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Isolation and Characterization of Bacteria from Refinery Effluent for Degradation of Petroleum Crude Oil in Seawater

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Abstract

Petroleum crude oil is transported on a global scale through marine vessels and barges by the sea route. Oil spills into the marine environment are known to cause long term effects on the health of marine life and human beings in addition to harming the eco-system. In this study, petroleum refinery effluent samples were collected and analyzed for their physico-chemical properties. Thirty two bacterial strains were isolated by direct isolation and post enrichment in crude oil. Among these, fourteen isolates could utilize petroleum hydrocarbons as sole carbon source on Bushnell Hass Agar plates supplemented with crude oil within 2 days. Out of these fourteen strains, four could decolorize 2,6-dichlorophenol indophenol within 36 h completely. They were identified as *Bacillus cereus* WD22, *Pseudomonas aeruginosa* WD23 and WDE11 (2 strains) and *Acinetobacter baumannii* OCB1 by 16sRNA sequencing. All four strains could tolerate salinity up to 4.0%w/v. The reduction in total petroleum hydrocarbons in sea water spiked with crude oil (1.0%v/v) supplemented with trace amounts of glucose and yeast extract was studied by gravimetric analysis. *P. aeruginosa* WD23 degraded 27.25% of supplied petroleum crude oil under limited nutrient conditions in seawater in 15 days.

Keywords: petroleum crude oil, sea water, petroleum refinery effluent, *Acinetobacter baumannii, Bacillus cereus, Pseudomonas aeruginosa*

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INTRODUCTION

The occurrence of oil spills is now a common issue of concern around the world. Some of the most well-known oil spills that have taken place in last four decades include the Exxon Valdez in Alaska (1989), the 'Gulf War' in Kuwait (1991), the Erica in France (1999); the Aegean Sea in Galicia, Spain (1992) and the Prestige in Spain and France (2002)[1]. Recently, oil spills have occurred in India such as in Goa, West Coast of India in 2005[2], Mumbai, Maharashtra in 2010[3] and Ennore, Tamil Nadu in 2017[4.] Also, the transfer of petroleum crude oil (PCO) from Arabian countries such as Saudi Arabia and Iran to India is by the sea route through tanker vessels.

The release of petroleum hydrocarbons into the marine environment has impact on marine ecosystems, adverse affects on human health and results in death of marine life as some of the polyaromatic hydrocarbons are known to be potent carcinogens, teratogens and mutagens[5]. The potential hazardous nature of components of PCO in oil spills continues to harm marine life and human health and damages the ecosystem until they are removed from the seawater. Study conducted by Han et al5 showed high concentration of polyaromatic hydrocarbons in the sea water at Ennore sea coast after 2-3 months of the oil spill, inspite of using physical methods to clear up the spill.

There are various physico-chemical methods employed for treatment of oil spills such as advection, diffusion, dissolution and emulsification but these methods offer more disadvantages over advantages[6]. Physical methods of treatment are expensive whereas the chemical methods are less efficient and alter the natural ecosystem[7].

Biodegradation of petroleum hydrocarbons in oil spills by indigenous microorganisms is presently gaining significance as a potential treatment method. Microorganisms utilize the components of PCO as a source of nutrition and convert them into simpler compounds. The growth of microorganisms in stressful conditions can be stimulated by addition of easily assimilated carbon and nitrogen sources[8]. Bioremediation offers benefits such as economy, reliability and eco-friendly nature over the physico-chemical methods[9].

The present study deals with the isolation and screening of petroleum hydrocarbon degrading bacteria from petroleum refinery effluent (PRE). The physico-chemical characteristics of PRE were studied. The best degraders were identified by 16S RNA sequencing and characterized. The ability of the identified bacteria to degrade petroleum hydrocarbons in seawater supplemented with PCO and minimal amount of glucose and yeast extract was determined by gravimetric analysis.

MATERIALS AND METHODS

Collection of PCO and PRE

PCO was collected from a petroleum refinery industry dumping yard situated in Mangalore, Karnataka, India (12.9951°N, 74.8094°E). PRE from refinery industry is subjected to treatment involving oil separation, activated sludge process and tertiary treatment post which it is sent to the refinery industry dumping yard. PRE was collected from the dumping yard in sterile plastic containers at two different seasons (winter and rainy).

Substrates and Chemicals

The redox indicator 6-dichlorophenol indophenol (DCPIP) was obtained from SRL (SRL chemicals, Bangalore, India). All other chemicals, solvents and reagents used in the study were of analytical grade, unless mentioned.

Physico-chemical characterisation of PRE

The physico-chemical characteristics of PRE at both seasons such as pH, total organic carbon (TOC), total dissolved solids (TDS), phosphates, nitrates, phenolics, conductivity and metal composition were analysed. The pH was determined by digital pH meter. The TOC, TDS, phosphates and nitrates were determined[7]. Conductivity was determined using Conductivity/ TDS meter (Wagtech WEDIST6). The metal composition was analysed by using standard atomic absorption spectrophotometer (Elico, India).

Quantitative Analysis of Bacteria

The quantitative analysis of bacteria in the PRE samples was performed to estimate the percentage of oil utilizing bacteria (OUB) in total viable count (TVC)[10].

Determination of TVC

PRE was diluted (1:1) and spread plated (50µl) onto nutrient agar medium. The plates were

incubated at 37±1°C for 24 – 48 h. The bacteria were enumerated and TVC was determined as CFU/ml.

Determination of OUB

Diluted PRE (50µl) was spread plated onto Bushnell Haas Agar (BHA) medium spread with PCO (100µl). The plates were incubated at 37±1°C for 24 – 48 h. The bacteria were enumerated and OUB was determined as CFU/ml.

Percentage of OUB in TVC

Percentage of OUB in TVC was calculated as per the following formula:

Percentage of OUB in TVC = (OUB / TVC) x 100 ...(1)

Isolation of bacteria from PRE

Isolation Post Enrichment in PCO

The bacteria in the PRE was enriched by addition of PRE (1 ml) to nutrient broth supplemented with 1.0%v/v PCO for 2 days followed by enrichment in Bushnell Haas (BH) broth with 1.0%v/v PCO as the sole carbon source for 6 days[10]. These steps were carried out three times. Enrichment was done at 27°C and 37°C. The enriched medium was spread plated onto nutrient agar medium and incubated at 37±1°C for 72h. Pure cultures of the isolates were sub cultured and stored as glycerol stocks at -20°C.

Growth of enriched isolates on Malachite Green Agar

Bacteria isolated post enrichment were streaked onto Malachite Green Agar, selective for *Pseudomonas aeruginosa* and incubated at 37±1°C for 48h.

Direct Isolation

PRE was serially diluted till 10^{-12} and spread plated onto nutrient agar plates. The plates were incubated at 37±1°C for 24 – 48h. Distinct bacterial colonies were isolated based on morphological characteristics and stored as glycerol stocks at -20°C.

Preliminary screening of PCO degraders

The isolates obtained from direct isolation as well as post enrichment were streaked on BH plates overlaid with 100µl of PCO and incubated at 37°C for 6 days. The isolates which grew on the agar plates were confirmed as hydrocarbon degraders. The zone of clearance around the degraders, grown on BHA plate was observed**.**

Screening of bacteria with good hydrocarbon remediating potential

Isolates showing zone of clearance within 2 days of incubation were selected for the estimation of oil degrading efficiency. The isolates were grown in BH broth supplemented with 1.0%v/v PCO along with DCPIP indicator (0.4%w/v) at 37±1°C for 5 days. During microbial oxidation of hydrocarbons, the electrons are transferred to DCPIP which becomes colourless (reduced) from blue (oxidised). This is estimated by measuring the absorbance at 600 nm[11].

Identification and Biochemical Characterization of best degraders

Best degraders were identified by 16S RNA sequencing. The 16S sequence obtained was analyzed by BLAST. Phylogenetic tree was constructed using neighbour joining method considering 16S sequences of bacteria showing maximum homology using Bioedit software. The 16S sequences were then deposited in NCBI-GenBank and accession numbers were obtained. Growth of identified bacteria at different culture conditions such as temperature (4°C, 25°C, 37°C and 45°C), pH (2.0 -12) and NaCl (4.0 – 12.0%w/v) was checked. Also, the different biochemical tests such as oxidase, catalase, urease, methyl red, Voges Proskauer, Indole, H_2S production, ortho-Nitrophenyl-β-galactoside (ONPG), nitrate reduction, esculin hydrolysis, utilization tests (citrate, lysine, ornithine, malonate) and sugar hydrolysis tests (arabinose, xylose, adonitol, rhamnose, cellobiose, melibiose, sachharose, raffinose, trehalose, glucose, maltose, fructose, sucrose and lactose) were performed[12].

Degradation of petroleum hydrocarbons of PCO in sea water by identified bacteria

Identified bacteria (16 h old, 5.0%v/v) containing 10^{10} CFU/ml cells were inoculated into sterile sea water (100 ml) supplemented with 1%v/v PCO, 1 g/L glucose and 0.05 g/L yeast extract at concentration. These were incubated at 27°C for 15 days at an agitation speed of 100 rpm. Sea water with the supplemented nutrients and without the inoculum was used as control.

After 15 days, the growth of bacteria was checked by measuring optical density at 600 nm. PCO from the culture broth as well as the control

was extracted with hexane in the ratio 20:1 in an extraction funnel. The solvent fraction was passed through anhydrous sodium sulfate to remove moisture content and was evaporated completely at 50°C[13]. The weight of the residual PCO was noted down. The percentage degradation of PCO was calculated as follows:

% Degradation = Wi – Wf/Wi x 100 ...(2)

Where Wi and Wf are the weights of added and residual PCO after 15 days of incubation

RESULTS AND DISCUSSION

Physico-chemical characterisation of PRE

Physico-chemical characterisation of treated PRE collected at summer (PRE1) and winter (PRE2) showed that all the parameters tested were high for PRE1 and low for PRE2 (Table 1). This is because the sump where the treated PRE is stored in the dumping yard is uncovered and rain water could have diluted the treated PRE.

The treated PRE were rich in TDS, TOC and phosphates, whereas the amount of nitrates was found to be relatively less. The phenol content was also measured to be low (Table 1). Out of the heavy metals tested, high concentration of magnesium and cadmium, low concentration of copper, nickel, manganese and zinc and trace amount of lead were found to be present. Mercury was observed at below detection limits

Table 1. Profile of PRE from refinery industry at Mangalore

Parameter	Amount	
	PRE1	PRE2
Physico chemical		
pH	7.3	7.9
Conductivity (mS)	3.05	1.4
TDS (mg/L)	1943	770
Inorganics		
Phosphates (mg/L)	48.22	31.8
Nitrates (mg/L)	0.061	0.016
Phenolics (mg/L)	1.29	BDL
Organics		
TOC (mg/L)	10.76	10.67
Heavy metals		
Mercury	BDL*	BDL
Lead	0.001	BDL
Cadmium	2.173	1.091
Manganese	0.232	0.075
Nickel	0.084	0.045
Magnesium	16.89	15.67
Zinc	0.082	0.045
Copper	0.103	0.045

Quantitative Analysis of Bacteria

TVC and OUB in PRE was more in summer than in the winter (Table 2). This is because the temperature of Mangalore in summer (29-30°C) is higher than in the rainy season (25-26°C). The TVC content in PRE in summer and winter was $1x10^5$ and $5x10^3$ CFU/ml respectively. The TVC content in untreated effluent from ONGC sites was found to be in the range $3x10^6 – 4x10^8$ CFU/ml[10]. The percentage average of OUB in TVC in PRE in summer and winter was 22.1% and 20% respectively.

Isolation post enrichment in PCO

Five isolates designated as 1, 2, 3, 4 and 5 were isolated post enrichment in PCO based on colony characteristic differences on the nutrient agar (Table 3). Isolates 1-3 and 4-5 were obtained from enrichment studies at 27°C and 37°C respectively. All the isolates were Gram negative bacilli. When they were grown in nutrient broth they produced a blue green pigment within 24 h of incubation. This is a characteristic of *P. aeruginosa,* a Gram negative bacillus which produces pyocyanin, a blue green phenazine pigment[14].

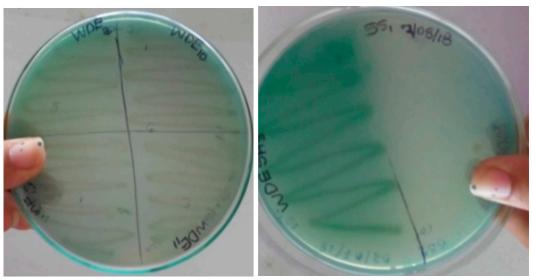

Fig. 1. Enriched isolates grow on Malachite Green agar, selective for *Pseudomonas aeruginosa*

Table 2. Bacterial load of PRE from refinery industry at Mangalore

Sample	TVC (CFU/ml)	OUB (CFU/ml)	%
PRE1	$1x10^5$	$2.21x10^4$	22.1
PRE2	$5x10^3$	$1x10^3$	20

Growth of enriched isolates on Malachite Green Agar

The enriched isolates grew on Malachite Green agar, selective for *P. aeruginosa* (Fig. 1). *P. aeruginosa* is known to exhibit different morphologies such as small-colony, rough small-colony, wrinkled, autoaggregating cells and rugose on solid medium[15]. Pyocyanin is a known potent antimicrobial inhibitor that can act against a wide range of microorganisms[16]. Bacteria belonging to

Table 3. Morphological and Colony characteristics of isolates obtained post enrichment in PCO

No.	Grams stain	Shape	Colony Morphology				
			Form	Elevation	Margin	Color$	Size#
1	-	B*	Circular	Convex	Entire	Cr	++
2	-	B	Circular	Flat	Entire	Cr	+
3	-	B	Circular	Convex	Entire	Cr	++
4	-	B	Circular	Convex	Entire	W	+++
5	-	B	Circular	Convex	Und*	P	+

*B – Bacilli
*Irregul – Irregular, Crater – Crateriform, Und – Undulate
$Colour: Cr – Cream, W – White, P – Pink
#Size: + – Very Small, ++ – Small, +++ – Big

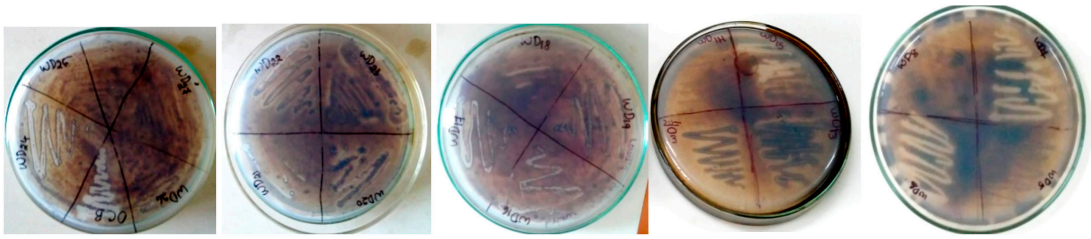

Fig. 2. Bacterial isolates exhibiting growth and zone of clearance on BH plates supplemented with PCO, at 2 days of incubation

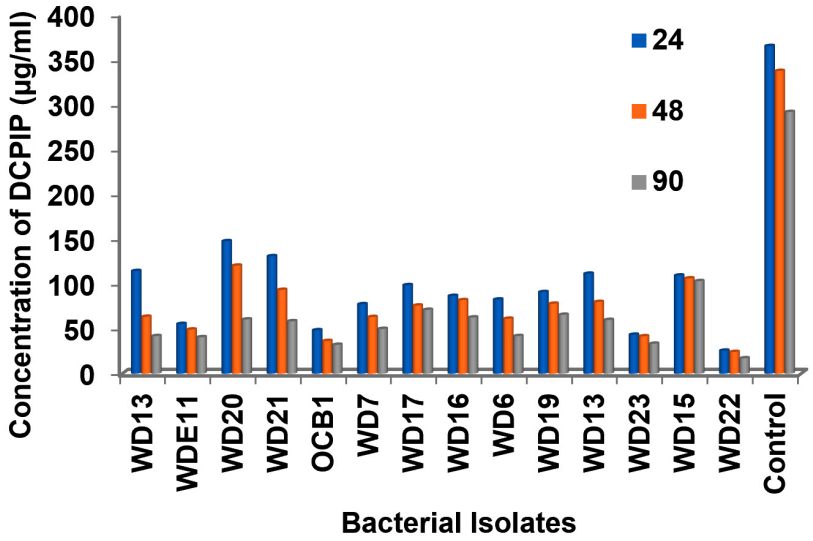

Fig. 3. Reduction in DCPIP concentration due to bacterial degradation of PCO

Pseudomonas genera are reported to be one of the most predominant polyaromatic hydrocarbons (PAH) degrading bacteria isolated from PAH contaminated environmental sites[17-19].This could be because pyocyanin, produced by *P. aeruginosa* inhibits most of the bacteria growing with it during selective enrichment. Hence, direct isolation of bacteria from PRE samples was carried out to observe the diversity of its bacterial population

Direct isolation

On direct plating of PRE samples on nutrient agar a total of 27 bacterial isolated were selected on the basis of difference in colony characteristics (Table 4). They were designated as WD1 to WD26 and OCB1. Isolates WD1-WD15 were obtained from PRE1 whereas the rest were isolated from PRE2.

On direct isolation of bacterial isolates (27), it was observed that bacterial population was dominated by Gram negative bacilli (16). Eighty four percent of isolated bacteria from Brazilian soil, water and marine water contaminated with PAH were found to be Gram negative[20]. The rest of the isolates were found to be Gram positive bacilli (5), Gram positive cocci (5) and Gram negative coccobacilli (1). Gram positive rods with pink, orange and yellow pigmentation could be carotenoid producing *Bacillus* species[21]. Majority of the bacteria isolated from petroleum refinery waste of IOCL oil refinery, Assam, India collected during June also belonged to *Pseudomonas* and *Bacillus* genera[20].

On comparison of colony characteristics of enriched isolates with direct isolates, it was

Table 4. Morphological and Colony charecteristics of isolates obtained from direct isolation

N.	Grams stain	Shape*	Colony Morphology				
			Form	Elevation	Margin	Colour$	Size#
WD1	-	B	Irregul	Crater	Und	O	+++
WD2	-	B	Circular	Convex	Entire	Cr	++
WD3	+	B	Circular	Flat	Curled	W	++
WD4	-	B	Circular	Flat	Entire	W	+
WD5	-	B	Circular	Raised	Entire	O	++
WD6	-	B	Irregul	Crater*	Und*	Cr	+++
WD7	-	B	Circular	Flat	Entire	Y with O centre	++
WD8	-	B	Circular	Convex	Entire	W with O center	+++
WD9	+	C	Circular	Raised	Curled	O	+++
WD10	-	B	Circular	Convex	Entire	W	+++
WD11	-	B	Circular	Flat	Entire	Cr	+
WD12	+	SC	Circular	Raised	Lobate	Bright Y	+
WD13	-	B	Irregul	Crater	Und	W	+++
WD14	+	B	Circular	Raised	Entire	Bright P	++
WD15	+	B	Irregul	Crater	Und	O with P center	++
WD16	-	B	Irregul	Crater	Lobate	W	+++
WD17	+	C	Circular	Convex	Entire	W mucoid	+
WD18	-	B	Circular	Convex	Entire	Cr	++
WD19	-	B	Circular	Convex	Entire	W with O center	+
WD20	-	B	Irregul	Crater	Und	W	+++
WD21	+	SC	Round	Raised	Lobate	Pale Y	+
WD22	+	B	Irregul	Crater	Lobate	Cottony W	+++
WD23	-	B	Circular	Convex	Entire	W with Bl center	+
WD24	-	B	Circular	Convex	Und	P	+
WD25	+	B	Circular	Raised	Lobate	Bright O	++
WD26	+	SC	Circular	Convex	Entire	Light Y with Bl center	++
OCB1	-	CB	Circular	Convex	Entire	Pale Y mucoid	+

*Shape B – Bacilli, C – Cocci SC – Staphylococci, CB - coccobacilli, *Irregul – Irregular, Crater – Crateriform, Und – Undulate
$Colour Cr – Cream, W – White, O – Orange, P – Pink, Bl – Black, Y -Yellow, #Size + – Very Small, ++ – Small, +++ – Big

observed that isolates 1, 2, 3, 4 and 5 had colony characteristics similar to WD2, WD11, WD18, WD13 and WD24 respectively. Hence they were re-designated as WDE2, WDE11, WDE18, WDE13 and WDE24 respectively.

Preliminary screening of PCO degraders

The bacterial isolates obtained post enrichment and from direct isolation were grown on BHA plates spread with PCO and observed periodically for 2 days till 6 days. Fourteen isolates (Fig. 2) grew on the BHA plate with in 2 days. The isolates WD6, WD7, WD24 and OCB1 gave the maximum zone of clearance. It was observed that WD22 and WD23 grew on the BH plates but failed to clear PCO around their colonies. Since, PCO is the sole carbon source that is supplied to the bacteria, all the bacteria could utilise PCO as the sole source of carbon. Surprisingly, only one

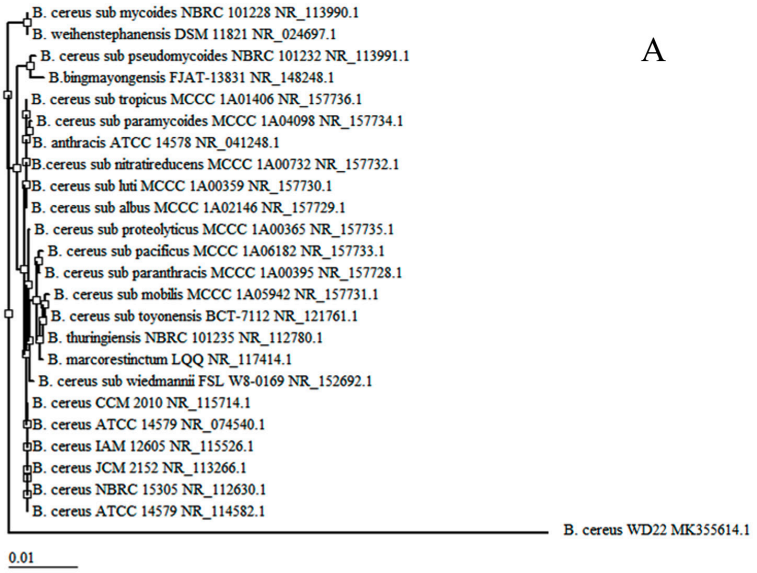

Fig. 4a. Phylogenetic tree showing the relationship of isolated WD22 with isolates showing homology as obtained from BLASTn

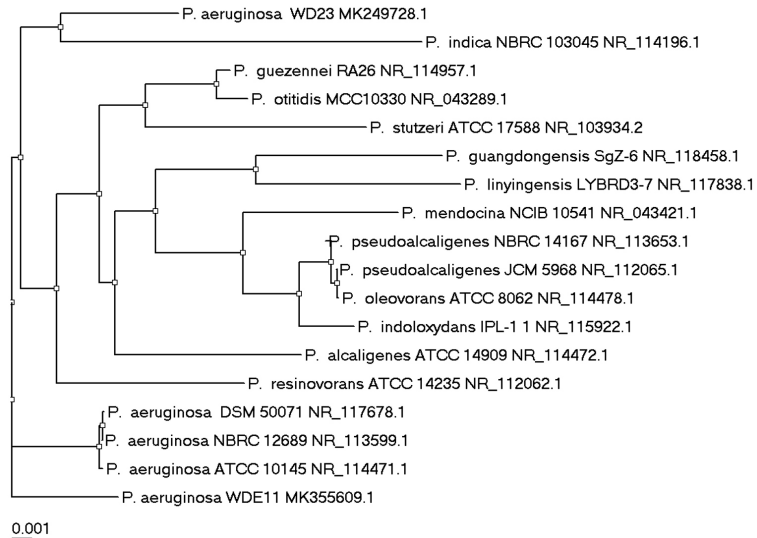

Fig. 4b. Phylogenetic tree showing the relationship of isolated WD23 and WDE11 with isolates showing homology as obtained from BLASTn

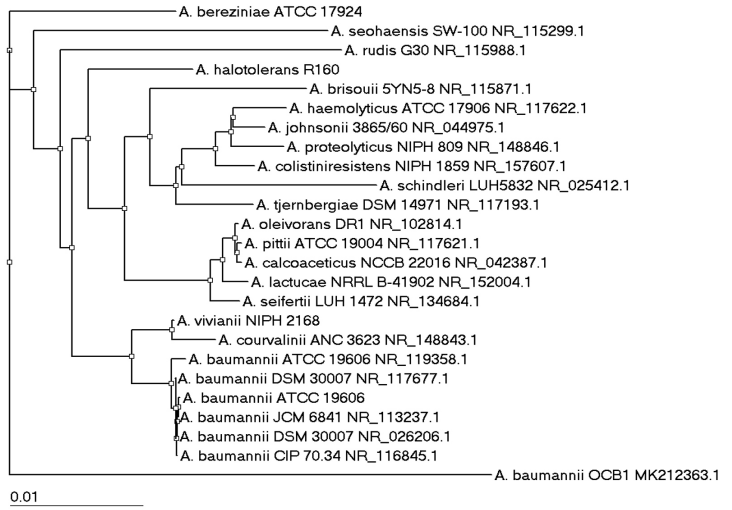

Fig. 4c. Phylogenetic tree showing the relationship of isolated OCB1 with isolates showing homology as obtained from BLASTn

enriched bacterial isolate (WDE11) grew with 2 days on the plate. Also, the rest of the isolates (19) could utilise PCO as sole carbon source by 6 days.

Screening of bacteria with good hydrocarbon remediating potential

The isolates which grew on BHA plates by 2 days were screened further by growing them in nutrient broth containing 1.0%v/v PCO and DCPIP (0.4%w/v). The reduction in color of DCPIP was measured spectrophotometrically at 600 nm. From Fig. 3 it was observed that culture WD22, WD23, WDE11 and OCB1 decolorised DCPIP rapidly within 24h. Complete decolorisation of DCPIP can be considered at a concentration of 30µg/ml. Similar studies showed bacterium HUB1 isolated from untreated petroleum refinery effluent could decolorize DCPIP at 120 h[10].

Table 5. Growth of isolated and identified best degraders of PCO at different cultural conditions Culture

Condition	OCB1	WD22	WD23	WDE11
Temp. (°C)				
4	-	-	-	-
10	-	-	-	-
25	+	+	+	+
37	+	+	+	+
45	+	+	+	+
pH				
2.0	-	-	-	-
4.0	++	-	+	+
6.0	+++	+++	+++	+++
8.0	+++	+++	+++	+++
10.0	+++	++	++	++
12.0	-	-	-	-
NaCl (%w/v)				
4	++	+	++	++
6	+	-	+	+
8	-	-	-	-
10	-	-	-	-
12	-	-	-	-

Table 6. Biochemical characterisation of isolated and identified best degraders of PCO

Tests	OCB1	WD22	WD23	WDE11
Biochemical Tests				
Oxidase	-	+	+	+
Catalase	+	+	+	+
Urease	+	+	+	+
Nitrate Reduction	-	-	-	-
H2S Production	+	+	+	+
ONPG	-	-	-	-
Phenylalanine	-	-	-	-
Esculin Hydrolysis	+	+	+	+
Methyl Red	+	-	-	-
Voges Proskauer	-	+	-	-
Indole	-	-	-	-
Utilisation Tests				
Lysine	-	-	-	-
Ornithine	-	-	-	-
Citrate	+	+	-	-
Malonate	-	+	-	-

Identification of best degraders of PCO

The identification of WD22, WD23, OCB1 and WDE11 by molecular sequencing of 16S RNA gene was performed and the gene was analyzed by BLAST and a phylogenetic tree was plotted using isolates showing maximum homology. The sequence of WD22 showed maximum similarity (80.78%) to all the strains of *B. cereus* isolates (Fig. 4a) hence was identified as *B. cereus* WD22. The isolates WD23 and WDE11 showed similarity to strains belonging to *Pseudomonas* genera with maximum similarity (97.67% and 98.05% respectively) to *P. aeruginosa* from BLAST analysis (Fig. 4b). They were identified as *P. aeruginosa* WD23 and *P. aeruginosa* WDE11. The isolate OCB1 showed similarity to strains belonging to *Acinetobacter* genera with maximum similarity of 86.67% to three strains of *A. baumannii* (Fig. 4c). Hence it was identified as *A. baumannii* OCB1. *Acinetobacter* strains are observed to be Gram negative coccobacilli and OCB1 also demonstrated the same morphology.

The identified isolates formed a separate clade in the phylogenetic tree that indicated significant differences from the isolates showing similarity through BLAST analysis. This could be because of evolution as they are found in PRE with

Table 7. Sugar hydrolysis results of isolated and identified best degraders of PCO

Sugar	OCB1	WD22	WD23	WDE11
Glucose	+	+	+	+
Maltose	+	+	-	-
Fructose	+	+	+	+
Sucrose	+	+	-	-
Lactose	-	-	-	-
Xylose	+	+	+	+
Arabinose	+	+	-	-
Raffinose	-	-	-	-
Rhamnose	-	-	-	-
Cellobiose	-	-	+	-
Melibiose	-	+	+	+
Saccharose	-	-	+	+
Adonitol	-	-	-	-
Trehalose	-	-	-	-

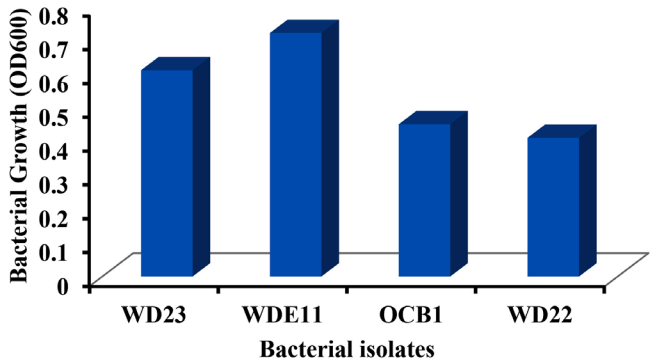

Fig. 5. Growth of isolates in sea water supplemented with PCO, glucose and yeast extract

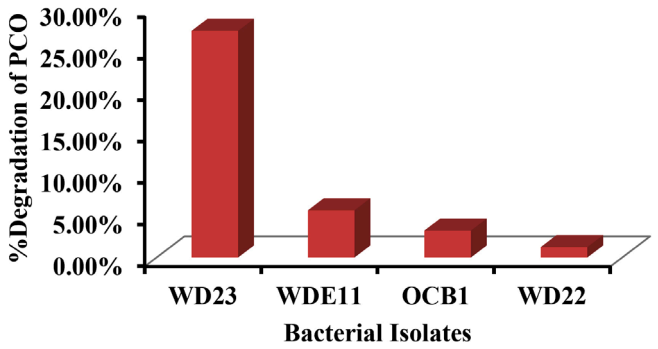

Fig. 6. Degradation of PCO by bacterial isolates in sea water supplemented with PCO, glucose and yeast extract

low nutrient conditions (Table 1). The partial 16s sequences of the isolated cultures i.e. *B. cereus* WD22, *A. baumannii* OCB1, *P.* WD23 and *P.* WDE11 have been deposited in NCBI-GenBank with the accession numbers MK355614, MK212363, MK249728 and MK355609 respectively.

Biochemical charecteristisation of best degraders of PCO

All the identified isolates grew at temperatures 27-45°C but failed to grow at lower temperatures (Table 6). All isolates except *B. cereus* WD22 grew at pH from 4.0 to 10.0. All isolates could tolerate NaCl concentration up to 4%v/v, hence they can be used for treatment of oil spills in sea water (NaCl% = 3.5). Most of the biochemical characteristics of all the identified isolates matched with the standard results (Table 6). All the isolates were positive for catalase, urease, hydrogen sulphide production and hydrolysis of esculin. None of the tested isolates could hydrolyse lysine and ornithine whereas only *B. cereus* WD22 could utilize malonate. All the isolates could hydrolyse glucose, fructose and xylose (Table 7). *P. aeruginosa* cannot hydrolyse maltose, sucrose and lactose and similar results were observed in this study

Degradation of petroleum hydrocarbons of PCO in sea water by identified bacteria

All the isolates were then grown in sea water supplemented with additional carbon and nitrogen sources and spiked with PCO for 12 days. The temperature was maintained at 27°C as in the marine environment as the average temperature of Indian seawater is observed to be between 25-28°C[5]. Spillage of oil into marine and fresh water environment causes an increase in carbon content and a drastic decrease in nitrogen content[23]. It has been reported that the addition of a simple carbon source such as glucose increased the biodegradation rate of total petroleum hydrocarbons (TPH) in contaminated samples[24]. Hence in the present study the growth of identified isolates and the biodegradation of PCO were studied under the presence of trace amounts of glucose and yeast extract.

Among the four isolates *P. aeruginosa* WD23 showed the maximum degradation of 1.0%v/v PCO (27.25%) whereas the least degradation was performed by *B. cereus* WD22 (1.25%) (Fig. 5). Even though *P. aeruginosa* WDE11 showed the maximum growth (OD600 = 0.72) it could degrade only 5.68% of PCO. This indicates that WDE11 utilised the supplied glucose and yeast extract as nutrients for its growth whereas on the other hand WD23 also degrades PCO when other nutrients are supplied. *A. baumannii* OCB1 and *B. cereus* WD22 showed moderate growth (OD600 = 0.45 and 0.41) with very low degradation of crude oil (Fig. 6).

CONCLUSIONS

In the present study, all the isolates obtained from PRE post enrichment in PCO from petrochemical refinery industry in Mangalore, India were *P. aeruginosa* strains. On direct isolation, other PCO degrading strains were obtained. Among the 32 isolates tested, four best degraders i.e *B. cereus* WD22, *A. baumannii* OCB1, *P. aeruginosa* WD23 and *P. aeruginosa* WDE11 were identified and characterised. *P. aeruginosa* WD23 could degrade 27.25% of PCO in sea water supplemented with glucose and yeast extract. From this study we can conclude that the bacteria isolated from petroleum hydrocarbon contaminated sites have the potential to be exploited for treatment of oil spills in sea water.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

DATA AVAILABILITY

All datasets obtained or studied during this study are incorporated in the manuscript.

ETHICS STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors.

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