Biodegradation of crude oil using marine *Bacillus* species from Vadinar coast, Gujarat, India

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The marine environment is open to large sources of toxic organic waste in the form of accidental oil spills. Therefore, it is important to study microbial degradation processes that help reduce the damage caused to the environment. Universally, oil spills produce enormous public anxiety and highlight the need for cost-effective, indigenous and environmentally acceptable bioremediation technologies. In recent times, advanced remedial techniques have been opted, such as solidifying, skimming, controlled burning and bioremediation. The present study aimed to isolate crude oil-degrading marine bacteria from Vadinar coastal area of Gujarat, India. Among seven isolates, three potential bacterial strains were chosen for crude oil and petroleum hydrocarbon (PHC) degradation, which were analysed by UV spectrophotometric and fluorometric analysis. These bacterial cultures were verified by 16S rRNA gene sequencing and identified as *Bacillus* species. Phylogenetic analysis was carried out to confirm the evolutionary relationship with existing oil-degrading species. In the present study, drop collapse, oil spreading and emulsification assay were performed to detect biosurfactant production. *Bacillus* sp. NM1 KT354277 was capable of degrading 50% of PHCs at the end 72 h for one week under rotary incubation in ONR7a medium. Among the studied strains, *Bacillus* sp. NM3 KT354278 showed promising lipase activity, viz. 60.72 and 61.19 U ml^{-1} for 2% of olive oil and tributyrin respectively. Thus, the present study explores indigenous marine isolates that could be utilized as a potential alternative for oil-spill remediation in future.

Keywords: Bioremediation, crude oil, lipase, marine bacterial isolates, spectrofluorometer.

OIL spills are known for their hazardous nature and threat to the marine environment. Occurrence of accidental incidence of oil pollution generally depend upon how it is handled. Hydrocarbons have a negative impact on the adjoining ecosystems[1,2]. Certain bacterial consortia exist in nature which thrive on hydrocarbons in oil-polluted sites, using them as the lone supply of energy and carbon for growth[3–5].

Physico-chemical or biological methods could be adopted for remediation of petroleum-contaminated sites. Shortcomings of physico-chemical approach have led to the exploitation of biological alternatives[6]. The shelf-life of petroleum pollution is dependent upon the amount, nature of the mixture and physiognomies of the affected the ecosystem[7]. Bacterial remediation has played a vital role in the management of petroleum contaminants in contrast to conventional approaches that rely upon human labour. Bacteriological remediation is economical and exhibits high competence without secondary pollution[8,9].

The bioavailability of petroleum hydrocarbons is constrained due to its hydrophobic nature. Surfactant alteration may boost oil mobility, thus improving degradation rates[10]. Naturally occurring microbial biosurfactants exert some influence on interfaces, which increases the insolubility of hydrocarbons and emulsifies the mixtures[11,12]. This assists in the growth of oil-degrading bacteria and also its ability to consume hydrocarbons[13,14]. Uniform dissolution of insoluble carbon source in the culture medium attained by the production of extracellular molecules supports the growth of microorganisms on hydrocarbons[15].

Recently, the northwest coastal stretch witnessed a drastic change in terms of increased anthropogenic and industrial activities. An array of industries, viz. oil refineries, thermal power plants, ports and jetties, mining, cement, fertilizer and chemical industries have emerged along the coastal belt. Vadinar is southern coastal belt of 20 km area located at the Gulf of Kuchchh which is marked by different maritime activities as thermal power plants and oil refineries. Various point-source outlets originate from nearby industries and drain into the coast. With rapid development, it is crucial to record the present-day status of water quality along this coastal stretch, which will serve as an index as well as a tool for coastal management.

The prime objective of the present study was to identify potential marine bacterial isolates capable of crude-oil degradation. Accordingly, microcosm experiments were

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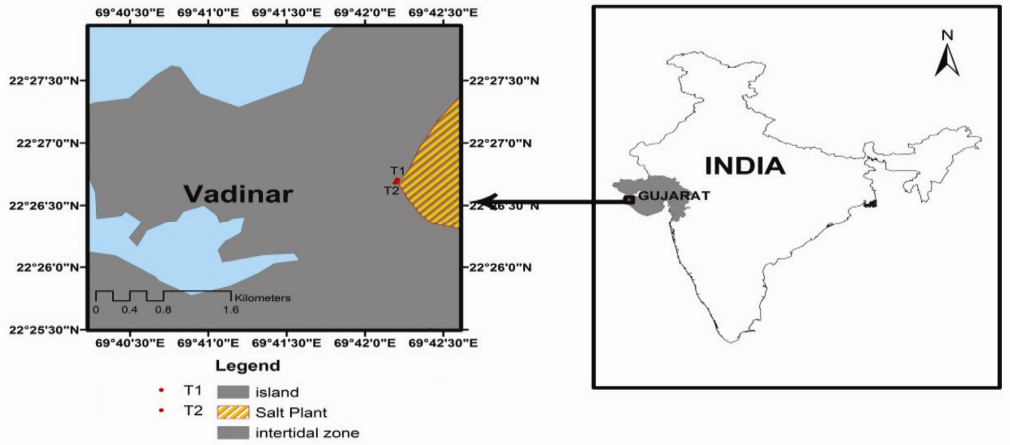

Figure 1. Map showing location of sampling sites (T1 and T2) in the Vadinar coastal area, Gujarat, India.

set up to evaluate the indigenous bacterial isolates from the coastal area of Vadinar for oil biodegradability purpose.

Materials and methods

Sampling

Sediment samples were collected from two sampling sites of Vadinar coastal area (22°26′40N; 69°42′11″E and 22°26′42″N; 69°42′12″E) (Figure 1). These sites are encircled by sensitive ecosystems like corals, mangroves, tidal incursions and small islands. Sediments cores (~500 g) from 5 to 15 cm below the surface were collected and transported in ice coolers for analysis.

Isolation and screening of crude-oil degrading bacteria

Isolation was carried out on ONR7a medium with 1% (v/v) of different types of crude oil as sole energy and carbon source[16]. ONR7a is composed of (l^{-1}) 22.79 g NaCl, 11.18 g $MgCl_2{\cdot}6H_2O$, 3.98 g Na_2SO_4, 1.46 g $CaC1_2{\cdot}2H_2O$, 0.72 g KCl, 0.27 g NH_4Cl, 89 mg $Na_2HP0_4{\cdot}7H_2O$, 83 mg NaBr, 31 mg $NaHCO_3$, 27 mg H_3BO_3, 24 mg $SrCl_2{\cdot}6H_2O$, 2.6 mg of NaF and 2 mg $FeCl_2{\cdot}4H_2O$. For solid medium, Bacto Agar (Difco) (15 g l^{-1}) was supplemented.

The sediments samples (10 g) were inoculated in flasks containing 100 ml of the medium, incubated for 7 days at 180 rpm and 30°C on a rotary shaker (GENETIX, India). Then 5 ml aliquots was transferred into fresh medium. During fourth stepwise enrichment, inoculums from the flasks were streaked to obtain well-isolated colonies on ONR7a agar medium. These isolated colonies were identified and characterized by morphological and biochemical tests using *Bergey's Manual*[17].

Extraction of DNA and 16S rRNA gene amplification

DNA was isolated using Qiagen DNeasy Blood and tissue kit, while 16S rRNA was amplified using universal bacterial primers: 16S (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′CGGTTACCTTGTTACGACTT-3′). Amplification was carried out under standard conditions using Eppendorf^{TM} Mastercycler^{TM} Pro PCR (Eppendorf, Germany). Amplified PCR product was purified using QIAquick PCR purification kit (Qiagen, Germany) and amplicons were sequenced using Sanger's dideoxy method (SciGenom Labs Pvt Ltd, India). The sequence homology was obtained using BLAST search with known and identified cultures of the NCBI database (http://ncbi.nlm.nih.gov/blast). CLUSTAL W V1.82 at the European Bioinformatics site served as an alignment tool. Phylogenetic tree was constructed by means of a neighbour-joining method using MEGA6 software[18] for calculating the evolutionary distance.

Crude-oil degradation assay

The isolated strains were incubated at 30°C for one week on a rotatory shaker (180 rpm) in ONR7a medium. Growth curve of the isolated strains was routinely assessed by quantifying turbidity at OD_{600} nm using UV–visible spectrophotometer (Shimadzu UV-1800, Japan). This assay was quantified by measuring optical density by solubilizing leftover crude oil in dichloromethane (DCM) along with blank at 420 nm (ref. 19).

Degradation of petroleum hydrocarbon

The medium containing 1% crude oil (Arab extra light crude oil) with bacterial culture was extracted with *n*-hexane to relocate petroleum hydrocarbons (PHCs) in the organic phase. This organic extract was concentrated after

drying. Fluorescence of the extract was quantified using Shimadzu RF-5301PC fluorescence spectrophotometer at excitation wavelength 310 ± 1 nm and emission wavelength 360 ± 1 nm. Samples were tested for quenching ability on dilution[20,21]. Blanks were maintained using the culture-free medium. The standard used was SAM (Saudi Arabian crude mixture) crude oil. All experiments were carried out thrice to establish uniformity and expressed in terms of mean values with standard deviation. The results are expressed as percentage of PHC degradation by bacterial isolates.

Drop collapse test

This test was carried out as prescribed by Plaza *et al.*[22]. In this method, a drop of supernatant from each bacterial isolate was taken onto a sterile glass slide. Different types of crude oil were supplemented onto the surface. If the drop on the supernatant became flat within a min on addition, the result was scored as positive, whereas if it remained beaded the result was scored as negative.

Oil spreading assay

Twenty microlitre of crude oil was dropped onto the surface of a petri dish containing 20 ml of distilled water[23]. Then 10 µl of cell-free culture broth was added to the oil surface. In the case of biosurfactants activity, oil will be displaced forming a clear zone and diameter indicates the degree of response. Negative and positive controls were maintained without surfactant and with Triton X-100 respectively.

Emulsification assay

Three millilitre of cell-free culture broth containing (g l^{-1}), 0.5 g K_2HPO_4, 1 g NH_4Cl, 2 g Na_2SO_4, 2 g KNO_3, 0.2 g $MgSO_4 \cdot 7H_2O$ and 0.002 g $FeSO_4 \cdot 7H_2O$ was mixed with 0.5 ml test oil, vortexed vigorously for 2 min and incubated at 37°C for 1 h at 400 nm using a spectrophotometer Shimadzu UV-1800 and the absorbance of aqueous phase was noted. Likewise, blanks were prepared with sterile growth medium. An absorbance of 0.010 units at 400 nm multiplied by dilution factor was considered as one unit of emulsification activity per ml (EU ml^{-1})[24].

Screening of lipase-producing bacteria

Secluded bacteria were plated onto solid tributyrin agar medium containing (g/l) peptone from meat 2.5, yeast extract 3.0, peptone from casein 2.5, agar–agar 15.0 and 10.0 ml tributyrin (glycerol tributyrate); pH was adjusted to 7.5. Clear zone around the colonies indicates lipase production[25].

Estimation of lipase activity

Lipase optimization for individual strains was tested by growth at 30°C on rotatory shaker (150 rpm) in medium (g/l) comprising glucose 2.0, yeast extract 5.0, sodium chloride 5.0, magnesium chloride 2.0, magnesium sulphate 1.0, calcium chloride 0.4 and potassium dihydrogen phosphate 1.0 supplemented with varying compositions of tributyrin/olive oil (0.5%–5%) at pH 7. Aliquots of the culture supernatant were withdrawn after 48 h and centrifuged at 10,000 rpm for 15 min at 4°C for lipase activity using colorimetric analysis[26]. In this method, *p*-nitrophenolpalmitate (*p*-NPP) was hydrolysed by lipase to give *p*-nitrophenol (*p*-NP), which showed yellow colour absorbance measured spectrophotometrically at 410 nm.

Enzyme solution of 100 µl was added in 900 µl freshly prepared substrate solution which contains one part of solution A (3.0 mM *p*-NPP) in 2-propanol and nine parts of solution B (50 mM Tris buffer (pH 8), 0.4% Triton X 100 and 0.1% Gum Arabic). The mixture was incubated at 37°C for 30 min. The activity was immobilized by 10 min boiling tailed by centrifugation at 8000 *g* for 10 min. The release of *p*-NP was measured at 410 nm against blank containing only buffer maintained at the same condition as above. One unit of enzyme is defined as the amount of enzyme that releases 1 µmol of *p*-NP from the substrate. Protein measurements were carried out by the Lowry's method using bovine serum albumin as standard[27].

Results

Isolation and identification of bacteria

Overall seven strains were initially screened for crude oil degradation by enriching them in ONR7a medium at 30°C for two weeks, among which three strains with efficient growth rate on crude oil were selected. These were characterized microbiologically (Table 1). The result of 16S rRNA sequence alignment performed by NCBI nucleotide BLAST search indicated that the isolated microbes NM1, NM3 and NM6 belonged to *Bacillus* species with GenBank accession numbers KT354277, KT354278 and KX404996 respectively. Construction of phylogenetic tree using MEGA6 software tool (Figure 2) showed the phylogenetic relationship between the isolates and other related oil-degrading microorganisms found in GenBank. The evolutionary history was traced using the neighbour-joining method. The bootstrap consensus tree was deduced from replicates to represent the evolutionary history of the taxa analysed. The evolutionary distances were computed using number of differences method and are given in the units of the number of base differences per sequence. Evolutionary analyses were done using MEGA6 software.

Table 1. Microbiological identification of crude oil degrading marine *Bacillus* species

Test	*Bacillus* sp. NM1 KT354277	*Bacillus* sp. NM3 KT354278	*Bacillus* sp. NM6 KX404996
Shape	Circular	Circular	Circular
Gram character	Gram-positive rod	Gram positive rod	Gram-positive rod
Amylase	–	–	+
Glucose utilization	+	+	–
Gelatinase	+	+	–
Catalase	+	+	+
Urease	+	+	+
Lysine utilization	+	–	–
Ornithine utilization	+	–	–
Nitrate reduction	–	+	–
H_2S production	+	+	–
Citrate utilization	–	–	+
Methyl red	+	–	+
Esculin hydrolysis	+	+	+
Arabinose	–	–	–
Xylose	–	–	–
Cellobiose	–	–	–
Saccharose	+	–	–
Glucose	+	–	–

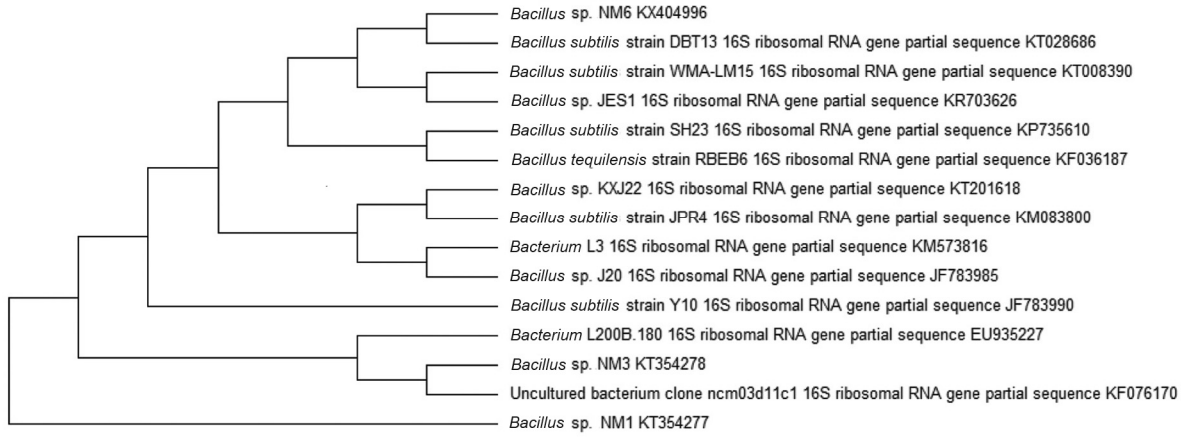

Figure 2. Evolutionary relationships of *Bacillus* sp. NM1 KT354277, *Bacillus* sp. NM3 KT354278 and *Bacillus* sp. NM6 KX404996 with existing oil-degrading microbes.

Growth rate and crude-oil degradation by bacterial isolates

All three bacterial isolates were cultivated in 1% of different types of crude oil – oil 1 (Arab extra crude oil), oil 2 (ONGC crude oil), oil 3 (vessel *MSC Chitra* bunker oil), oil 4 (Mahul Fish Merchant Society Ltd) and oil 5 (BPA oil sample) at 30°C for about one week on a rotary shaker. At a regular interval of 24 h microbial growth and using different types of crude oil, biodegradation was analysed spectrophotometrically. As reported in Table 2, *Bacillus* sp. NM1 KT354277 shows the highest percentage of crude-oil (oil 2) biodegradation (50%) among the other isolated bacteria (Figure 3). Accidental decrease in the percentage of oil degradation by isolates was observed after 72–96 h. This may depend on the half-life exhibited by the compounds present in crude oil. Apart from degradation, losses may be due to volatilization or its partial conversion to bound residues[28]. Microorganisms possess several different mechanisms by which they degrade crude oil compounds, utilizing them as carbon and nitrogen sources. The pattern of degradation varies for different degrading microorganisms as they possess diverse catabolising enzymes[29].

Degradation of petroleum hydrocarbons

All three bacterial isolates were grown in 1% crude oil (Arab extra light crude oil) for one week in a rotary incubator at 30°C. At a regular interval of 24 h, PHC degradation was analysed by fluorescence spectrophotometer. The bacterial isolate NM1 shows the highest percentage of PHC degradation (52%) at the end of 72 h among all isolated bacteria (Figure 4).

Table 2. Growth rate and crude oil degradation by *Bacillus* species

Crude oil used	Rate of crude oil degradation (%)		
	Bacillus sp. NM1 KT354277	*Bacillus* sp. NM3 KT354278	*Bacillus* sp. NM6 KX404996
Oil 1 (Arab extra crude oil)	42.65	38.03	24.35
Oil 2 (ONGC crude oil)	50	38.81	28.42
Oil 3 (Vessel *MSC Chitra* bunker oil)	26.13	35.22	31.95
Oil 4 (Mahul Fish Merchant Society Ltd)	42.22	23.69	19.32
Oil 5 (BPA oil sample)	46.87	26.53	24.86

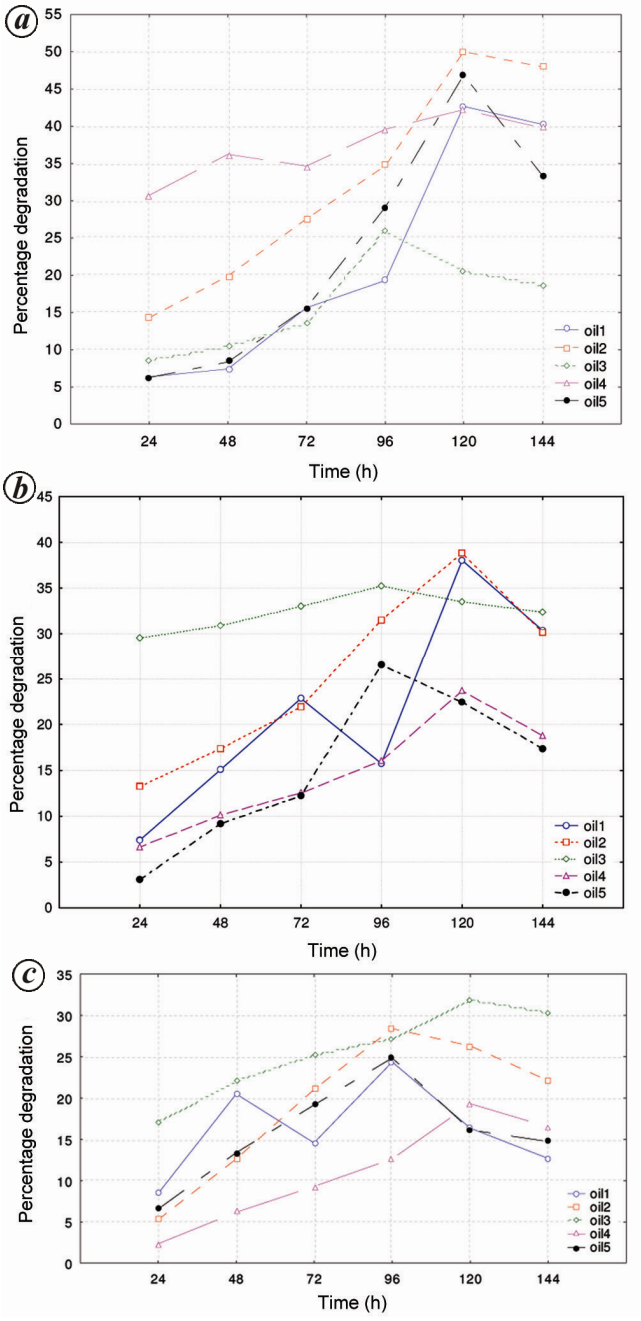

Figure 3. Degradation of different types of crude oil by *Bacillus* species at 30°C for 7 days in controlled condition: ***a***, *Bacillus* sp. NM1 KT354277; ***b***, *Bacillus* sp. NM3 KT354278; ***c***, *Bacillus* sp. NM6 KX404996.

Drop collapse test, oil spreading assay and emulsification assay

Plaza *et al.*[22] proposed the drop collapse technique as a quantitative method to assess biosurfactant production[30]. However the present study involves the use of this technique for qualitative estimation. Morikawa *et al.*[23] illustrated the proportionality between the expanse of displacement by surfactant and its concentration. These results confirmed the presence or absence of the surface active compound in the cell-free culture broth. Emulsification activity was employed as a criterion for primary screening of biosurfactants producing-bacteria. Table 3 indicates that all the three bacterial isolates show a varied degree of response for the mentioned tests.

Lipase activity

Lipase activity of *Bacillus* sp. NM1 KT354277, *Bacillus* sp. NM3 KT354278 and *Bacillus* sp. NM6 KX404996 was estimated (Table 4). *Bacillus* sp. NM1 KT354277 showed lipase activity of 58.72 U ml^{-1} using olive oil (2%) as a substrate, and 56.45 U ml^{-1} of using tributyrin (2%). *Bacillus* sp. NM3 KT354278 was found to have the highest lipase activity of 60.72 and 61.19 U ml^{-1} for 2% olive oil and tributyrin respectively, whereas in the case of *Bacillus* sp. NM6 KX404996 lipase activity was moderate for both the substrates.

Discussion

Yakubu[31] found that microorganisms with oil-degrading potential are widely dispersed across nature and have been isolated in multiple ecosystems. Similar kinds of studies have been reported for microbial capability exploiting oil and its derivatives as a source of carbon and energy[32,33]. Recently, Rodriguez-R *et al.*[34] reported that the successional patterns of microbial communities respond to crude oil agitation, supporting the specialization disturbance hypothesis during degradation.

In the present study, different crude oil degrading bacteria were isolated from the coastal area of Vadinar. Three among the seven isolated strains were selected for the study. Efficiency of crude-oil degradation of the

Table 3. Drop collapse, oil spreading assays and emulsification activity

Bacterial isolate	Crude oil used	Drop collapse test[a]	Oil spreading assay[b]	Emulsification assay[c]
Bacillus sp. NM1 KT354277	Oil 1	+++	+	++
	Oil 2	++	++	+++
	Oil 3	NA	++	+
	Oil 4	++	+	++
	Oil 5	+++	+	NA
Bacillus sp. NM3 KT354278	Oil 1	+	++	+
	Oil 2	++	NA	++
	Oil 3	++	+	NA
	Oil 4	NA	+	+++
	Oil 5	+++	NA	+
Bacillus sp. NM6 KX404996	Oil 1	+++	++	++
	Oil 2	++	+	+++
	Oil 3	+	++	++
	Oil 4	++	NA	+
	Oil 5	+	NA	+

[a]NA, No drop collapse activity; +++, Drop collapse within 1 min; ++, Drop collapse after 1 min; +, Drop collapse after 3 min of bacterial culture addition. [b]NA, No oil spreading activity; +, Oil spreading with a clear zone of 0.5–1.0 cm; ++, Oil spreading with a clear zone of 1.1–2.0 cm; +++, Oil spreading with a clear zone of 2.1–2.5 cm. [c]NA, No emulsification; +++, Emulsification activity $D_{400} \geq 3$, ++, $D_{400} \geq 2$; and +, $D_{400} \geq 1$.

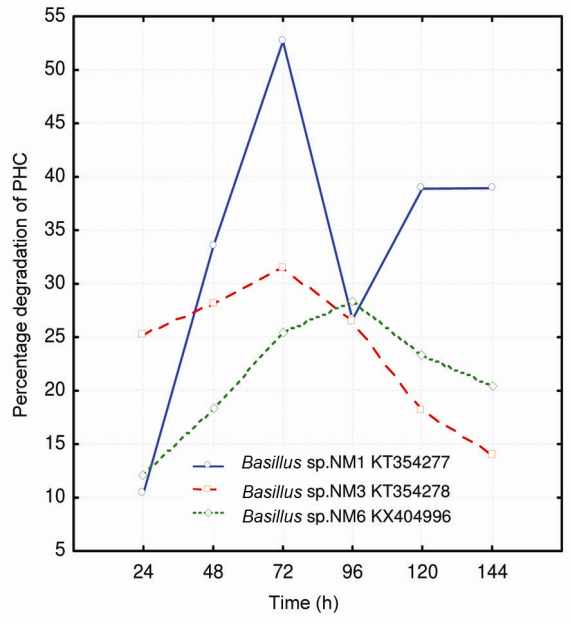

Figure 4. Degradation of petroleum hydrocarbon by *Bacillus* species at 30°C for 7 days in controlled condition.

specific bacterial cultures was established, and the results indicate that *Bacillus* sp. NM1 KT354277 shows maximum degradation of 50% within 120 h of incubation. Okerentugba and Ezeronye[35] suggested that active degraders may be isolated from an environment containing high loads of hydrocarbon; our findings also support this result.

The survival of microorganisms in oil is a decisive aspect in the degree of biodegradation of hydrocarbons either in soil or in the liquid phase[36]. *Bacillus* sp. NM1 KT354277 showed the highest percentage of PHC degradation (52%) at the end of 72 h. Bodour *et al.*[37] suggested the drop collapse test to determine biosurfactants production in a natural ecosystem. Curiosity in microbial surfactants has been cumulative in recent years, since they have several benefits in contrast to chemical surfactants counting on lower toxicity, better compatibility and effectiveness at extreme conditions. Majority of surfactants are of bacterial origin reported as *B. amyloliquefaciens* An6 (ref. 38) and *B. subtilis*, *B. stearothermophilus* and *B. thermoglucosidasius*, *B. coagulans*, *Candida* and *Rhodococcus*[39,40]. Recently, Nerurkar *et al.*[41] reported isolation of lipase from marine bacteria *Bacillus sonorensis* for bioscouring of impurities from cotton fabric. In the present study, the highest lipase activity of 58.72 and 56.45 U ml^{-1} was attained using olive oil (2%) and tributyrin (2%) respectively. Olive oil may be considered an economically viable substrate for lipase production.

Biosynthesis of biosurfactants was also explored by means of drop collapse and oil spreading tests[30]. Selection of these conventional methods has been attributed to their advantages like counting ease, low cost and quick execution[42,43]. The oil spreading technique lacks sensitivity in detecting minute concentrations of biosurfactants in view of the fact that all bacterial isolates that were positive by drop collapse method did show the same by the oil spreading technique.

Emulsification assay is a subsidiary method to examine the production of biosurfactants. Ojo[44] studied that the presence of oil-degrading organisms in the polluted soil and water is clear indication that the indigenous microbes were carrying out their metabolic action, liable for the bioremediation[44].

Table 4. Estimation of lipase activity using different substrates

Lipid source	Lipid concentration (%)	Lipase activity (U ml^{-1})	Protein (mg ml^{-1})	Dry weight (mg ml^{-1})	Specific activity (U mgl^{-1})
Bacillus sp. NM1 KT354277					
Olive oil	0.5	45.972 ± 0.38	0.302	3.15	152.22
	1	46.819 ± 0.62	0.648	4.16	72.25
	2	58.722 ± 1.42	0.664	8.97	88.43
	5	40.372 ± 1.40	0.994	5.82	40.61
Tributyrin	0.5	41.097 ± 0.22	0.741	4.12	55.46
	1	49.555 ± 0.09	0.708	6.34	69.99
	2	56.458 ± 3.86	0.644	10.18	87.66
	5	31.916 ± 0.34	0.159	8.94	200.72
Bacillus sp. NM3 KT354278					
Olive oil	0.5	40.930 ± 0.90	0.286	5.34	134.11
	1	43.236 ± 0.33	0.437	4.28	98.93
	2	60.722 ± 0.31	0.397	9.69	152.95
	5	35.5 ± 0.16	0.437	7.16	81.23
Tributyrin	0.5	38.055 ± 0.37	0.572	3.42	66.52
	1	52.791 ± 0.54	0.771	4.21	68.47
	2	61.194 ± 0.34	0.461	8.17	132.74
	5	39.875 ± 1.59	0.604	7.01	66.01
Bacillus sp. NM6 KX404996					
Olive oil	0.5	34.625 ± 0.39	0.604	3.57	57.32
	1	45.694 ± 0.87	0.731	5.61	62.50
	2	61.5 ± 1.09	0.700	9.87	87.85
	5	36.44 ± 0.42	0.517	7.67	70.48
Tributyrin	0.5	36.319 ± 1.51	0.700	4.64	51.88
	1	53.722 ± 0.21	0.413	5.82	130.07
	2	54.777 ± 0.41	0.922	6.12	59.41
	5	37.166 ± 1.51	0.350	5.17	106.18

±, Standard deviation (based on triplicates).

Thus, the overall results indicate that the studied bacterial isolates can prevent the contamination of oil-polluted areas in future. The present study shows that *Bacillus* species with efficient emulsification activity, drop collapse test and oil spreading assay can be used for marine oil bioremediation. Among the many disasters caused by anthropogenic activities, marine oil spills are one of the most hazardous, which threaten the marine environment. Thus, the efficient rate of crude oil degradation by bacterial isolates used in present study may be a suitable and promising method of pertaining such marine microorganisms in oil contaminated areas and deliver new acumens for a better perceptive of the potential significances of bio-treatments on the oil fate in polluted marine waters and also immobilized lipase isolated from indigenous strains can be used for commercial applications.

Conflict of interest statement: There is no conflict of interest.

1. Head, I. M., Jones, D. M. and Roling, W. F., Marine microorganisms make a meal of oil. *Nature Rev. Microbiol.*, 2006, **4**, 173–182.
2. Emtiazi, G., Saleh, T. and Hassanshahian, M., The effect of bacterial glutathione S-transferase on morpholine degradation. *Biotechnol. J.*, 2009, **4**, 202–205.
3. Hassanshahian, M., Emtiazi, G. and Cappello, S., Isolation and characterization of crude-oil-degrading bacteria from the Persian Gulf and the Caspian Sea. *Mar. Pollut. Bull.*, 2012, **64**, 7–12.
4. McGenity, T. J., Folwell, B. D., McKew, B. A. and Sanni, G. O., Marine crude-oil biodegradation: a central role for interspecies interactions. *Aquat. Biosyst.*, 2012, **8**, 10.
5. Wafula, D., Chauhan, A., Green, S. J., Gragg, R. and Jagoe, C., A survey of deepwater horizon (DWH) oil-degrading bacteria from the eastern oyster biome and its surrounding environment. *Front. Microbiol.*, 2014, **5**, 149.
6. Okoh, A. I., Biodegradation alternative in the cleanup of petroleum hydrocarbon pollutants. *Biotechnol. Mol. Biol. Rev.*, 2006, **1**, 38–50.
7. Atlas, R. M. and Bartha, R., Hydrocarbon biodegradation and oil spill bioremediation. *Adv. Microb. Ecol.*, 1992, **12**, 287–338.
8. Beolchini, F., Rocchetti, L., Regoli, F. and Dell'Anno, A., Bioremediation of marine sediments contaminated by hydrocarbons: experimental analysis and kinetic modeling. *J. Hazard. Mater.*, 2010, **182**, 403–407.
9. Wardlaw, G. D., Nelson, R. K., Reddy, C. M. and Valentine, D. L., Biodegradation preference for isomers of alkylated naphthalenes and benzothiophenes in marine sediment contaminated with crude oil. *Org. Geochem.*, 2011, **42**, 630–639.
10. Laha, S., Liu, Z., Edwards, D. A. and Luthy, R. G., Surfactant solubilization of phenanthrene in soil–aqueous systems and its effects on biomineralization. *Adv. Chem. Ser.*, 1995, **244**, 339–361.

11. Bordoloi, N. K. and Konwar, B. K., Bacterial biosurfactant in enhancing solubility and metabolism of petroleum hydrocarbons. *J. Hazard. Mater*., 2009, **170**, 495–505.
12. Banat, I. M., Biosurfactants production and possible uses in microbial enhanced oil recovery and oil pollution remediation: a review. *Bioresour. Technol*., 1995, **51**, 1–12.
13. Ron, E. Z. and Rosenberg, E., Biosurfactants and oil bioremediation. *Curr. Opin. Biotechnol*., 2002, **13**, 249–252.
14. Bao, M. T., Wang, L. N., Sun, P. Y., Cao, L. X., Zou, J. and Li, Y. M., Biodegradation of crude oil using an efficient microbial consortium in a simulated marine environment. *Mar. Pollut. Bull*., 2012, **64**, 1177–1185.
15. Cappello, S., Santisi, S., Calogero, R., Hassanshahian, M. and Yakimov, M. M., Characterization of oil-degrading bacteria isolated from bilge water. *Water Air Soil Pollut*., 2012, **223**, 3219–3226.
16. Dyksterhouse, S. E., Gray, J. P., Herwig, R. P., Lara, J. C. and Staley, J. T., *Cycloclasticus pugetii* gen. nov., sp. nov., an aromatic hydrocarbon-degrading bacterium from marine sediments. *Int. J. Syst. Bacteriol.*, 1995, **45**, 116–123.
17. Krieg, N. R., *Bergey's Manual of Determinative for Bacteriology*, Williams and Wilkins, New York, 1998.
18. Tamura, K., Stecher, G., Peterson, D., Filipski, A. and Kumar, S., MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol. Biol. Evol*., 2013, **30**, 2725–2729.
19. Rahman, K. S. M., Banat, I. M., Thahira, J., Thayumanavan, T. and Lakshmanaperumalsamy, P., Bioremediation of gasoline contaminated soil by a bacterial consortium amended with poultry litter, coir pith and rhamnolipid biosurfactant. *Bioresour. Technol*., 2002, **81**, 25–32.
20. *Manual for Monitoring Oil and Dissolved/Dispersed Petroleum Hydrocarbons in Marine Waters and on Beaches*, UNESCO, Paris, 1984, vol. 35.
21. Law, R. J., Kelly, C. A., Graham, K. L., Woodhead, R. J., Dyrynda, P. E. and Dyrynda, E. A., Hydrocarbons and PAH in fish and shellfish from southwest wales following the sea empress oil spill in 1996. In International Oil Spill Conference, UK, 1997, vol. 1, pp. 205–211.
22. Płaza, G. A., Zjawiony, I. and Banat, I. M., Use of different methods for detection of thermophilic biosurfactant-producing bacteria from hydrocarbon-contaminated and bioremediated soils. *J. Pet. Sci. Eng*., 2006, **50**, 71–77.
23. Morikawa, M., Hirata, Y. and Imanaka, T., A study on the structure–function relationship of lipopeptide biosurfactants. *Biochim. Biophys. Acta*, 2000, **1488**, 211–218.
24. Patil, J. R. and Chopade, B. A., Studies on bioemulsifier production by *Acinetobacter* strains isolated from healthy human skin. *J. Appl. Microbiol*., 2001, **91**, 290–298.
25. Smibert, M. and Krieg, N. R., Systematics: general characterization. In *Manual of Methods for General Bacteriology*, American Society for Microbiology, Washington DC, 1981, pp. 409–443.
26. Pera, L. M., Romero, C. M., Baigori, M. D. and Castro, G. R., Catalytic properties of lipase extracts from *Aspergillus niger*. *Food Technol. Biotechnol*., 2006, **44**, 247–252.
27. Lowry, O. H., Rosebrough, N. J., Fax, A. L. and Randall, R. J., Protein measurement with the folin phenol reagent. *J. Biol. Chem.*, 1951, **193**, 265–275.
28. Johnsen, A. R., Wick, L. Y. and Harms, H., Principles of microbial PAH-degradation in soil. *Environ. Pollut*., 2005, **133**, 71–84.
29. Penet, S., Vendeuvre, C., Bertonicini, F., Marchal, R. and Monot, F., Characterization of biodegration capacities of environmental microflorae for diesel oil by comprehensive two-dimensional gas chromatography. *Biodegradation*, 2006, **17**, 577–585.
30. Bodour, A. A. and Miller-Maier, R. M., Application of a modified drop-collapse technique for surfactant quantitation and screening of biosurfactant-producing microorganisms. *J. Microbiol. Methods*, 1998, **32**, 273–280.
31. Yakubu, M., Biodegradation of Lagoma crude oil using pig dung. *Afr. J. Biotechnol.*, 2007, **6**, 2821–2825.
32. Magot, M., Indigenous microbial communities in oil fields. In *Petroleum Microbiology*, American Society of Microbiology Press, Washington DC, 2005, pp. 21–34.
33. Khan, J. A. and Asthana, A., A study on oil degradation potential of *Bacillus megaterium* isolated from oil contaminated sites in Lucknow. *Arch. Appl. Sci. Res*., 2011, **3**, 513–517.
34. Rodriguez-R, L. M., Overholt, W. A., Hagan, C., Huettel, M., Kostka, J. E. and Konstantinidis, K. T., Microbial community successional patterns in beach sands impacted by the Deepwater horizon oil spill. *ISME*, 2015, **9**, 1928–1940.
35. Okerentugba, P. O. and Ezeronye, O. U., Petroleum degrading potentials of single and mixed microbial cultures isolated from rivers and refinery effluent in Nigeria. *Afr. J. Biotechnol*., 2003, **2**, 288–292.
36. Ramos, J. L., Duque, E. and Ramos-Gonzalez, M. I., Survival in soils of an herbicide-resistant *Pseudomonas putida* strain bearing a recombinant TOL plasmid. *Appl. Environ. Microbiol*., 1991, **57**, 260–266.
37. Bodour, A. A., Drees, K. P. and Maier, R. M., Distribution of biosurfactant-producing bacteria in undisturbed and contaminated arid southwestern soils. *Appl. Environ. Microbiol*., 2003, **69**, 3280–3287.
38. Ayed, H. B., Jemil, N., Maalej, H., Bayoudh, A., Hmidet, N. and Nasri, M., Enhancement of solubilization and biodegradation of diesel oil by biosurfactant from *Bacillus amyloliquefaciens* AN6. *Int. Biodeterior. Biodegrad.*, 2015, **99**, 8–14.
39. Hamid, N. S. A., Zen, H. B., Tein, O. B., Halifah, Y. M., Saari, N. and Bakar, F. A., Screening and identification of extracellular lipase-producing thermophilic bacteria from a Malaysian hot spring. *World J. Microbiol. Biotechnol*., 2003, **19**, 961–968.
40. Haba, E., Bresco, O., Ferrer, C., Marques, A., Busquets, M. and Manresa, A., Isolation of lipase-secreting bacteria by deploying used frying oil as selective substrate. *Enzyme Microb. Technol.*, 2000, **26**, 40–44.
41. Nerurkar, M., Joshi, M. and Adivarekar, R., Bioscouring of cotton using lipase from marine bacteria *Bacillus sonorensis*. *Appl. Biochem. Biotechnol*., 2015, **175**, 253–265.
42. Youssef, N. H., Duncan, K. E., Nagle, D. P., Savage, K. N., Knapp, R. M. and McInerney, M. J., Comparison of methods to detect biosurfactant production by diverse microorganisms. *J. Microbiol. Methods*, 2004, **56**, 339–347.
43. Ibrahim, M. L., Ijah, U. J. J., Manga, S. B., Bilbis, L. S. and Umar, S., Production and partial characterization of biosurfactant produced by crude oil degrading bacteria. *Int. Biodeterior. Biodegrad*., 2013, **81**, 28–34.
44. Ojo, O. A., Petroleum-utilization by native bacterial population from a wastewater canal Southwest Nigeria. *Afr. J. Biotechnol*., 2006, **5**, 333–337.

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