ejpmr, 2015,2(5), 541-555.

SJIF Impact Factor 2.026

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 3294-3211

EJPMR

ISOLATION, IDENTIFICATION AND CHARACTERIZATION OF OIL DEGRADING MARINE BACTERIA FROM SANDY SHORE OF SHIVAJI PARK, MUMBAI.

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Article Received on 12/07/2015 **Article Revised on 03/08/2015** **Article Accepted on 24/08/2015**

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ABSTRACT

Marine ecosystem is known for abundance of life represented by micro, meso and macro organisms exhibiting a variety of biodiversity. The composition, spatial distribution and survival techniques of these organisms rely on the niche they prefer. Marine microbes viz. bacteria are of great interest due to variety of applications and their well-defined requirements such as seawater, especially sodium for growth. Although marine microorganisms are not well identified taxonomically, preliminary studies indicated that the applications of these microbes made a promising frontier in the area of drug development, food industry, and biological products like biosurfactants and complex processes like bioremediation. Marine oil pollution due to oil slicks was remediated artificially by chemical and biologically tailored components. The current study is aimed at isolating, identifying and characterizing marine bacterial species capable of Degrading different types of oils from the sandy shores of Mumbai coast.

KEYWORDS: Oil Degrading Marine Microbes, Mumbai Sand, Shivaji Park, Biosurfactant, *P. aeruginosa*, *S. aureus*, *Bacillus* spp, *Micrococcus* spp, *Aeromonas* spp.

INTRODUCTION

Marine biotechnology is one of the most interesting fields of research in today's life. Due to their ability to withstand extreme conditions and easy availability marine bacteria are considered for research. Bacteria isolated from coastal marine waters, sea shore sand are capable of degrading organic complex pollutants like diesel, petrol, PAHs etc. (Jyothi et al., 2011, Teli Nikhil et al., 2013, Chithra et al., 2014, Pawar et al., 2013, Sharma et al., 2001,

Arpita et al., 2015, Kostka et al., 2011).These microorgansims with their diverse structure, physiology and metabolism survive in extreme halophilic, thermophilic and acidophilic conditions. Halophiles are a class of extremophiles with interesting characters such as adaptability to harsh hyper saline conditions, resistance to denaturing effects of salts and competition for water. Due to this reason they grow over a wide range of salt concentrations viz hyper saline brine in arid, coastal and deep-sea locations as well as in artificial salters used to mine salts from the sea along with halotolerants (Shiladitya, 2001). An important ecological function of the halophilic bacteria is to bioremediate, recycle of nutrients, degrade and / or detoxify chemical substances including metals, petroleum products, aliphatic and aromatic hydrocarbons, industrial solvents, pesticides and their metabolites (Chandrakant et al., 2011). The toxic effects of oil pollution on biota and environment are devastating (Elliot, 1997). There is a need to identify bacteria which can naturally synthesize surfactants that are capable of enhancing the bioremediation with lower toxicity. The present study has been undertaken to isolate marine bacteria from coast of Mumbai, Maharashtra, to characterize and to evaluate their ability to degrade different types of oils.

MATERIALS AND METHODS

Surface sea water and (episamic) sand samples were collected from coast of Shivaji Park area of Mumbai, Maharashtra. Water samples were collected in sterile containers at 20 meters distance from the shore and at a depth of about 30cm, and surface sand away from off shore. Collected samples were carefully added with sterile Artificial Sea Water (ASW), and transported to the laboratory in an ice box for bacterial isolation and characterization procedures.

Mineral salts media was used for the bacterial collection and isolation along with Nutrient agar. Gram's staining, Cultural, biochemical tests were performed based on Bergey's manual. Oil spread test and Oil degrading tests were performed as per the standard procedures to characterize biosurfactant production ability and oil degrading capacity of isolates. The chemicals used were of analytical grade obtained from Hi media and Loba, Mumbai.

Samples collected from marine waters and sand samples were serially diluted up to 10^{-6} using sterile saline. Enumeration of the bacterial colonies in each diluted sample was made using pour plate technique and Nutrient agar media. The plates were incubated at 27^{o}C for 48 hr.

Isolation of Oil degrading bacteria

Isolated bacterial colonies from Nutrient Agar were initially enriched in the MSM media broth. A loopful from the initial growth was spread on to the sterile mineral salts medium (MSM) agar plate. The plates and the MSM broth were incubated at 27^{o}C for 48 hr. The colonies with clear circular zones against a cloudy field were identified as oil-degrading bacteria (Z. Liu, 1995). The composition of Mineral salts medium (MSM) has 170 mg of KH_2PO_4, 435 mg of K_2HPO_4, 668 mg of $Na_2HPO_4.7H_2O$, 85 mg of NH_4Cl, 22.5 mg of $MgSO_4$ $.7H_2O$, 27.5 mg of $CaCl_2$, and 0.25 mg of $FeCl_3.6H_2O$ per liter of de ionized water, pH 7.2. Sterilization of MSM was carried by autoclaving at 121°C for 15 min and supplemented with 1% (v/v) filter sterilized hydrocarbons (test compound oil i.e. Edible oil / Engine Oil / Bunker Oil) to serve as carbon and energy source for bacteria and later isolated on MSM media plates.

Pure cultures of morphologically distinguishable isolates were obtained by subsequent sub-culturing thrice on Nutrient agar slants. Pure cultures of bacterial isolates were reconfirmed on MSM oil plates which were used to study the colony characteristics.

Identification of bacterial isolates

Based on Oil degrading capacity of the microorganisms, further tests such as Gram's staining, cultural and biochemical tests were performed on the selected isolates. Genus level bacterial identification was based on their colonial morphology, cellular morphology and biochemical characteristics according to the taxonomic scheme of Bergey's Manual of Determinative Bacteriology (Bergey's manual of determinative bacteriology, 1994). Oil spread test, Oil degrading tests were considered to characterize the biosurfactant production ability and oil degrading capacity of isolates.

Tests performed

a. Gram staining procedure

a) A loop full of microbial colony from a 24hr culture slant was smeared on clean glass slide and heat fixed.

b) Flood was smeared with Crystal violet and incubated for 30 seconds.

c) Drained off the CV on the slide and washed with running tap water.

d) Smear was flooded with Gram's Iodine and the slide was washed with water after 30 seconds incubation.

e) Decolorize the slide with alcohol and wash with tap water continuously until no color

remains.

f) Counter stain the slide with Saffranin for 60 seconds, wash with tap water and dry.

g) The slide was then observed under a microscope with a 100X oil immersion lens.

b. Cultural Characteristics

The cultural characters of the selected isolates were determined by studying the following parameters.

i) Temperature tolerance: The growth of the isolates was observed at different temperatures between 4°C and 45°C. Tubes with 5ml NB broth were inoculated with 0.1ml of 48hrs. Culture and incubated at different temperatures for 2 days. The tubes were observed for visible turbidity at 24hr intervals and optimum temperature for growth was recorded.

ii) pH tolerance: The pH tolerance of the selected isolated was tested. A pH range between 4-10 was considered. Tubes of sterile NB broth with different pH grades was prepared and inoculated with a 48hr old culture. The turbidity of the tubes was observed after 2 days incubation at 37°C.

iii) Tolerance to salt: As suggested by Novick and Tyler (1985), different salt concentrations of sterile NB broth were prepared by supplements of respective salt concentration of 1.2%, 2.5%, 5%, 10%, and 20%. Tubes were inoculated with a 48hr culture and incubated for 2 days at 37°C. The turbidity of the tubes was observed.

c. Biochemical characterization

Characterization of the isolates were carried out by performing various biochemical tests adopting standard protocols as described in "Practical Medical Microbiology Manual" (Mackie and McCartney, 1989), unless otherwise specially mentioned. The procedure in all the cases was suitably modified by using NB medium or supplementing the required media with 1.2% NaCl to facilitate the growth of halophilic bacteria. Known positive and negative organisms for the various biochemical tests performed were included as controls.

i) Sugar utilization test

Sugar utilization levels of the selected isolated was determined as mentioned by Novick and Tyler, 1985. Phenol red (0.005%) was considered as indicator due to its less toxicity towards marine bacteria than bromothymol blue. Artificial seawater supplemented with 0.1% NH4Cl, 0.005Mphosphate buffer (pH 7.0) was used as basal medium for sugar utilization and 10µl of

Phenol Red was added for every 5ml media. 5% stock solutions of various carbohydrates were prepared and sterilized at 10 lbs. The stock solutions were added to 5ml of the basal medium to get a final concentration of 0.5% of the respective sugars. The tubes were inoculated with 0.1ml of 48hr old culture (~10^5 cells/ml) and incubated at 37°C for 24-48hrs. The utilization of sugars along with the production of acid/gas was recorded.

ii) Indole test

Selected isolates were checked for their ability to convert the amino acid tryptophan to indole was tested by adding 0.5ml of Kovac's reagent to 48hr old culture of the isolates in peptone water supplemented with 1.2% NaCl. The production of red color in the alcohol layer indicates a positive Indole reaction.

iii)Methyl Red (MR) test

Bacteria's ability to ferment glucose and produce acid was determined using Glucose Phosphate Peptone medium (2P) supplemented with 1.2% NaCl. Methyl red indicator (2-3 drops) was added to 48hr old culture tubes and results were recorded immediately. Appearance of bright red color in the medium indicated a positive reaction. *E. coli* and *K. pneumoniae* were used as positive and negative controls respectively for MR and VP tests.

iv)Voges – Proskauer (VP) test

The selected isolates ability to produce acetoin as the end product was detected by adding 0.5ml of O' meara reagent to 48hr old culture tubes of the marine isolates in GPP media. The development of eosin pink color indicates a positive reaction.

v) Citrate utilization test

The ability of the organism to utilize citrate as the sole source of carbon and energy for growth and an ammonium salt as the sole source of nitrogen was determined by streaking Isolates on Simmon's Citrate Agar slant (pH 6.8) and incubating for 96hrs at 37°C. A positive test indicated change in the color of the medium from green to blue and appearance of a streak of growth. *K. pneumoniae* and *E. coli* were used as positive and negative controls for VP and Citrate tests.

vi)Catalase Test

The catalase test primarily useful in differentiating between genera, it is also valuable in speciation of certain gram positives from gram negative organisms. The production of

catalase enzyme, which mediates the release of oxygen from hydrogen peroxide, was tested by picking up single colony from a 48hrs old plate culture and introducing into 1ml of 37% of hydrogen peroxide. The appearance of effervescence / gas bubbles from the surface of the liquid indicated a positive test. *Staphylococcus aureus* and *Streptococcus pneumoniae* were used as positive and negative controls for the test respectively.

vii)TSI slant test

Ability of bacteria to use any of the triple sugar and to produce acid or alkali in 8hrs was tested by streaking on TSI slant and incubating for 8- 24hrs at 37°C. Acid production was indicated by yellow coloration after 24hrs in slant and alkali was indicated by initially yellow and later by development of brown coloration in slant.

viii)Urease test

This test was used to distinguish urease-positive bacteria from other Enterobacteriaceae spps. The production of urease enzyme was tested by streaking the culture on Christensen's medium supplemented with 1.2% NaCl (pH 6.8-6.9). The appearance of purple pink color growth indicated a positive reaction. *Proteus vulgaris* and *E.coli* were used as positive and negative controls for Urease tests.

ix) Oxidase test

The oxidase test is a biochemical reaction that assays for the presence of cytochrome oxidase, an enzyme sometimes called indophenol oxidase. Production of oxidase enzyme was tested by dipping the TMPD discs in 48hrs old culture. Color change of white disc to purple indicated positive results.

x) Coagulase test

The coagulase test differentiates strains of Staphylococcus aureus from other coagulase-negative species. Production of coagulase enzyme to clot rabbit blood was tested by incubating single colony from a 48hrs old plate culture and introducing into 1ml of rabbit blood. *Staphylococcus aureus* and *Streptococcus epidermidis* were used as positive and negative controls for the test respectively.

d. characteristics test

1. Oil Spreading test

Oil spreading assay was performed for the selected isolates as by Morikawa et al., 2000. The

petriplate base was filled with 50 ml of distilled water. On the water surface, 20 µl of corresponding oil and 10 µl of culture supernatant were added respectively. The culture was introduced at different spots on the oil which is coated on the water surface. The occurrence of a clear zone indicates positive result (Plaza G., 2006, Vanessa Walter et al., 2000).

2. Oil degradation test: (zone of clearance) (mm)

A colony from pure culture was incubated on to the sterile mineral salts medium (MSM) plate, consisting of 170 mg of KH_2PO_4, 435 mg of K_2HPO_4, 668 mg of $Na_2HPO_4.7H_2O$, 85 mg of NH_4Cl, 22.5 mg of $MgSO_4$ $.7H_2O$, 27.5 mg of $CaCl_2$, and 0.25 mg of $FeCl_3.6H_2O$ per liter of deionized water, 20 gm of agar pH 7.2. The medium without hydrocarbons was sterilized by autoclaving at 121°C for 15 min. The medium was supplemented with 1% (v/v) filter sterilized hydrocarbons (test compound oil i.e. Edible oil/Engine Oil/Bunker Oil) to serve as the only source of carbon and energy. This ability of degrading different oils was determined at various temperatures. i.e. at 45^oc, 37^oC, 25^oC for 7 days. Clear circular zones around the colonies of the oil-degrading bacteria were measured (Z. Liu, 1995).

RESULTS

Most of the marine isolates found to be slow growers and formed discrete colonies only after 2 days incubation at 27°C on Nutrient agar plates. The initial isolation of bacterial colonies was followed by observing oil degradation, colony morphology and pigment production.

Identification of Bacterial Isolates

Oil degrading microorganisms

After isolation of the bacterial cultures onto MSM media (1% test compound oil i.e. Edible oil/Engine Oil/Bunker Oil), six different types of bacteria showed zone of clearance with different types of oils. The isolates were designated as Isolate 5, 7, 9, 19, M1, M2.

Of these, isolates 9 and M1 showed high capability to degrade vegetable oils and bunker crude oil, and M1 and M2 in the case of Engine oil. When compared with clearance zones 9 and M1 showed higher level of deteriorating the vegetable oils than the engine and bunker crude oils (Table - 1).

These isolates were selected for further cultural and biochemical characterization.

Table - 1: Clearance zones of isolates (mm±SD) after an incubation of 48hrs at 27° C

Type/ Isolates	5	7	9	19	M1	M2
Vegetable Oil	5 ± 1.50	4 ± 0.85	6 ± 0.24	4 ± 0.27	6 ± 1.33	4 ± 0.67
Engine Oil	3 ± 0.42	2 ± 0.50	3 ± 1.01	1 ± 0.20	5 ± 0.67	5 ± 0.98
Bunker Crude Oil	1 ± 0.07	-	2 ± 0.26	1 ± 0.49	2 ± 0.85	1 ± 0.03

Morphological characters of marine isolates

Morphological characters of selected six isolates indicated that two gram negative and four gram positive of which two were spore forming rods.(Table - 2)

Table - 2: Colony morphology and Gram staining observations of selected isolates

Isolates	Colony Morphology	Microscopic Observation
5	Off white large colonies, irregular shape, dry, flat 5-6 mm in diameter with lobate margine	Spore forming Gram + ve rods
7	White colonies, circular, with entire margins minute in diameter	Spore forming Gram +ve rods
9	Yellow pinhead colonies, circular, with entire margins minute in diameter	Gram +ve cocci
19	Off white colonies, circular, with entire margins measuring 1-4 mm in diameter.	Gram -ve bacilli
M1	Fluorescent Cream colonies, circular, with entire margins measuring 1-4 mm in diameter.	Gram -ve bacilli
M2	Circular, pinhead, convex colonies with entire margins	Gram +ve cocci

Cultural characteristics: The cultural characters of the distinct morphological isolates were studied to determine the conditions of growth. Growth of the isolates was studied at different temperatures, pH and tolerance to salt concentration (Table - 3).

Isolates 5, 9, M1 and M2 showed wide temperature tolerance ranging from 27-45°C. Whereas, isolates 7 and 19 could not grow at 4°C and 45°C but showed growth at 27-37 °C.

Isolates 5, 9, and M1 showed wide range of salt tolerance from 1.2 to 10%, followed by 19 and M2 up to 5% and least tolerance was observed with isolate 7 (only up to 5%).

Isolate 9 could grow at varied range of pH from 5-10 including highly alkaline pH, whereas similar results were observed with isolate M1 which grew in highly acidic pH. Isolates 5 and 7 showed moderate pH tolerance ranging from pH 5 – 9, followed by isolates 19 and M2.

Table - 3: Cultural characteristics of isolates with physical parameters

Isolate	Temp (°C)				Salt tolerance (%)					pH						
	4	27	37	45	1.2	2.5	5.0	10	20	4	5	6	7	8	9	10
5	-	+	+	+	+	+	+	+	-	-	+	+	+	+	+	-
7	-	+	+	-	+	+	-	-	-	-	+	+	+	+	+	-
9	-	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+
19	-	+	+	-	+	+	+	-	-	-	+	+	+	+	-	-
M1	-	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-
M2	-	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+

Biochemical characterization: Initial characterizations of the isolates were carried out by conducting sugar utilization tests, IMViC and other biochemical tests. Based on the morphological and biochemical characters attempts were made to categorize the isolates up to genus level.

Table - 4: Biochemical analysis of selected isolates

Isolate	5	7	9	19	M2	M1
Sucrose	+	-	+	+	+	-
Glycerol	+	+	+	+	+	+
Glucose	+	+	+	+	-	+
Galactose	+	+	+	+	-	+
Fructose	+	+	+	+	+	+
Starch	+	-	+	+	-	-
Mannitol	-	-	+	-	-	-
Indole	-	-	-	-	-	-
MethylRed	-	-	-	-	+	-
Voges Proskaeur	+	-	-	-	-	+
Citrate	+	-	+	+	-	+
Urease	-	+	+	+	+	+
Oxidase	-	-	-	+	+	+
Catalase	-	+	+	+	+	+
Coagulase	+	V	+	-	-	-
TSI	A/A	K/A	A/K	V	K/NC	K/K

(Key: A/A – Acid/Acid, K/A- Alkaline/ Acid, A/K- Acidic/Alkaline, V- Variable, K/NC- Alkaline/ no consistency K/K- Alkaline/ Alkaline)

Identification of the isolates: Based on morphological, biochemical and Selective tests, Isolates were tried to be classified under given genus as isolate 5 and 7 belonged to Bacillus genus and were named as *Bacillus* spp I and *Bacillus* spp II respectively, Isolate 9 as *Staphylococcus aureus*, Isolate 19 as *Aeromonas* spp, M1 as *Pseudomonas aeruginosa*, and M2 from *Micrococcus* spp.

Special Characteristic tests

Screening for Biosurfactant Activity: (mm±SD)

If biosurfactant is present in the supernatant, the oil is displaced and a clearing zone is formed when respective culture supernatant is added on the corresponding test compound oil loaded on water surface at room temperature and allowed to settle for 2-3hrs. The diameter of this clearing zone on the oil surface correlates to surfactant activity, also called as oil displacement activity. For pure biosurfactants, a linear correlation between quantity of surfactant and clearing zone diameter is given.

All the isolates showed better displacement with vegetable oil followed by Engine oil and least activity in bunker oil. *P. aeruginosa*, *Bacillus* spps I and *S. aureus* showed remarkable results with all types of oils .Higher level of activity was noticed by *Pseudomonas aeruginosa* (13mm). *Micrococcus* spps showed the least biosurfactant activity. (Table- 5)

Table - 5: Selected isolates and their biosurfactant activity (mm ± SD)

Type/ Isolates	*Bacillus spps I*	*Bacillus spps II*	*Staphylococcus. Aureus*	*Aeromonas spps*	*Pseudomonas aeruginosa*	*Micrococcus spps*
Vegetable Oil	12 ± 0.1	9 ± 1.06	12 ± 0.03	10 ± 0.61	13 ± 0.16	8 ± 0.94
Engine Oil	6 ± 0.40	3 ± 0.85	6 ± 0.90	6 ± 0.54	7 ± 0.49	6 ± 1.60
Bunker Crude Oil	4 ± 1.09	1 ± 0.67	2 ± 0.71	2 ± 0.62	4 ± 1.64	1 ± 0.40

Oil degradation test: (zone of clearance) (mm): The utilization of the hydrocarbons resulted in increase in cell densities with a visual gradual reduction in the oil content and complete disappearance of the oil around the colonies with prolonged incubation of 7 days. This ability of degrading different oils was determined at temperatures ranging from 25 ^{o} C to 45^{o} C (Table- 6).

Table- 6: Zone of clearance (mm±SD) exhibited by the selected isolates at different temperature conditions on different oils

	Temperature (^{o}C)	*Bacillus spps I*	*Bacillus spps II*	*Staphylococcus aureus*	*Aeromonas spps*	*Pseudomonas aeruginosa*	*Micrococcus spps*
Vegetable Oil	25	11 ± 2.1	9 ± 0.42	12 ± 0.13	10 ± 0.81	13 ± 2.22	10 ± 0.51
	37	16 ± 1.8	15 ±0.62	19 ± 1.59	15 ± 0.29	20 ± 1.53	11 ± 0.81
	45	5 ± 0.54	-	8 ± 0.21	-	7 ± 0.75	9 ± 0.03
Engine Oil	25	5 ± 3.12	3 ± 1.1	4 ± 1.02	3 ± 1.32	5 ± 2.38	3 ± 1.67
	37	9 ± 0.64	7 ± 0.72	9 ± 0.91	6 ± 0.70	10 ± 0.34	8 ± 0.95
	45	3 ± 0.45	-	4 ± 0.65	-	3 ± 1.05	2 ± 0.58
Bunker Crude Oil	25	2 ± 1.01	-	1 ± 0.21	1 ± 0.04	2 ± 1.09	1 ± 0.36
	37	4 ± 0.79	1 ± 0.38	3 ± 0.11	2 ± 0.26	5 ± 0.27	3 ± 0.74
	45	2 ± 0.01	-	2 ± 0.40	-	3 ± 0.85	1 ± 0.19

All the isolates could utilize oil and degrade it remarkably at 37^{o}C compared to at 4^{o}C and 45^{o}C. *P. aeruginosa* showed highest oil degrading capacity followed by *S. aureus*, *Bacillus* spps I, *Aeromonas* spps, *Bacillus* spps II and least by *Micrococcus* spps. *Bacillus* spps II and *Aeromonas* spps could not grow at 45^{o}C and hence no oil degrading activity was observed (Table- 6).

During the current study six out of 22 bacterial species indicated oil degradation capacities. Highest oil degrading capacity out of six was of *P. aeruginosa* indicated highest zone of oil degradation 20 ± 1.53 mm at 37°C in vegetable oil. Though it can tolerate higher temperature up to 45° C, its optimum activity was observed at 37 $^{\circ}$C in vitro. These gram negative bacteria as well showed higher biosurfactant activity (13 ± 0.16mm)

S. aureus, gram positive cocci isolated from marine sands showed comparable efficiency in biodegradation procedures 19 ± 1.59 mm. The conditions for the action were found to be 37° C. However, it can withstand temperature from 27 $^{\circ}$to 45°, it has salt tolerance up to 10% and pH tolerance range is 5 – 10. It also indicated a biosurfactant activity of (12 ± 0.03 mm) diameter in vegetable oil indicating it as second best preferred bacteria from marine environment.

Other Gram positive bacteria represented by *Bacillus* spp I can also be considered for bioremediation studies. Bacterial species is characterized by moderate action (16 ± 1.8 mm) at temp. 37° C with veg oil and is able to thrive in similar conditions. Surfactant activity was also found to be high at 37° C.

Aeromonas species (Gram negative) and Bacillus species II showed similar level of bioremediation at 37° C optimum temp. However, the surfactant activity *Aeromonas* spp was more than that of *Bacillus* spp II. The abiotic requirements of *Bacillus* species II were different from that of *Aeromonas* spp. During the study *Micrococcus* found to have lower levels of biodegradation capacity when compared to other species. Salt tolerance of *Micrococcus* spp was observed to be similar to that of *Aeromonas* spp and was found higher than that of *Bacillus* spp II.

DISCUSSION

Marine ecosystems have capacity to balance marine ecological balance. The sandy coasts filter as well as accumulate organic and inorganic material from terrestrial and marine

environments. They act as biocatalytic filter and comprise biofilms of highly diverse microbial communities – microbial niches. The microbes have capability to remove and degrade the dissolved or particulate organic matter. Thus they play an important role in recycling organic matter, which has prominence in biochemical cycle of carbon and nutrients in shallow water (Gobet et al, 2011, Hunter et al, 2006, Huettel et al., 2000 and 1996)

Microbial populations that consist of strains that belong to various genera have been detected in petroleum-contaminated soil or water (Sorkhoh et al., 1995). Of these, Gram-positive bacteria may play a key role in PAH degradation on contaminated tropical beaches (Zhuang, 2003). Earlier studies indicated an isolation of around 68 halophilic isolates capable of hydrocarbon degradation capabilities from marine shores (Anand et.al., 2013). PAH-degrading bacteria belonging to the Gram-positive nocardioforms and spore-forming Paenibacillus groups have recently been isolated from the rhizosphere of salt marsh plants (Daane et al., 2001). During the current study, around 6 bacterial species were identified as capable of oil degradation and can produce biosurfactants amongst 22 species from intertidal sandy beaches. The Characterization studies indicated of the 6 isolates 4 belonged to gram positive and 2 gram negative, represented by *Bacillus spp I, Bacillus spps II, Staphylococcus aureus and Aeromonas spp.* This could be due to that gram positive bacteria have a stronger cell envelope than gram negative bacteria and this allows them to thrive in the highly variable intertidal environment.

Results from the current study indicated that Gram negative microbes also had the capacity to produce biosurfactants and to degrade oils. Thus it can be suggested that Gram negative and Positive both play an important role in degrading harmful organic matter. Sorkhoh and co-workers (1995) observed a sequential change of the composition of the oil-degrading bacteria over a period of time in sand samples that were contaminated with oil. Studies conducted on degradation of edible oil, where *P. aeruginosa* (Bharathi P. Et al, 2012) and *S. aureus* is proven to be effective (Prasad, M.P et al.,2011). The results of the current study indicated that the temperature found to be an essential parameter for oil degrading activity (37^{o}C).

Biosurfactants or microbial surfactants are surface-active biomolecules that are produced by a variety of microorganisms. Oil contaminated environment contain large amount of hydrocarbons and represent the occurrence of biosurfactant producing microorganisms The current study indicated that marine ecosystem also comprised such organisms naturally represented by *P. aeruginosa, S. aureus, Aeromonas spp. Bacillus spp.1 and 2* as well as

Micrococcus spp. Priya and Usharani (2009) reported a similar kind of study where *Pseudomonas aeruginosa* recorded higher biosurfactant activity than *Bacillus subtilis*. Since most of the isolates from the shore sample indicated good quality of biosurfactant activity and oil degradation capabilities, these isolates can be used for industrial applications.

CONCLUSION

It is evident from the current study that not only marine water, but also the marine coastal sands of Mumbai, Shivaji Park can be used for isolation of oil degrading microbes. These marine microbes can be proven useful source of biological products; biosurfactant being one of the major products. Extraction procedure of these biosurfactants needs to be studied and validated for this purpose. Also, one of the major concerns of environment being organic biodegradation can be achieved successfully by just overlaying these microbes. Six out of twenty two types represented remarkable oil degrading capacity, not only with Bunker or crude oil, but also with vegetable oil. Thus oil polluted environment can be remediated using certain types of marine microbes.

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