



Isolation and molecular identification of hydrocarbon degrading bacteria from oil-contaminated soil

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Abstract

The spills of hydrocarbon due to the petrochemical industry are major contaminants in the environment. Bioremediation is an effective, economical and environmentally sound treatment. The purpose of our study was to isolate, screen and identified the hydrocarbon degrading bacteria from oil polluted soil. Three oil contaminated soil were collected from Arzew oil refinery, North-west of Algeria. Sixteen bacterial strains were isolated using mineral salt media supplemented with 1% of crude oil; these isolates were screened for their best degradation abilities. Four selected bacterial strains designated as (P2.3, P2.2, S15.1 and E1.1) were identified on the basis of morphological, biochemical and molecular characterization using 16S rRNA gene sequence analysis. The sequences were compared to the closest relative species in the GenBank database of National Centre for Biotechnology Information. The growths rates of the selected isolates were determined using spectrophotometer at 600nm. Based on the partial 16S rRNA gene sequencing and phylogenetic analysis; the isolates were identified as *Pseudomonas aeruginosa* P2.3, *Achromobacter xylosoxidans* P2.2, *Staphylococcus haemolyticus* S15.1 and *Enterococcus faecalis* E1.1. Results indicated that the isolates strains had effectively utilize crude oil as sole carbon source. Linear increase in Optical Density (OD) was observed between days 4 and 10. *Pseudomonas aeruginosa* P2.3 and *Achromobacter xylosoxidans* P2.2 showed the highest growth in media with crude oil. This study indicates that the contaminated soil samples contain a diverse population of hydrocarbon degrading bacteria and these strains could be used for the bioremediation of oil contaminated soil.

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Introduction

Petroleum-based products are the major source of energy for industry and daily life. Leaks and accidental spills occur regularly during the exploration, production, refining, transport, and storage of petroleum and petroleum products (Das and Chandran, 2010). Release of oil into the environment whether accidentally or due to human activities is one of the main causes of water and soil pollution (Holliger *et al.*, 1997). The amount of natural crude oil seepage was estimated to be 600,000 metric tons per year (Kvenvolden and Cooper, 2003).

Uncontrolled releases of petroleum compounds that are carcinogenic, mutagenic and are potent immunotoxicants into soil and groundwater poses a serious threat to human, plant and animal health (Lin and Singh, 2008). Physical and chemical methods to reduce hydrocarbon pollution are expensive (Erdogan *et al.*, 2012).

Biodegradation by natural populations of microorganisms represents one of the primary mechanisms by which oil pollutants can be removed from the environment (Das and Chandran, 2010) and is considered to be cheaper than other remediation technologies (Leahy and Colwell, 1990). Petroleum hydrocarbon can be degraded by various

microorganisms such as bacteria and fungi (Hamzah *et al.*, 2010). More than 100 species representing 30 microbial genera had been shown to be capable of utilizing hydrocarbon (Atlas, 1981), Bartha and atlas found 22 genera of bacteria, 1 algal genus, and 14 genera of fungi had been demonstrated to utilize petroleum hydrocarbon as a source of carbon (Das and Chandran, 2010). Bacteria are the most active agents in petroleum degradation, and they work as primary degraders of spilled oil in environment (Rahman *et al.*, 2003; Das and Chandran, 2010). In northwest Atlantic coastal waters and sediment Mulkins-Phillips and Stewart found that hydrocarbon-utilizing bacteria representing genera of *Nocardia*, *Pseudomonas*, *Flavobacterium*, *Vibrio*, and *Achromobacter* (Mulkins-Phillips and Stewart, 1974). The main goal of the present study was to isolate and identify petroleum hydrocarbon-degrading bacteria from different oil-contaminated soils at Arzew refinery.

Materials and methods

Study area

The Algerian Petroleum Company (Sonatrach) RA1Z refinery is located 40 km from the city of Oran (North-west of Algeria), in an industrial area near the town of Arzew, about 1.7 km west of the Mediterranean (Fig. 1).



Fig. 1. Map of Algeria showing Arzew oil refinery and the site of the contaminated soil (S1: Sampling site 1; S2: Sampling site 2; S3: Sampling site3).

Petroleum hydrocarbon

Petroleum hydrocarbon used in this study was crude oil obtained from Arzew oil refinery.

Soil samples

Oil-contaminated soil was aseptically collected from three sites in Arzew oil refinery (Fig. 1). Soil samples were obtained at two different depths (5 cm and 10cm) (Fardoux *et al.*, 2000). Around 250 g of soil were collected per sample in sterile plastic bags and transported immediately in cold storage containers to laboratory. Soil samples were crushed and sieved through 2mm pore size (Pétard, 1993; Fardoux *et al.*, 2000). There were placed in sterile bags were stored at 4°C for further study (Chaussod *et al.*, 1986; Chaussod *et al.*, 1992; Fardoux *et al.*, 2000).

Soil characterization

Physicochemical analyses were performed in the laboratory of the “National Institute of Soils, Irrigation and Drainage (INSID of Ksar Chellala, Algeria)”, according to the methodology described in (Aubert, 1978). The soil texture was determined using the international Robinson pipette method. Soil pH was measured in a soil–water ratio of 1:2.5 by electrometric method, electrical conductivity (Cd) was determined in a 1:5 soil to distilled water ratio, the total petroleum hydrocarbons (TPH) content was determined gravimetrically.

Enrichment and isolation of petroleum hydrocarbon degrading bacteria

Petroleum hydrocarbon degrading bacteria were isolated from oil contaminated soil as described above. Media used in this study were mineral salts medium (MSM), which contained per liter: 0.8 g K₂HPO₄, 0.2 g KH₂PO₄, 0.1 g KCL, 0.025 g Na₂MoO₄·2H₂O, 0.014 g Na₂FeEDTA and 1.0 g NH₄NO₃, 0.2 g MgSO₄·7H₂O and 0.06 g CaCl₂·H₂O with pH 7 (Mancera-Lopez *et al.*, 2007).

The medium was supplemented with 1% (V/V) filter-sterilized crude oil as the sole source of carbon (Verma *et al.*, 2006; Hamzah *et al.*, 2010; Erdogan *et al.*, 2012; Jyothi *et al.*, 2012). For enrichment procedure: 10g oil-polluted soil samples was added to

100 ml sterile liquid MSM and incubated for 7 days at 30°C on an orbital shaker at 180 rpm in the dark (Lin and Singh, 2008; Erdogan *et al.*, 2012).

After one week incubation, 1 ml of enriched media was transferred into fresh MSM and incubated at the same conditions. After four consecutive transfers (each with an incubation interval of one week) (Salam *et al.*, 2011; Guermouche *et al.*, 2013), 100 µl of culture were plated on MSM agar (20 g⁻¹) and supplemented with 1% filter-sterilized crude oil and incubated at 30°C for 3 to 5 days (Verma *et al.*, 2006; Meenakshisundaram and Bharathiraja, 2014). Single colonies were streaked onto Luria Bertani agar plates and tryptic soy agar plates, incubated at 30°C overnight, and stored at 4°C until further use. For long-term preservation, pure isolates were maintained in 25% glycerol at -20°C.

Screening and biochemical characterization of isolates

The purified isolates were screened for their ability to grow on crude oil, as described by (Palanisamy *et al.*, 2014).

The screened isolate were identified on the basis of colony morphology, gram staining and biochemical characteristics according to the Bergey's Manuel (Holt *et al.*, 1994).

Molecular characterization

DNA extraction and PCR amplification of 16S rRNA gene

The total DNA of four selected isolates was extracted using genomic DNA purification kit according to the manufacturer instructions (Thermo Fisher Scientific). The quality of extracted genomic DNA was evaluated by UV spectrophotometry at 260 and 280nm. The 260/280 ratio gives an idea of the quality of the DNA solution. The DNA concentration was measured by Pico-Green method using Quant-iT™ Pico-Green™ dsDNA assay kit (Thermo Fisher Scientific). The Extracted DNA was stored at -20°C.

Bacterial 16S rRNA gene was amplified using the following two primers: Forward primer 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and Reverse

primer 1492R (5'-TACGGYTACCTTGTTACGACTT-3'). Each PCR reaction mixture contained (10µl) 1µl DNA template, 0.1 µl Q5® High-Fidelity DNA Polymerase (New England Bio-labs Ltd), 0.2mM dNTP, 1X buffer 5X Q5 (New England Bio-labs Ltd) and 0.2µM of each primer. Conditions for PCR amplification were as follows:

Initial denaturation for 30 sec at 98°C, followed by 30 cycles at 98°C for 10 sec, 62°C for 15 sec and 72°C for 30 second and a final extension at 72°C for 2 min. PCR products were visualized on 1% agarose gel electrophoresis.

Sequencing of 16S rDNA and phylogenetic analysis

The purified PCR product was sequenced by Sanger dideoxy method using Big-Dye Terminator v3.1 Cycle Sequencing Kit in 96-well plate on 3730 DNA Analyzer (Applied Biosystems). Sequences were then compared to those in Gen Bank database in the National Centre for Biotechnology Information (NCBI) using the website for BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>).

The sequences were aligned using Clustal W. The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei, 1987), The evolutionary distances were computed using Jukes-

Cantor method (Jukes and Cantor, 1969) and a phylogenetic tree was constructed by MEGA6 (Tamura *et al.*, 2013).

In addition, all 16S rRNA genes sequences were confirmed and classified using the RDP Naïve Bayesian rRNA classifier tool.

Characterization of bacterial degradation potential by turbidometry

The biodegrading activities of each isolates were obtained by using MSM broth with 1% of crude oil as the sole carbon source and incubated at 30°C for 15 days (Boboye *et al.*, 2010).

The growth of the bacterium was measured by taking the optical density (OD) readings at 600nm for 15 days at regular 2-day intervals by a spectrophotometer, against sterile mineral salt medium as a blank (Jyothi *et al.*, 2012; Meenakshisundaram and Bharathiraja, 2014). All experiments were performed in duplicate (Lin and Singh, 2008).

Results and discussion

Physical and chemical characterization of soil samples

Soil sampling properties are presented in Table 1.

Table 1. Physicochemical characteristics of the four soil samples.

Parameters	Samples			
	1	2	3	
Texture	Clay %	9.69	8.66	21.71
	Coarse silt %	9.18	1.53	21.21
	Fine silt %	3.04	8.05	4.25
	Coarse Sand%	18.79	29.07	20.66
	Fine Sand %	59.29	52.70	32.16
	Texture	Loamy Sand	Sandy Loam	Sandy Clay Loam
Color	Brown	Darkbrown	Brown	
Water content (%)	08	3.35	5.36	
PH	7.08	7.09	7.85	
CE (dS/m à 25° C)	0.28	0.25	0.53	
TPH content (g/kg de sol)	24	28	30	

Table 2. Morphological characteristics of the four oil degrading bacterial isolates cultured on tryptic soy agar medium.

Isolates	Colony color	Colony size	Colony form	Colony elevation	Colony margin	Gram stain	Cell shape
P 2.3	Beige	Small	Circular	Convex	Entire	Gram -	Cocci
P 2.2	Light-yellow	Small	Circular	Raised	Entire	Gram -	Rods
S15.1	White	Small	Circular	Convex	Entire	Gram +	Cocci
E1.1	White	Small	Circular	Raised	Entire	Gram +	Cocci

+: positive; -: negative.

Granulometry analysis shows that sand is the predominant fraction. Thus, the sample has sandy and loamy nature (Fig. 2). The soil pH ranges from neutral to slightly alkaline, with water content between 3% to 8% and an electrical conductivity less than 0.6ds/m. Therefore, the sample has a low salinity. The hydrocarbon content was presented in supplementary Fig. 3. According to Greene *et al.* (2000), the sandy and loamy texture facilitates fluids

circulation; that contain nutrients and oxygen which are accessible to microorganisms in the medium (Bouderhem *et al.*, 2016).

The optimal pH for the development of microorganisms ranged from 7 to 8. Kaboré-Ouédraogo *et al* indicated that a pH between 5 and 7.8 is favorable for the degradation of hydrocarbons in the soil (Kaboré-Ouédraogo *et al.*, 2010).

Table 3. Biochemical characteristic of the P2.3 and P2.2 isolates.

Strains	Tests																	Probable organism												
	Respiration	Motility	Catalase	Oxidase	NO ₃	TRP	GLU (fermentation)	ADH	URE	ESC	GEL	PNPG	GLU	ARA	MNE	MAN	NAG		MAL	GNT	CAP	ADI	MLT	CIT	PAC	Pyoverdine	Pyocyanine	Growth at 42 °C	Growth at 4 °C	Identity (%)
P2.3	Obligate Aerobe	+	+	+	+	-	+	+	-	+	+	-	-	-	+	+	-	-	+	+	+	+	+	+	+	+	+	+	99.9%	<i>Pseudomonas aeruginosa</i>
P2.2	Aerobe	+	+	+	+	-	-	-	-	-	-	-	+	-	-	-	-	-	+	+	+	+	+	+	-	-	-	+	94.5%	<i>Achromobacter xylooxidans</i>

(+): positive reaction; (-): negative reaction.

The total hydrocarbon content of our sample is greater than the intervention value cited by Dutch standard (40mg PAH/kg soil and 0.1 mg volatile hydrocarbon/kg soil). So, our soil samples are highly polluted by hydrocarbon. Comparative study of oil biodegradation in clayey and sandy mangrove soils shows that, the microorganism activity is related to the soil water content (Scherrer and Mille, 1989).

The physicochemical properties were the most dominant factor of bacterial community distribution and abundance, followed by geographical location.

The first dominant phylum of the site with sandy or sandy loam soil texture was *Proteobacteria* (Liao *et al.*, 2015).

Isolation, screening, biochemical and molecular characterization of isolates

A total of 78 bacterial colonies with different morphologies were isolated on MSM agar (Fig. 4). Sixteen bacterial isolate were screened with the best degradative abilities on crude oil, the macroscopic

and microscopic studies showed three types of bacteria (50% bacilli gram-, 31cocci gram+ and 19% bacilli gram+). Four bacterial isolates (P2.3, P2.2, S15.1 and E1.1) were selected for this study. Results in Table 2 show the morphological characteristic of these isolates.

Table 4. Biochemical characteristic of the S15.1 and E 1.1 isolates.

Strains	Tests																			Probable organism									
	Oxidase	Catalase	Motility	Respiration	GI 11	FRU	MNE	MAL	LAC	TRE	MAN	XLT	MEL	NIT	VP	RAF	XYL	SAC	MDG		NAG	ADH	URE	Coagulase	DNase	Hemolysis	FSC	Growth at pH 9.6	Growth at 6.5% NaCl
S15.1	-	+	-	Facultative Anaerobe	+	+	-	+	+	+	-	-	-	+	-	-	-	+	-	+	+	+	-	-	-	+	ND	ND	Staphylococcus Haemolyticus
E1.1	+	-	-	Facultative Anaerobe	+	+	+	+	+	+	+	-	-	+	+	-	-	+	-	ND	ND	ND	ND	ND	+	+	+	+	Enterococcus faecalis

(+): positive reaction; (-): negative reaction.

Table 5. Identification, Gen Bank accession number and sequence similarity of Bacterial isolate from oil contaminated soil (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>).

Isolate code	Closest relative	Query cover (%)	Identity (%)	Identification	GenBank Accession Number
P2.3	<i>Pseudomonas aeruginosa</i>	100%	100%	<i>Pseudomonas aeruginosa</i> P2.3	MF767436
P2.2	<i>Achromobacter xylosoxidans</i>	100%	95%	<i>Achromobacter xylosoxidans</i> P2.2	MF620099
S15.1	<i>Staphylococcus haemolyticus</i>	100%	99%	<i>Staphylococcus haemolyticus</i> S15.1	MF620098
E1.1	<i>Enterococcus faecalis</i>	100%	100%	<i>Enterococcus faecalis</i> E1.1	MF620100

The colonies were circular, smooth, elevated and approximately 1 to 3 mm in diameter. More biochemical tests were carried out to identify the isolates strains as shown in Table 3.

After morphological characterization, biochemical test and bio-Mérieux API kits (20NE, STAPH), the four isolates (P2.3, P2.2, S15.1 and E1.1) were respectively tentatively identified as *pseudomonas*

aeruginosa, *Achromobacter xylosoxidans*, *Staphylococcus haemolyticus* and *Enterococcus faecalis*.

Genomic DNA was isolated from all isolates, a PCR product of 1500bp of the four isolates were analyzed on 1% agarose gel electrophoresis (Fig. 5). Based on the partial 16S rRNA gene sequencing and phylogenetic analysis (Fig. 6), the isolates (P2.3, P2.2, S15.1 and E1.1) were identified compared to the

closest relative species in the GenBank database (Table 5). The 16S rRNA gene sequence revealed that the strain P2.3 was highly similar (100%) with *Pseudomonas aeruginosa* DSM50071 (NR11678), *Pseudomonas aeruginosa* ATCC10145 (NR114472)

and *Pseudomonas aeruginosa* NBRC12689 (NR113599). Thus P2.3 strain was identified as *Pseudomonas aeruginosa* P2.3. This was supported (95% confidence) by RDP Naïve Bayesian rRNA classifier tool.

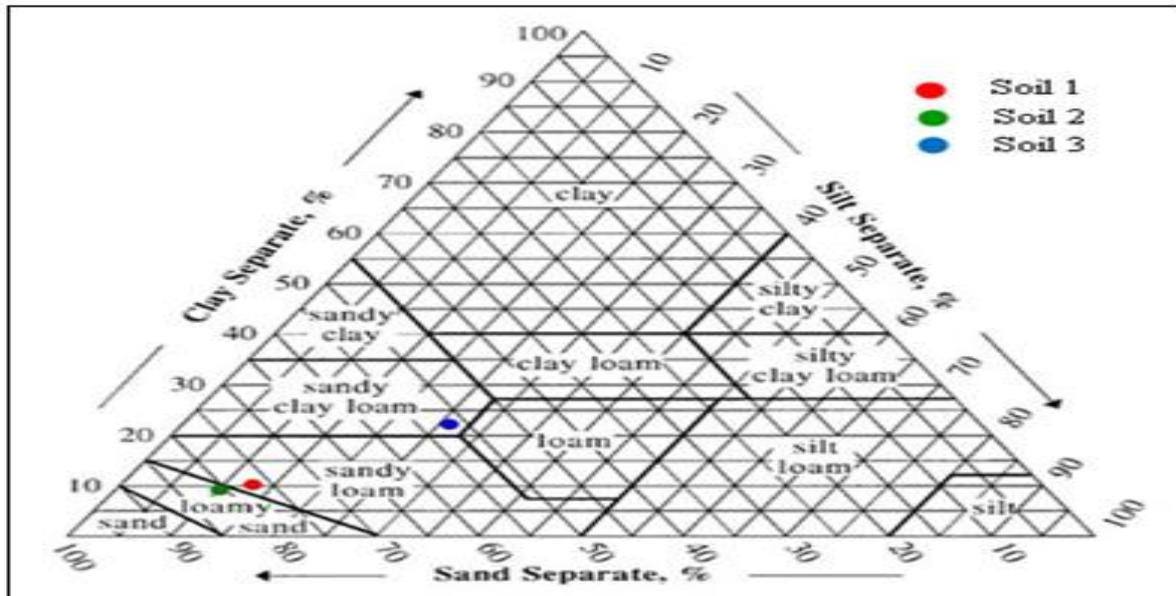


Fig. 2. Localization of soils studied on the texture triangle.

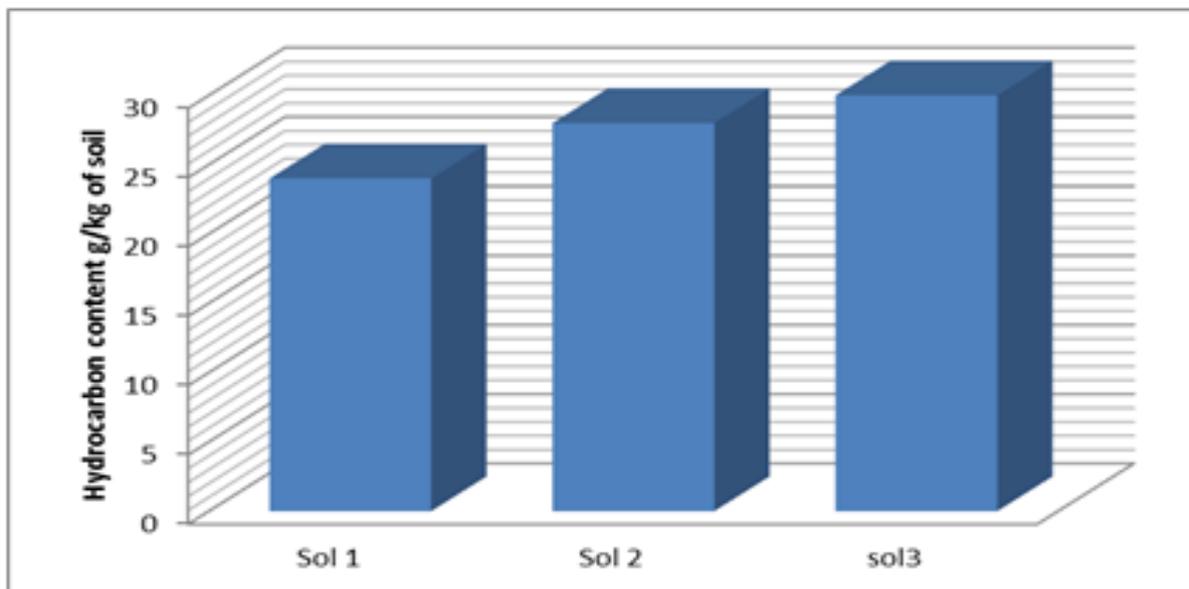


Fig. 3. The total petroleum hydrocarbons (TPH) content in the different samples.

The P2.2 isolate has 95% identity with *Achromobacter xylosoxidans* and classified as the order Burkholderiales, Family Alcaligenaceae and genus *Achromobacter* with RDP classifier tool (95% confidence). The phylogenetic analysis (fig. 6) revealed that the isolate P2.2 formed a monophyletic

group with *Achromobacter xylosoxidans* NBRC 15126 (NR113733) and *Achromobacter xylosoxidans* LGM1863 (NR118403) with high Bootstrap value, 100%. S15.1 was identified as *Staphylococcus haemolyticus* S15.1 (100% identity) with *Staphylococcus haemolyticus* SM131 (NR036955)

and *Staphylococcus haemolyticus* JCM2416 (NR113345). The E1.1 strain showed 100% sequence similarity with *Enterococcus faecalis* ATCC19433 (NR115765), *Enterococcus faecalis* NBRC 100480 (NR113901) and *Enterococcus faecalis* JCM 5803 (NR040789).

Similarly, Chadli *et al.* (2013) isolated many strains of *Pseudomonas* and *staphylococcus*. Furthermore, AL-

Adwan(2010) isolated sixteen bacterial genera include *Staphylococcus* sp and *Pseudomonas* sp from AL-Rwuayashid oil contaminated soil. Sibi *et al.* (2015) isolated *pseudomonas aeruginosa* and *Achromobacter*. Moreover, de Vasconcelos *et al.*(2009) isolated *Achromobacter xylosoxidans*. Obuotor *et al.* (2016), Boontawan and Boontawan. (2011) isolated also *Enterococcus faecalis*.

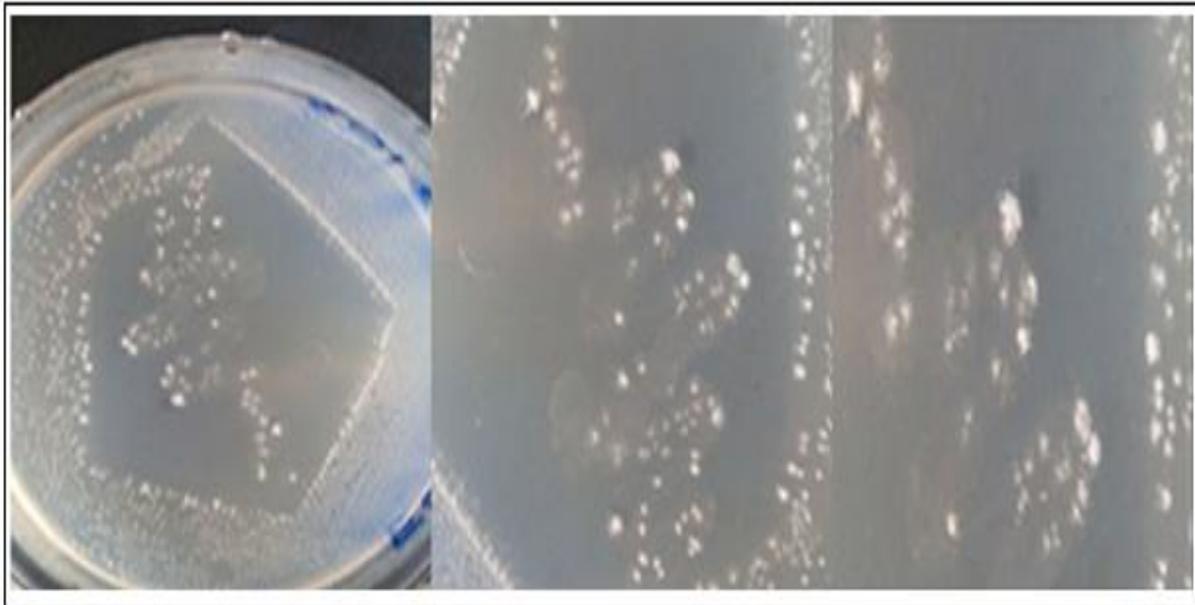


Fig. 4. Bacterial colonies on MSM agar with crude oil.

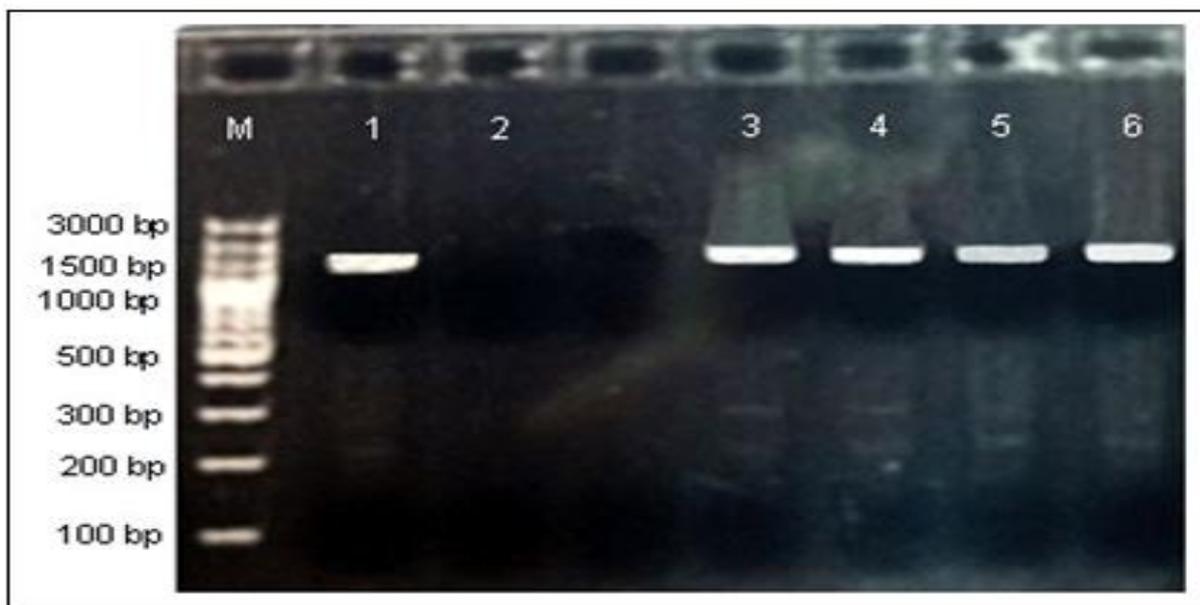


Fig. 5. Agarose gel electrophoresis of PCR amplification product of bacterial isolates using 16S rDNA primers: lane M: Gene Ruler 100 bp Plus DNA Ladder (Thermo Scientific), lane 1: positive control with *Pseudomonas aeruginosa* ATTC 27853, lane 2: negative control without DNA, lane3: isolate P3.2, lane 4: isolate P2.2, lane 5: isolate S15.1, lane 6: isolate E1.1.

Determination of bacterial degradative activity by turbidometry

Following a 15 days incubation of the four isolates on 1% crude oil as sole source of carbon and energy, the bacterial growth was evaluated by measuring optical density (600nm) at regular intervals (2 days), as

indicator for utilization of hydrocarbon (Ebrahimi *et al.*, 2012). Our results (Fig. 7) show that all the isolates had effectively utilized crude oil; the level of degradation differs between isolates (Jyothi *et al.*, 2012).

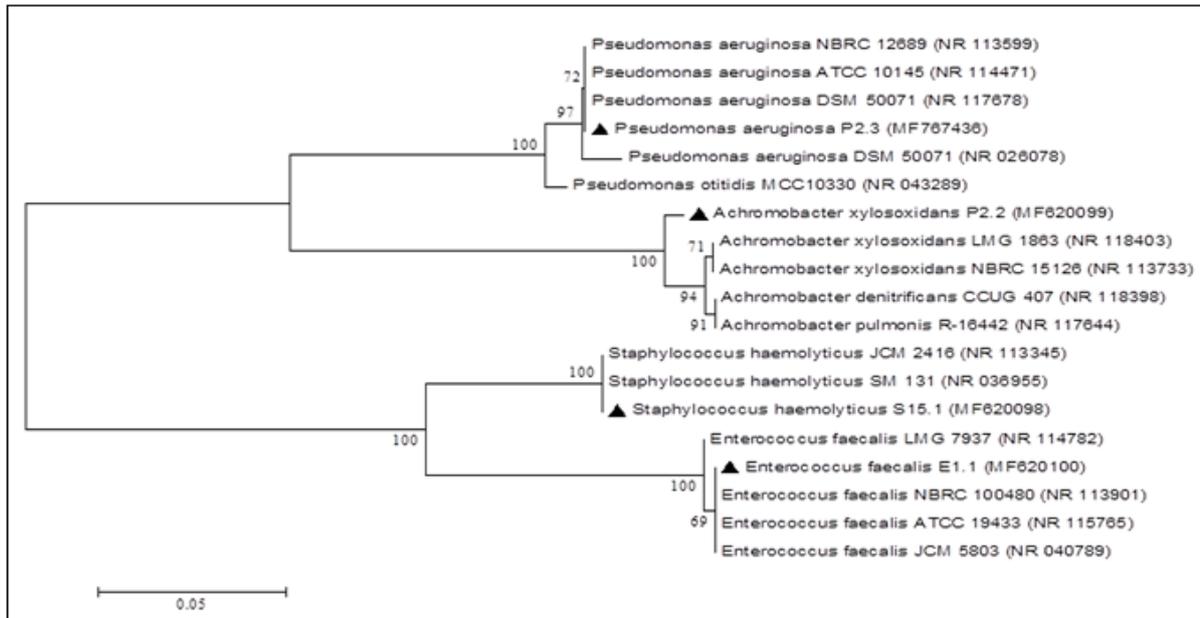


Fig. 6. Phylogenetic tree based on the partial 16S rRNA gene sequences of isolates (P2.3, P2.2, S15.1 and E1.1) and related species found by a BLASTn database search. The evolutionary history was inferred using the Neighbor-Joining method. The evolutionary distances were computed using the Jukes-Cantor method. The percentages of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. GenBank accession numbers are listed after species between parentheses.

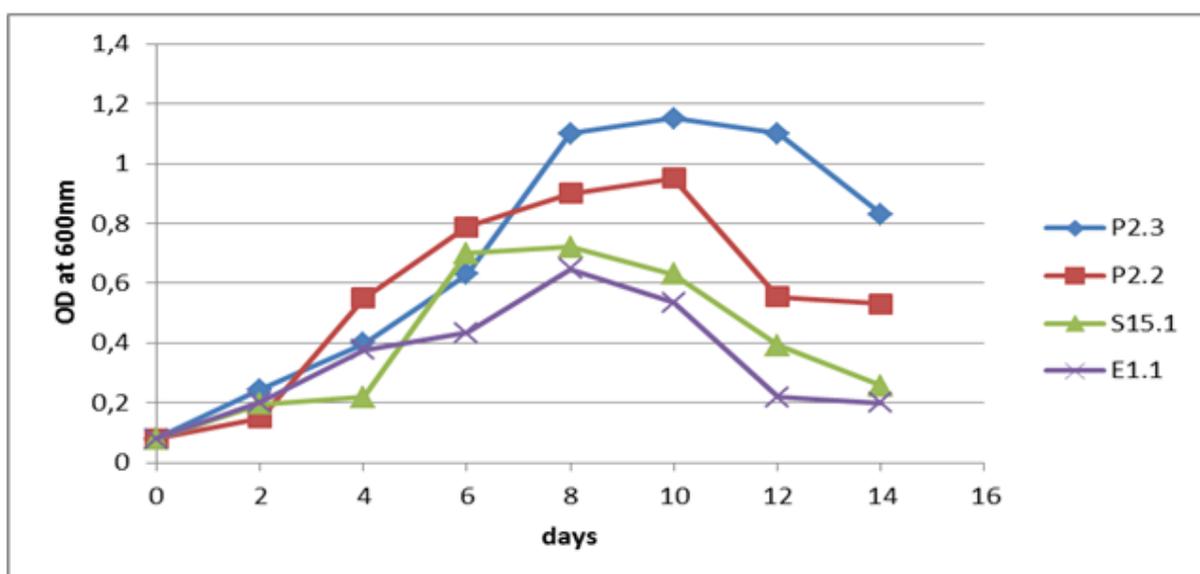


Fig .7. Growth curve (OD values) of P3.2, P2.2, S15.1 and E1.1 in MSM broth with 1% crude oil for a15 days of incubation.

Linear increase in OD was observed between days 4 and 10 of strains *Pseudomonas aeruginosa* P2.3 and *Achromobacter xylosoxidans* P2.2 but *Staphylococcus haemolyticus* S15.1 and *Enterococcus faecalis* E1.1 attained the stationary phase at day 8. *Pseudomonas aeruginosa* P2.3 and *Achromobacter xylosoxidans* P2.2 had the highest growth in the medium with crude oil while *Staphylococcus haemolyticus* S15.1 and *Enterococcus faecalis* E1.1 had the lowest ability to degrade crude oil. Similarly, Sibi and his colleagues. (2015) reported that *Pseudomonas aeruginosa* and *Achromobacter* spp showed the highest growth in petrol and diesel containing media. In another study realized by Obuotor *et al.* (2016) *Pseudomonas aeruginosa* was able to utilize spent engine oil better than all other isolates. According to Das and Mukherjee. (2007), *Pseudomonas aeruginosa* strains were more efficient than *Bacillus subtilis* strains isolated from a petroleum contaminated soil from north-east India. On the other hand, Boontawan and Boontawan. (2011), found that *Enterococcus faecalis* showed a high oil degradation performance.

Conclusion

In this current study, four bacterial strains were isolated from different oil contaminated site in Arzew oil refinery. These strains have significant ability to utilize a crude oil as sole source of carbon and energy. On the basis of morphological, biochemical and molecular characterization, these new isolates were identified as *Pseudomonas aeruginosa* P2.3, *Achromobacter xylosoxidans* P2.2, *Staphylococcus haemolyticus* S15.1 and *Enterococcus faecalis* E1.1. To our knowledge, except the *Pseudomonas aeruginosa* P2.3, the other three strains were isolated in the Arzew area for the first time. This study reported that *Pseudomonas aeruginosa* P2.3 and *Achromobacter xylosoxidans* P2.2 exhibited the best growth and had the highest levels of petroleum degradation. The ability of this strain to degrade hydrocarbon in oil polluted site suggests that they could be used for the treatment of other oil wastes in soil and water. Additional works will be suggested in our future research in order to determine the optimum environmental and biological factors favorable for biodegradation potential.

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References

- AL-Adwan MM.** 2010. Molecular Detection and Identification of Oil Degrading Bacteria from Oil-contaminated Soil in Al-Rwuyashid area (The Northeast Part of Jordan), thesis, University of Yarmouk, Jordan, 29-32.
- Atlas RM.** 1981. Microbial degradation of petroleum hydrocarbons: an environmental perspective. *Microbiological reviews* **45**, 180-209.
- Aubert G.** 1978. Méthodes d'analyses des sols, Centre régional de documentation pédagogique de Marseille, 191.
- Boboye B, Olukunle O, Adetuyi F.** 2010. Degradative activity of bacteria isolated from hydrocarbon-polluted site in Ilaje, Ondo State, Nigeria. *African Journal of Microbiology Research* **4**, 2484-2491.
- Boontawan A, Boontawan P.** 2011. Isolation and characterization of *Jatropha* oil degradation by *Enterococcus faecalis* and *Burkholderia cenocepacia* W-1 under anaerobic condition. *African Journal of Biotechnology* **10**, 13841-13851.
<https://doi.org/10.5897/AJB10.1720>
- Bouderhem A, Ould EL Hadj KHelil A, DJrarbaoui AN, Aroussi A.** 2016. The Use of Terrestrial Indigenous Bacterial Strains In the Bioremediation of Oil Contaminated Soils. *Advances in Environmental Biology* **10**, 259-265.
- Chadli A, Baba Hamed MB, Kihal M.** 2013. Characterization of indigenous and adapted hydrocarbon degrading bacteria isolated from landfill leachate from ain temouchent engineered landfill, Algeria. *Journal of Environmental Science and Engineering* **A2**, 537-548.

- Chaussod R, Nicolardot B, Catroux G.** 1986. Mesure en routine de la biomasse microbienne des sols par la méthode de fumigation au chloroforme. *Science du sol* **2**, 201-211.
- Chaussod R, Zuvia M, Breuil MC, Hétiér JM.** 1992. Biomasse microbienne et statut organique des sols tropicaux: exemple d'un sol vénézuélien des Llanos sous différents systèmes de culture. *Cahiers ORSTOM. Série pédologie* **27**, 59-67.
- Das K, Mukherjee AK.** 2007. Crude petroleum-oil biodegradation efficiency of *Bacillus subtilis* and *Pseudomonas aeruginosa* strains isolated from a petroleum-oil contaminated soil from North-East India. *Bioresource technology* **98**, 1339-1345.
- Das N, Chandran P.** 2010. Microbial degradation of petroleum hydrocarbon contaminants: an overview. *Biotechnology research international* **2011**, 1-13.
- De Vasconcellos SP, Crespim E, da Cruz GF, Senatore DB, Simioni KCM, dos Santos Neto EV, Marsaioli AJ, de Oliveira VM.** 2009. Isolation, biodegradation ability and molecular detection of hydrocarbon degrading bacteria in petroleum samples from a Brazilian offshore basin. *Organic Geochemistry* **40**, 574-588.
- Ebrahimi M, Sarikhani M, Fallah R.** 2012. Assessment of biodegradation efficiency of some isolated bacteria from oil-contaminated sites in solid and liquid media containing oil-compounds. *Internal Research Journal of Applied Basic Sciences* **3**, 138-147.
- Erdogan EE, Sahin F, Karaca A.** 2012. Determination of petroleum-degrading bacteria isolated from crude oil-contaminated soil in Turkey. *African Journal of Biotechnology* **11**, 4853-4859.
<https://doi.org/10.5897/AJB10.2239>
- Fardoux J, Fernandes P, Niane-Badiane A, Chotte J.** 2000. Effet du séchage d'échantillons d'un sol ferrugineux tropical sur la détermination de la biomasse microbienne: Comparaison de deux méthodes biocidales de référence. *Etude et Gestion des Sols* **7**, 385-394.
- Greene EA, Kay JG, Jaber K, Stehmeier LG, Voordouw G.** 2000. Composition of soil microbial communities enriched on a mixture of aromatic hydrocarbons. *Applied and environmental microbiology* **66**, 5282-5289.
- Guermouche M, Bensalah F, Gray N.** 2013. Application of molecular methods as a biomarker in bioremediation studies. *International Journal of Biotechnology Applications* **5**, 147-154.
- Hamzah A, Rabu A, Azmy R, Yussoff NA.** 2010. Isolation and characterization of bacteria degrading Sumandak and South Angsi oils. *Sains Malaysiana* **39**, 161-168.
- Holliger C, Gaspard S, Glod G, Heijman C, Schumacher W, Schwarzenbach RP, Vazquez F.** 1997. Contaminated environments in the subsurface and bioremediation: organic contaminants. *FEMS Microbiology Reviews* **20**, 517-523.
- Holt JG, Krieg NR, Sneath PHA, Stanley JT, William ST.** 1994. *Bergey's Manual of Determinative Bacteriology*, William and Wilkins, Baltimore.
- Jukes TH, Cantor CR.** 1969. Evolution of protein molecules. In Munro HN, Editor, *Mammalian Protein Metabolism*, Academic Press, New York.
- Jyothi K, Surendra Bk, Nancy CK, Amita K.** 2012. Identification and Isolation of Hydrocarbon Degrading Bacteria by Molecular Characterization. *Helix* **2**, 105-111.
- Kaboré-Ouédraogo PW, Savadogo PW, Ouattara CAT, Savadogo A, Traoré AS.** 2010. Etude de la Bio-dépollution de Sols contaminés par les Hydrocarbures au Burkina Faso. *Journal de la Société Ouest-Africaine de Chimie* **30**, 19-28.
- Kvenvolden K, Cooper C.** 2003. Natural seepage of crude oil into the marine environment. *Geo-Marine Letters* **23**, 140-146.

- Leahy JG, Colwell RR.** 1990. Microbial degradation of hydrocarbons in the environment. *Microbiological reviews* **54**, 305-315.
- Liao J, Wang J, Huang Y.** 2015. Bacterial community features are shaped by geographic location, physicochemical properties, and oil contamination of soil in main oil fields of China. *Microbial ecology* **70**, 380-389.
- Lin J, Singh C.** 2008. Isolation and characterization of diesel oil degrading indigenous microorganisms in Kwazulu-Natal, South Africa. *African Journal of Biotechnology* **7**, 1927-1932.
- Mancera-Lopez M, Rodriguez-Casasola M, Rios-Leal E, Esparza Garcia F, Chavez Gomez B, Rodriguez-Vazquez R, Barrera-Cortes J.** 2007. Fungi and Bacteria Isolated from Two Highly Polluted Soils for Hydrocarbon Degradation. *Acta Chimica Slovenica* **54**, 201-209.
- Meenakshisundaram M, Bharathiraja C.** 2014. Isolation and molecular identification of hydrocarbon degrading bacteria from oil contaminated soils from Tamilnadu. *Indian Journal of Applied Research* **4**, 39-42.
- Mulkins-Phillips G, Stewart JE.** 1974. Distribution of hydrocarbon-utilizing bacteria in Northwestern Atlantic waters and coastal sediments. *Canadian Journal of Microbiology* **20**, 955-962.
- Obuotor T, Sakariyau A, Bada B.** 2016. Enhanced Biodegradation of Spent Engine Oil Contaminated Soil using Organic Wastes. *Applied Environmental Research* **38**, 27-38.
- Palanisamy N, Ramya J, Kumar S, Vasanthi N, Chandran P, Khan S.** 2014. Diesel biodegradation capacities of indigenous bacterial species isolated from diesel contaminated soil. *Journal of environmental health science and engineering* **12**, 142.
- Pétard J.** 1993. Les methodes d'analyse. I. Analyses des sols. Notes techniques, laboratoire commun d'analyses 5, Orstom Nouméa.
- Rahman K, Rahman TJ, Kourkoutas Y, Petsas I, Marchant R, Banat I.** 2003. Enhanced bioremediation of n-alkane in petroleum sludge using bacterial consortium amended with rhamnolipid and micronutrients. *Bioresource technology* **90**, 159-168.
- Saitou N, Nei M.** 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* **4**, 406-425.
- Salam LB, Obayori OS, Akashoro OS, Okogie GO.** 2011. Biodegradation of bonny light crude oil by bacteria isolated from contaminated soil. *International Journal of Agriculture and Biology* **13**, 245-250.
- Scherrer P, Mille G.** 1990. Biodegradation of crude oil in experimentally-polluted clayey and sandy mangrove soils. *Oil and Chemical Pollution* **6**, 163-176.
[https://doi.org/10.1016/S0269-8579\(05\)80022-X](https://doi.org/10.1016/S0269-8579(05)80022-X)
- Sibi G, Pant G, Simaria C.** 2015. Characterization and Evaluation of Polycyclic Aromatic Hydrocarbon (PAH) Degrading Bacteria Isolated from Oil Contaminated Soil. *Applied Microbiology: Open Access* **1**.
<https://doi.org/10.4172/2471-9315.1000104>
- Tamura K, Stecher G, Peterson D, Filipski A, Kumar S.** 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution* **30**, 2725-2729.
- Verma S, Bhargava R, Pruthi V.** 2006. Oily sludge degradation by bacteria from Ankleshwar, India. *International biodeterioration and biodegradation* **57**, 207-213.