

RT	Compound name	Lipid classification
21.231	7,9-di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	Ester
21.825	Cycloundecane, 1,1,2-trimethyl-	Fatty acid
21.954, 22.860	Heneicosane	Fatty acid
22.104	1-Nonadecene	Fatty acid
22.968	Succinic acid, dec-2-yl 4-cyanophenyl ester,	Ester
23.117	beta.-alanine, n-pentafluoropropionyl-, dodecyl ester	Ester
23.476	Bis(2-ethylhexyl) maleate	Ester
23.642	Octadecanoic acid	Ester
25.878	n-Tetracosanol-1	Fatty alcohol
27.277	Octacosanol	Fatty alcohol
29.379	Stigmastane	Sterols

Discussion

Klebsiella pneumoniae sp. VITGK5 isolated from crude oil contaminated motor garages sites in Vellore, Tamil Nadu, India showed crude oil degradation and biosurfactant production potential. Hydrocarbon-degrading microorganisms were isolated with the help of the enrichment technique as reported earlier[24]. Lipase assay, hemolytic assay, emulsification index, oil spreading assay and drop collapse test were routinely used for screening of biosurfactant production by microorganisms.

Multiple studies have reported the use of emulsification index as a screening technique for biosurfactant producers. Drop collapse and oil dispersion are the other two tests that have been widely used for biosurfactants screening[18]. All three methods are direct and based on the surface activity of the agents. In this study, emulsification index, drop collapse and oil dispersion were utilized for screening the microorganisms for biosurfacatant production. Only few reports are available on the effect of salinity on the stability of biosurfactants. Ahamed et al[1] used the emulsification index to study the extracted biosurfactants stability under varying concentrations of salinity. In our study, the isolate *Klebsiella pneumoniae* sp. VITGK5 showed the higher emulsification index, drop collapse and oil dispersion.

The biosurfactant produced by VITGK5 was extracted through acidification and liquid-liquid extraction methods. The extracted biosurfactant was characterized by FTIR and GC-MS analysis. The use of both these techniques for the characterization of biosurfactants produced by *Klebsiella pneumoniae* species was reported earlier[19]. FTIR spectrum revealed the functional groups present in the biosurfactant. Presence of lipid compounds was identified from the GC-

MS spectrum, confirming the lipid nature of the extracted biosurfactant. The biosurfactant produced by the isolate VITGK5 was identified as glycolipopeptide. The observed peaks in the GC-MS spectrum showed the presence of degraded hydrocarbon compounds along with biosurfactants. Previously Jain et al[8] reported the production of glycolipopeptides by *Klebsiella* species. Nwaguma et al[19] reported the presence of a phospholipid biosurfactant in *Klebsiella pneumoniae* IVN51. *Klebsiella* species is one of the predominant genera among hydrocarbon degraders and is a potent biosurfactant producer. The results of our study were supported by previous reports that *Klebsiella pneumoniae* is the potent organism for effective hydrocarbon-degradation and biosurfactant production.

Bhattacharya et al[6] reported the hydrocarbon degradation potential of *Klebsiella* spp. and it was known to survive in harsh conditions and environments. They are known to degrade an array of pollutants like halogenated aromatic compounds and nitroaromatic compounds. It was reported that *Klebsiella pneumoniae* possesses four catabolic genes (*todC1, ndoB, xylE and alkB1*) responsible for hydrocarbon degradation[26]. *Klebsiella pneumoniae* AWD5 has been reported to possess gene clusters for PHCs utilisation and also code for enzymes capable of cleaving of aromatic rings[25]. Kazemzadeh et al[13] reported hydrocarbon degradation potential of *Klebsiella variicola* SKV_2 to an extent of 93.3% within 3 weeks.

In another study, *Klebsiella variicola* was able to achieve 83% hydrocarbon removal within two weeks with 4% of crude oil (V/V) which served as a sole carbon source. *Klebsiella pneumoniae* strain VM18 degrades (62.84%) diesel oil after 35 days of incubation[23]. *Klebsiella pneumoniae* strain YSA-9 has been shown to be very effective in the degradation of a wide range of hydrocarbons with the formation of new compounds[32]. *Klebsiella* sp. possesses genes for *alkB* (alkane-1-monooxygenase) and genes for beta oxidation reported to be responsible for hydrocarbon degradation[27]. *Klebsiella pneumoniae*-K05 has been reported to be very effective in degrading hydrocarbons[22].

Klebsiella pneumoniae SJK1 has been shown to degrade (40.5%) phenantherene (1.25g/L) after 12 days of incubation evidenced by the detection of phthalate, dibutyl phthalate, succinic acid and hexadecenoic acid as intermediates of phenanthrene degradation[9]. *Fusarium equiseti* showed 97.8% and NIOSN-M126 (*Penicillium citrinum*) showed 100% PAHs degradation[4]. The GC-MS data of the degraded crude oil showed *Klebsiella penumoniae* sp. VITGK5 possess higher degradation capability on oil within 5 days of incubation.

Conclusion

Klebsiella species has been well-documented for hydrocarbon degradation through *biosurfactant* production. In our study *Klebsiella pneumoniae* sp. VITGK5 isolated from motor oil contaminated soil showed effective degradation of oil within 5 days of incubation. The oil degrading ability of VITGK5 is due to the biosurfactant (glycolipopeptide) produced by them. Biosurfactant-mediated oil degradation is an eco-friendly method that needs to be explored further to treat oil pollution in seas and other water bodies caused by oil spills, oil drilling and other related activities.

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