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Phenotypic, Molecular Characterization and Evaluation of Effectiveness for the Bioremediation of Oil-Degrading Bacteria Isolated from Different Habitats in the United Arab Emirates

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Abstract

Objective: To isolate potential hydrocarbon degraders from different habitats within the United Arab Emirates (UAE) contaminated with Hydrocarbon Compounds (HC) and assess their degradation potential by a rapid qualitative method. **Methods/Analysis**: Eight different oil contaminated samples were collected for isolation of Hydrocarbon Degrading Bacteria (HDB) on the surface of nutrient agar plates where the appearance of bacterial colonies was observed and then phenotypically and biochemically characterized. Isolates were screened for HC degradation by the "whole plate diffusion" method after observation of the bacterial growth around the hole. PCR analysis was carried out to detect the key degrading gene, alkane hydroxylase gene (A*lkB*) in the positive HDB. **Findings:** Results indicated the recovery of 19 isolates from different HC contaminated samples with nine isolates namely (2A, 1D, So1, S1A, S3, KF1, SO2, AJ1 and 2B) were identified as positive degraders for one or more of the tested hydrocarbon compounds. The majority of these isolates were able to utilize heptane as a sole carbon source for their survival while the isolates SO2, S1A and S3 were the most potent as indicated by their growth around the agar hole-plate. Degradation of hydrocarbon compounds by indigenous microbial communities to marine environment was shown to be successful with a significant biodegradation role can be played by the recovered isolates. PCR analysis of the positive degraders for the presence of A*lkB* gene showed two groups with different band size products; group 1 (G1) (~330 bp) and group 2 (G2) (multiple of 330 pb). **Novelty/Improvements:** This study confirmed the HC degradation after detecting the marker *AlkB* gene in the positive degraders, and the use of th "Hole plate diffusion method" as a rapid qualitative evaluation method for bacterial HC degradation by bacteria.

Keywords: *AlkB* gene, Degradation, Hydrocarbon, Oil Spills, PCR

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1. Introduction

Environmental exposure to oil pollution resulting from human anthropogenic activities such as exploration, production and transportation operations is a widespread problem that is frequently encountered in the environment. Introduction of oil components to different environmental habitats of water and soil can cause significant and long lasting damage to the surrounding ecosystem. In many cases, however, the oil dispersants have been found to be more toxic and responsible for more long lasting damage than the original oil spill.

There is a regular trend of small-scale natural seepages of oil wherever there are shallow deposits of oil. These seepages provide a constant and small-scale supply of hydrocarbons. Over biological time, it is assumed that bacteria will naturally evolve to exploit this environmental niche. These bacteria therefore are potentially an effective and natural treatment for anthropomorphic oil spills and have the potential to provide a wholly natural approach to oil spill cleanup. In this study, we attempt to locate and identify them and test their effectiveness to break up oil by simple and rapid method with a promising gall in mind of implementing appropriate steps of action to clean up these polluted sites by bioremediation which is a natural or managed biological degradation of environmental pollution and considered as an important option for restoration of oil-polluted environments[1].

In the last few years, a number of oil spill accidents have been reported which have affected the UAE Gulf and Indian Ocean coastlines resulting in marine damage and loss of tourism. Particularly significant spills occurred at Dubai 2001, Khorfakkan[2]. The Federal Government of the United Arab Emirates (UAE) already has in place environmental regulations to monitor the oil spill problem and set appropriate response strategies to minimize the immediate and long-term damage[3]. The UAE is a member of the International Maritime Organization (IMO) since 1980 and has as one of its main goals the development of a regulatory framework for shipping to prevent and minimize marine pollution resulting from oil contamination[4].

1.1 Previous Work

In the literature, researchers have been able to identify a diverse range of Hydrocarbon Degrading Bacteria (HDB) such as *Pseudomonas, Marinobacter, Micrococcus* and *Alcanivorax*[5], which have been isolated and identified for their capability to degrade petroleum hydrocarbons in various habitats. It has been observed that since crude oils are a very varied mixture of thousands of different hydrocarbons, individual species tend to degrade only a limited range of hydrocarbons. Successful biodegradation techniques therefore need to employ a bacterial mixture or consortium for succession removal of different hydrocarbon pollutants.

Indigenous microbes utilize the available hydrocarbon to grow and thrive as part of the natural biodegradation process. However, these bacteria account for less than one percent of the total microbial community in a typical unpolluted environment, rising to about 10% in response to hydrocarbon pollution[6]. Since the composition of crude oil spills varies, with alkanes, cycloalkanes, aromatic compounds and petroleum hydrocarbons[5] being present, this tends to limit the nutritional source for a specific microbial community resulting in slow growth and slow bioremediation. However, once the community has been stimulated via supply of hydrocarbon food, the native hydrocarbon degrading bacteria population increases, causing a major change in the diversity of the microbial community. This leads to the concept of pre-producing the necessary bacteria and storing them for deployment in the initial phase of an oil spill. Initial spraying of an oil spill with these bacteria and nutrients would kick-start the natural bioremediation process reducing the need for the environmentally harmful dispersants.

Molecular studies had identified several genes and enzymes involved in the fundamental process of hydrocarbon biodegradation and the relative metabolic pathways. Some species are potent degraders of crude oil, including *Pseudomonas aeruginosa* and *Alcanivorax borkumensis.* These have been reported previously to possess an alkane monooxygenase ALKB (*AlkB*) gene. *AlkB* gene is one of the key genes involved in the aerobic degradation

of alkanes. This is important because alkanes represent a significant proportion of crude oil, reaching up to 50%[7]. Initial steps include the activation of alkanes by the addition of oxygen (oxidation) to make it more water soluble which also introduces reactive sites for further breakdown of the hydrocarbons[8].

The aim of the present study is to isolate and identify indigenous hydrocarbon degrading bacteria from different environmental habitats of the UAE. The ability of these recovered bacteria to degrade different hydrocarbon compounds (pentane, hexane, heptane, tetradecane and diesel) as individual species and as a consortium has also been investigated.

2. Materials and Methods

2.1 Location, Sampling and Sample Processing

In total, eight different oil contaminated samples were collected from various sites for the isolation of HDB; 2 seawater samples and 1 surface sediment sample were collected from Ajman and Khorfkkan (UAE) harbor in 500 mL sterile Duran bottles (Table 1). Another sample of water exposed to heavy oil contamination sample was obtained from Abu Dhabi. Crude oil samples were collected from three different oil producing wells (SB-161 and SB-226) and provided by Khalifa University. All the collected samples (Table 1) were transported to laboratory and stored at 4°C for further study and analysis. In addition, a soil sample that has been regularly exposed to diesel contamination was included in this investigation. Briefly a soil sample of ~ 2 Kg was collected from City of Sharjah/UAE, placed in a plastic bag and then transferred to the lab. The soil sample was spread on a plastic tray of 40 cm length x 30 cm width and kept at room temperature under laboratory conditions and regular spraying with diesel for ~ 5 years.

2.2 Determination of Bacterial Population, Isolation and Identification

All samples were inoculated as 0.5% (vol/vol) of water, 0.5% (wt/vol) of sediment, or 1% (wt/vol) of soil and incubated at 28°C for 2-4 weeks with continuous shaking in a

Table 1. Description of the collected samples and their locations

Sample Number	Name	Abbreviation	Description	Location
1	ADCO	AD	Crude oil, Black, viscus	Abu Dhabi
2	SB-161	1	Crude oil, Black, low viscosity	Abu Dhabi
3	SB-226	2	Crude oil, Black, low viscosity	Abu Dhabi
4	Water oil AD	O	Polluted water with heavy oil	Abu Dhabi
5	Sedimented oil	S	Oil contaminated sludge	Khorfakkan harbor
6	AJM water	AJ	Diesel contaminated water	Ajman harbor
7	KFN water	KF	Diesel contaminated water	Khorfakkan harbor
8	Soil	SO	Soil treated with Diesel	UOS lab

rotary shaker (Fisher Roto Rack Model 343) at 120 rpm in Erlenmeyer flasks (250 mL) containing 100 mL nutrient broth (NB) with at least 40% headspace to promote sufficient aeration. The soil sample was incubated under the same conditions but for 30 minutes only. The samples were incubated until turbidity was observed, and then serially diluted up to 1 x 10^{-6}. Aliquots of 0.1 mL from each dilution were spread over the surface of nutrient agar (Himedia, India) plates. The plates were incubated at 28°C for 2 days and thereafter the appearance of different bacterial colonies on the surface of agar plates was observed. Morphological features such as color, size, form, margin, and elevation of each colony were determined. Phenotypically, different isolated colonies were characterized based on their morphological characteristics and staining reaction according to[9]. As a confirmatory test, bacterial isolates were streaked on Eosin Methylene Blue (EMB) agar media selective for Gram-negative bacteria.

Isolated bacteria were biochemically identified using VITEK automated microbial identification system (VITEK 2 compact) and based on Gram-positive (GP) and Gram-negative (GN) colorimetric reagents card. The VITEK 2 database contains a large set of well-characterized microorganism strains that were tested under different culture conditions and in which some of them were derived from clinical and industrial sources. Forty-seven biochemical tests including Beta-Galactosidase, H_2S production and lipase were performed for Gram-negative bacteria, while 43 biochemical tests including Cyclodextrin, phosphatase and urease were applied for Gram-positive bacteria[10].

2.3 Hydrocarbon Degradation Test

Screening for hydrocarbon degrading bacteria was carried out using the "hole plate diffusion" method[11] where diesel, pentane, hexane, heptanes, and tetradecane were used as the sole hydrocarbon source.

Pure single colonies representing each isolate were inoculated by a sterile loop into 5 ml sterile NB, incubated in a rotary shaker at 120 rpm and 28°C for 48-72 hours until turbidity was observed. Bacterial cultures were centrifuged (Heraeus Megafuge 16R centrifuge) at 3000 rpm for 8 minutes, cell pellets were collected and washed three times with sterile 0.85% normal saline and then suspended in 1 ml saline.

Washed bacterial suspension free of nutrient broth residues was plated on mineral salt agar medium (MSM) plates[12], composed of; (g/L): NaCl 2.5 g, K_2HPO_4 4.74 g, KH_2PO_4 0.56 g, $MgSO_4.7H_2O$ 0.5 g, $CaCl.6H_2O$ 0.1 g, NH_4NO_3 0.5 g, agar 20 g and supplemented with 0.001% (wt/ vol) yeast extract and the pH adjusted to 7.1. A 6mm diameter plug was removed from 20 mL MSM agar plates and 50 µL of each hydrocarbon source was placed in the well. Similarly, plates with holes filled with 50 µL sterile distilled water were used as a control. All plates were incubated at 28°C for 7 to 12 days for bacterial growth observation.

Based on the screening test, bacterial isolates with a potential of hydrocarbon degradation were inoculated as consortia in 5 mL NB, incubated 48 hours and further analyzed using the same screening procedure.

3. Molecular Detection of *AlkB* Gene

3.1 Genomic DNA Extraction

Isolates with efficient degrading capability were chosen for DNA genomic extraction. A fresh overnight bacterial culture was used for total Genomic DNA recovery using PureLink® Genomic DNA Kit (Bioline) according to the manufacturer's instructions. The purity and quality of extracted DNA was examined in 1 % (wt/vol) agarose gel electrophoresis in TAE buffer.

3.2 *AlkB* gene Primer and PCR Amplification

For the detection of the key degrading gene, alkane hydroxylase gene (*AlkB*), forward and reverse primers, ALK-F: 5'-TCGAGCACAACCGCGGCCACCA-3' and ALK-R: 5'-CCGTAGTGCTCGACGTAGTT-3', were selected for A*lkB* amplification according to the method[13]. Polymerase chain reaction (PCR) was carried out in a total volume of 25 µL containing: 9.5 µL Nuclease free water, 1

µL genomic DNA, 1 µL forward primer and 1 µL reverse primer with 12.5 µL PCR mixture (Taq PCR Master Mix, Qiagene). Amplification was performed, starting with initial temperature of 94°C for 5 minutes followed by 30 cycles of 94 °C for 1 minute, 58 °C for 1 minute and 72 °C for 1 minute, with a final extension at 72 °C for 5 minutes. The PCR reaction was performed in Thermal cycle (S1000™) and held at 4 °C then finally stored at -20 °C for further use. The resulting PCR products were examined in 2 % (wt/vol) agarose gel electrophoresis along with 100 bp ladder (Promega, USA), the gel is further visualized using Gel Doc-It ™ imagining system.

4. Results

4.1 Bacterial Isolation

Nineteen morphologically different bacterial isolates were identified from the 8 samples. Samples required different periods to show bacterial growth as indicated by turbidity. Although nutrient media used in the isolation provides all the supplements needed for bacterial growth, it took from 2 weeks up to 1 month for turbidity to be observed. Crude oil samples took approximately one month of incubation to observe turbidity. Spreading of turbid samples resulted

Table 2. Morphological characteristics and Gram staining for the different 19 isolates recovered from different habitats

Sample	Isolate No.	Isolate abbreviation	Shape	Margin	Elevation	Colony surface	Size	Color	Gram staining
SB-161	1	1A	Circular	Entire	Flat	Smooth	Small	Translucent	-
	2	1B	Circular	Entire	Raised	Smooth	Medium	Dark creamy	+
	3	1C	Circular	Entire	Raised	Smooth	Pin point (very small)	White	-
	4	1D	Circular	Entire	Convex	Smooth	Pin point (very small)	Light orange	+
AJM	5	AJ1	Punctiform	Entire	Flat	Smooth	Pin point (very small)	Translucent	+
KFN	6	KF1	Irregular	Undulate	Raised	Wrinkled/ Dull	Small	Transparent	-
SB-226	7	2A	Circular	Entire	Flat	Smooth	Pin point	Translucent	-
	8	2B	Circular	Entire	Raised	Smooth	Pin point	Light creamy	-

Table 2 Continued

ADCO	9	AD1	Circular	Entire	Flat	Smooth	Pin point (very small)	Yellow	+
	10	AD2	Circular	Entire	Raise	Smooth	Pin point (very small)	Translucent	+
Sediment	11	S1A	Circular	Entire	Raised	Smooth	Medium	Translucent	+
	12	S1B	Circular	Entire	Raised	Smooth	Medium	Light creamy	-
	13	S2	Circular	Entire	Flat	Smooth	Pi point (very small)	Light creamy	+
	14	S3	Circular	Entire	Raised	Smooth	Pin point (very small)	White	+
	15	S4	Circular	Entire	Flat	Dull/ Smooth	Pin point (very small)	Light yellow	-
Soil	16	SO1	Irregular	Undulate	Flat	Smooth	Medium	Creamy	+
	17	SO2	Circular	Entire	Convex	Smooth	Small	Light creamy	+
	18	SO3	Irregular	Undulate	Flat	Smooth	Small	Translucent	+
Water OIL	19	O2	Circular	Entire	Flat	Shiny/ Smooth	Small	Translucent	-

in the isolation of 19 different types of colonies (Table 2). For example, spreading of SB-226 resulted in the isolation of two types of colonies based on morphology. Also, three different colonies; SO1, SO2 and SO3 were isolated from the diesel treated soil.

4.2 Phenotypic Characterization of the Isolates

Based on Bergey's Manual[9], the morphological feature and gram staining for the 19 recovered isolates are shown in (Table 2). Results of Gram staining showed that eight

isolates were Gram negative and 11 isolates were Gram positive. Growth of the Gram-negative isolates on EMB agar selective media confirmed the gram reaction.

4.3 Biochemical Identification

The isolates were biochemically identified by the VITEK 2 automated microbial identification system. Based on the conducted tests, isolates SO1, S4, 1D, 1C, AD1, S1A, KF1, S3 and 2A were identified in a percentage that ranged between 90-99 %. On the other hand, SO2 had low discrimination identification where 2 choices were available such as *Micrococcus luteus* and *Kocuria varians* by a percentage of 50-50 %. This system of identification revealed the following: SO1 (*Staphylococcus aureus* 93 %), S4 (*Acinetobacter haemolyticus* 90 %), 1D (*Kocuria kristinae* 92 %), 1C (*Yersinia enterocolitica group* 96 %), AD1 (*Micrococcus luteus/lylae* 99 %), S1A (*Leuconostoc mesenteroides ssp. Cremoris* 90 %), KF1 (*Rhizobium radiobacter* 99 %), S3 (*Staphylococcus hominis* 96 %), and 2A (*Pseudomonas stutzeri* 97 %). SO2 was identified as low discrimination organism (*Micrococcus luteus/lylae* 50 % / *Kocuria varians* 50 %). Isolates 2B, 1B & AJ1 were unidentified.

4.4 Hydrocarbon Degradation Test

Screening for hydrocarbon degrading bacteria was done

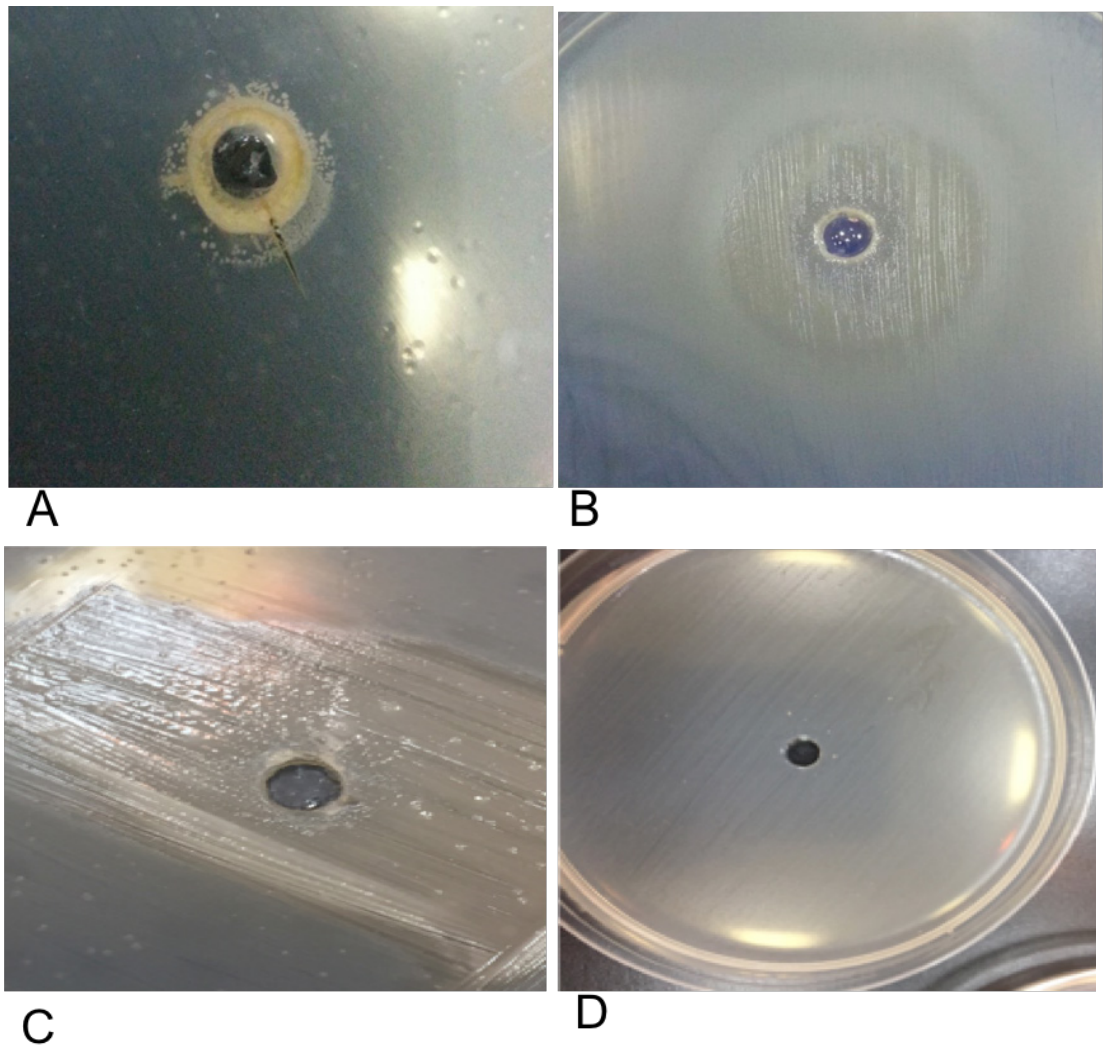

Figure 1. Screening test for SO2 isolate growing on different hydrocarbon compounds. SO2 showed concentrated growth around the hole-containing A) hexane, B) tetradecane C) diesel D) Control plate with sterile distilled water.

by the "hole plate diffusion method" using diesel, pentane, hexane, heptane, and tetradecane as the sole hydrocarbon source. SO2 isolate showed growth around the hole that contains hexane, tetradecane and diesel when compared to the control (Figure 1). The same result was obtained for S1A isolate in response to heptane and diesel (data not shown). Similarly, AJ1, SO1, and 1D isolates showed concentrated growth around the hole that contains heptane (Figure 2). Also, growth of SO1 and 2B isolates around the hole that contained tetradecane was observed (Figure

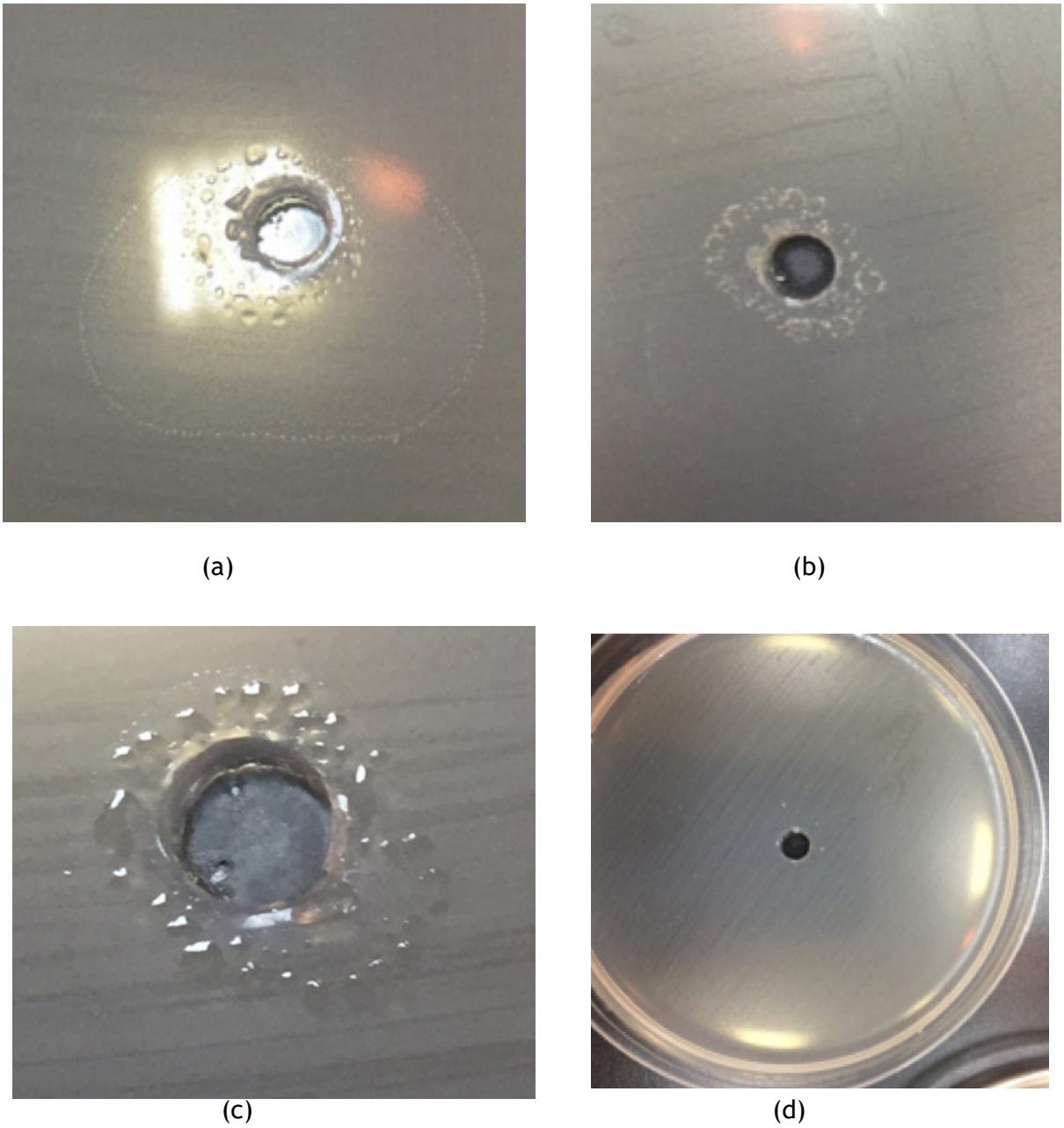

Figure 2. Screening test for AJ1, SO1, and 1D isolates showed concentrated growth around the hole-containing heptane. A) AJ1, B) SO1, C) 1D, D) Control plate with sterile distilled water.

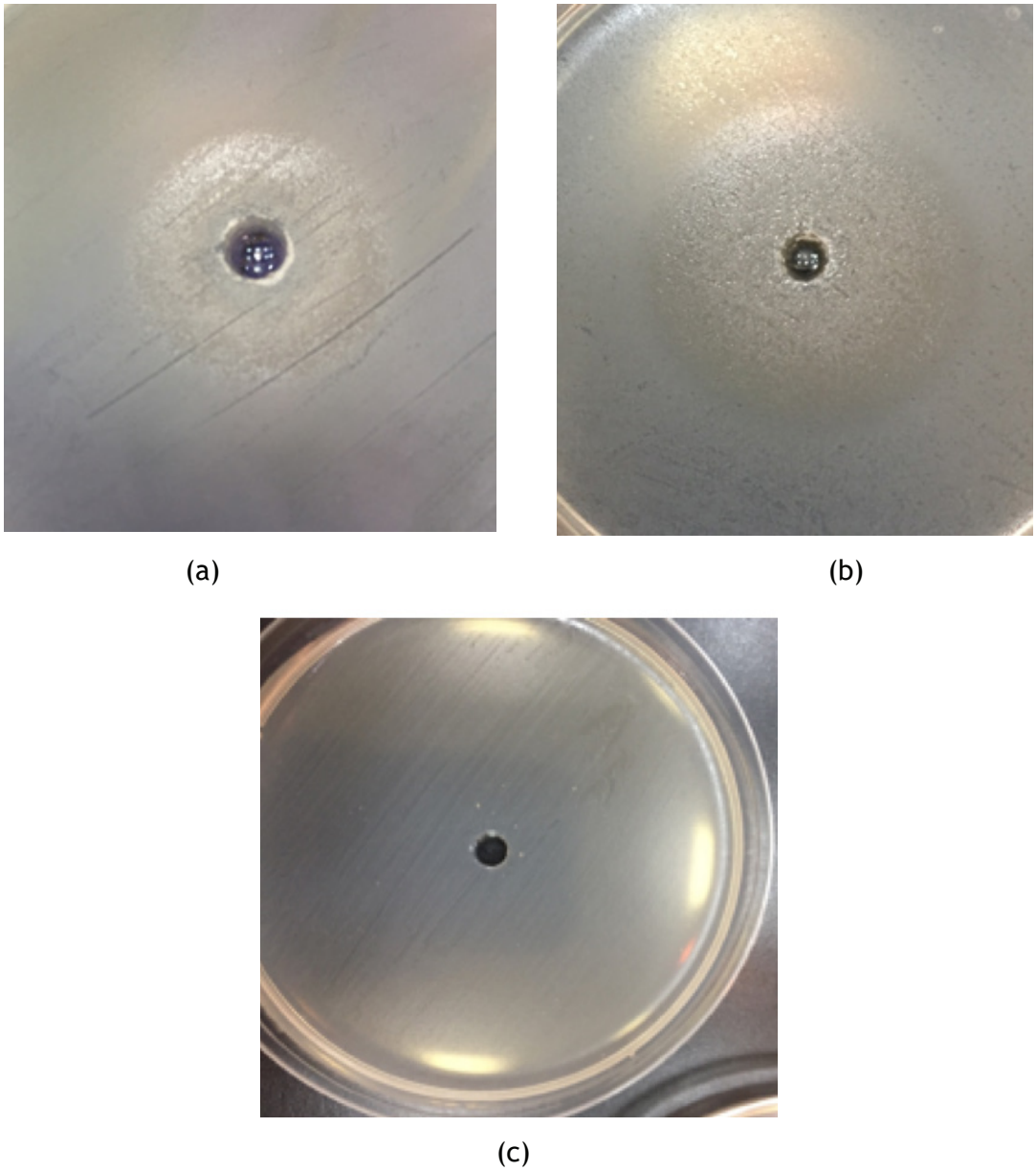

Figure 3. Screening test for SO1 and 2B isolates showed concentrated growth around the hole-containing tetradecane. A) SO1, B) 2B, C) Control plate with sterile distilled water.

3). In contrast, 2A (tetradecane and heptane), S3 (heptane and diesel) and KF1 (heptane) showed distant growth from the hole (data not shown). Contrary, no growth was observed in plates that contained pentane (data not shown).

Based on the screening test, bacterial isolates (KF1, AJ1, 2A, S3, 1D, SO2, and S1A) showed a potential for hydrocarbon degradation. The growth of a consortium of these isolates was further observed around the hole containing diesel, tetradecane and heptane (Figure 4).

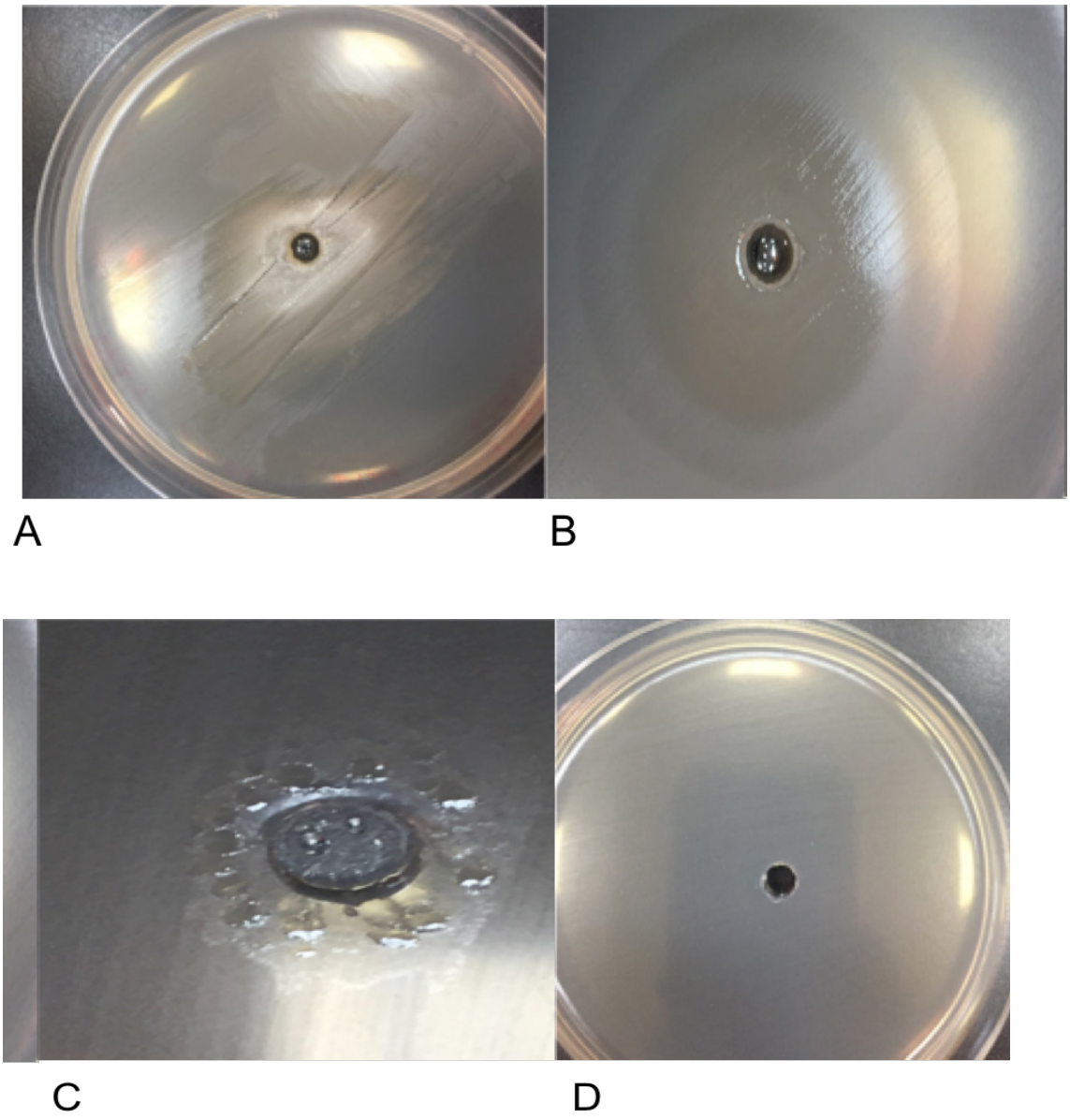

Figure 4. Screening test for bacterial consortium growing on different hydrocarbon compounds. Growth of bacterial consortium around the hole-containing A) diesel B) tetradecane and C) heptane D) Control plate with sterile distilled water.

4.5 Molecular Detection of A*lkB* gene

Genomic DNA was successfully extracted for KF1, S3, 2A, 1D, SO1, AJ1 and S1A isolates. Analysis of the positive hydrocarbon degraders isolates for the presence of *AlkB* gene by PCR showed two groups with different band size products; group 1 (G1) (~330 bp) and group 2 (G2) (multiple of 330 pb) (Figure 5).

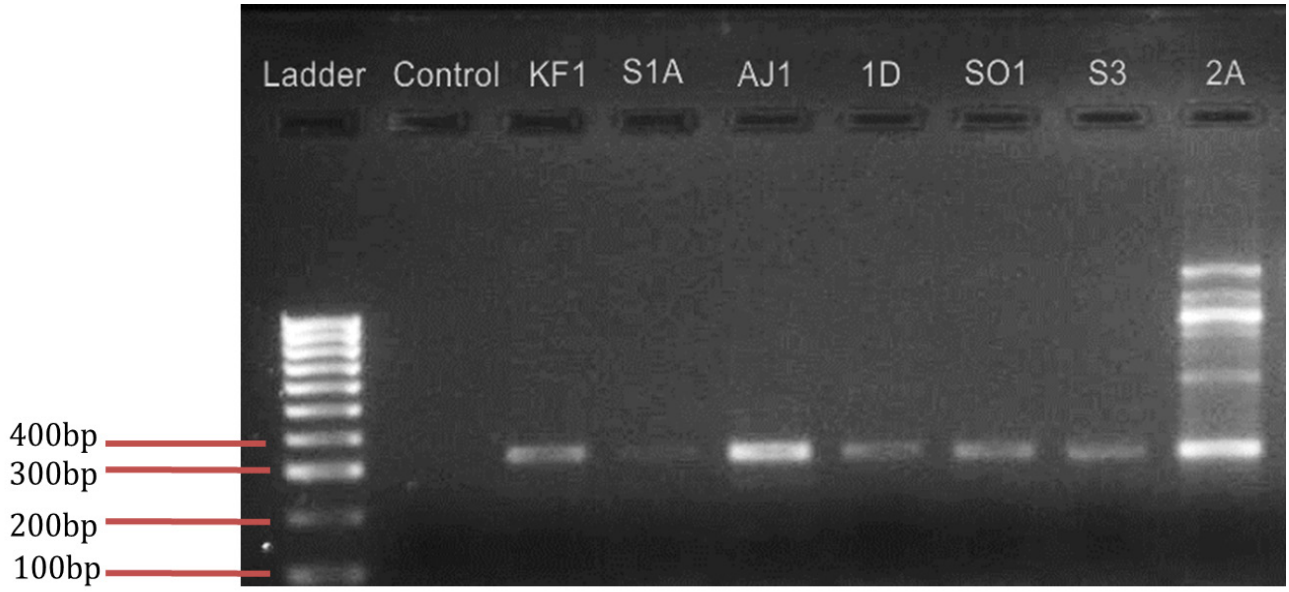

Figure 5. Agarose gel (2%) electrophoresis of PCR amplification of alkane hydroxylase genes from the positive hydrocarbon degrading isolates' with AlkB primers. Lane 1: 100 bp molecular weight marker. Lane 2: negative control; Lane 3: KF1; Lane 4: S1A; Lane 5: AJ1; Lane 6: 1D; Lane 7: SO1; Lane 8: S3; Lane 9: 2A. All isolates (lane 3-8) showed a band with approximate size of 330 bp or multiple of 330 bp in isolate 2A (lane 9).

5. Discussion

Oil spills is a major problem that affects aquatic and terrestrial environments and has a dramatic negative effect in the whole ecological system balance. Current practices such as dispersants do little to deal with the crude oil spills. However, evidence shows that long after they break up the oil and oil has gone; dispersants persist, causing environmental damage over a period of several years[14]. This problem has led to extensive research to find the least harmful solution of treatment of oil spills using physical and biological methods instead of conventional chemical methods. Consistent with the UAE's environmental strategy, bioremediation is a promising technology that stimulates the natural biodegradation process by bacteria for efficient clean up and biodegradation. It is an effective alternative to chemical and physical methods. Native indigenous microbial community in such polluted environment have been studied for several years, from Red Sea coast, Persian Gulf, Gulf of Mexico, and from Ennore Creek, Pensacola Florida beach, for their capability to naturally utilize and mineralize some of crude oil and petroleum derivatives[15-18].

Pure cultures represented by 19 different bacterial isolates were morphologically variable in term of shape, color, elevation and other characteristics as presented in (Table 2). Isolation of different bacteria from the same sample indicates the diversity and the possibility of these bacteria to work as consortium in a real environment. The presence of all members of the microbial community in any of the tested samples may explain the cooperative action between the mixed bacterial populations using the available hydrocarbon sources to survive.

Biochemical tests were conducted to understand more about the physiology of the isolates and to identify them accurately. Results revealed that some isolates were identified in a percentage that ranged between 90 -99 %, while others had low discrimination identification with a percentage of 50 %. Additional tests and observation may help in determining the suitable identification between the available choices of low discrimination identification[10]. Isolates 2B, 1B and AJ1 were unidentified, which

may suggest that previously unidentified strains exist that deserve to be investigated in future.

In addition to identification, these biochemical tests provided a background of some of the enzymes possessed by the isolates. Furthermore, two of the identified isolates (***S3***-*Staphylococcus hominis* ***and 2A***-*Pseudomonas stutzeri*) were found to be reported in other studies concerning hydrocarbon biodegradation. For example, *P. stutzeri* ***had*** environmentally metabolic activities including the biodegradation of oil derivatives, aromatic and non-aromatic carbon[19]. In addition, *S. hominis* isolated from the soil of a petroleum station had the ability to degrade synthetic diesel[20].

All of the 19 isolates were screened for their capability to degrade various types of hydrocarbons; short chain (pentane, heptane), aromatic (hexane), alkanes (tetradecane) hydrocarbons and diesel. Mineral salts media that lack any nutritional source assayed bacterial response to each hydrocarbon source. Hydrocarbons provide a major source of carbon and electron otherwise nutrient starvation will suppress microbial growth. Hence, bacterial growth observed for (2A, 1D, SO1, SO2, S1A, S3, KF1, AJ1, 2B) isolates had resulted from utilizing the hydrocarbon substrates. Results showed that isolates S1A, S3 and SO2 are the most potent bacteria with capability to utilize two or four sources of hydrocarbons (diesel, hexane, tetradecane, and heptane).

Bacterial isolates with the ability to degrade hydrocarbons were tested as a consortium providing the first glance at the possible metabolic interaction in the microbial community. These results support the hypothesis of successful bioremediation depends on appropriate cooperative action of microbial consortia and environmental condition[21]. Interestingly, bacteria isolate from crude oil (1D, 2A, 2B) with the capability of degrading different hydrocarbon compounds are promising agents for bioremediation process. This finding provides evidence that hydrocarbon-degrading bacteria could be isolated not only from polluted environment but also from the crude oil itself if suitable nutrients were supplied.

In general, the microbial community responds to environmental stresses by adapting to the polluted environment and shifting to hydrocarbon tolerating bacteria or hydrocarbonoclastic bacteria[22]. These findings explained the concentrated growth of (1D, SO1, SO2, S1A, AJ1, 2B) isolates observed around the holes containing hydrocarbon, where these bacteria suggested to be hydrocarbonoclastic bacteria. Distribution of growth of the other isolates (KF1, 2A, S3) in the plate away from the hydrocarbon source can be explained as a result of less toxicity or the low molecular of the tested hydrocarbon compound (Figure 4C). Hydrocarbon concentration is another important factor of degradation efficiency, some bacteria cannot tolerate the higher concentration, and this can explain the phenomena of bacterial growth distant from the hole. These results are further confirmed by comparing the growth with the control; no bacterial growth was observed in the absence of hydrocarbon. Our overall results are consistent with evidence that the degradation process can take place without the addition of nourishment, depending only on the available substrates[22].

Alkane hydroxylase gene (A*lkB*) is a very important gene that has been recorded in several bacteria linked to hydrocarbon degradation including *Alcanivorax borkumensis*, *Pseudomonas aeruginosa*, *Mycobacteria* and *Rhodococcus genera*[7,8]. There are currently over 250 *alkB* gene homologues found in diverse bacterial species in which a large portion of these genes was detected in oil-contaminated environments[8]. PCR product was located between 300 and 400 bp on the gel (Figure 3) representing the A*lkB* gene as it has been reported previously[13]. The presence of A*lkB* gene in all of the seven hydrocarbon degraders suggests that they undergo an alkane oxidation pathway for alkane biodegradation. The isolate 2A showed a PCR product of multiple of 300-330 bp which may imply that (i) A*lkB* gene is multiplicated (duplicated, triplicated, or quadruplicated), in this strain in order to produce more alkane hydroxylase enzyme and be more efficient for alkane degradation, or (ii) different related A*lkB* genes are clustered together under the control of the same promoter that might be involved in alkane degradation rather than a single gene[23] explained the presence of four alkane monooxygenases in one strain of

Rhodococcus spp. (assuming that all four oxidize alkanes) is that each alkane monooxygenase is specific for a certain range of alkanes. For example, *Pseudomonas putida* GPo1 AlkB does not act on alkanes longer than C12, while the *P. rugosa* AlkBs do not efficiently oxidize alkanes shorter than C12[24]. As the *P. stutzeri* strain studied here oxidizes heptanes, tetradecane, and diesel, the multiple AlkB homologues may each cover a part degradation of specific carbon number range of the tested hydrocarbon compound.

6. Conclusions

This study confirmed the presence of oil-degrading bacteria in different habitats in UAE with the ability to recover more HDB from sediment samples. Isolation of HDB from the polluted sites and testing them individually or as a consortium indicates the importance of the microbial community members in working together in order to achieve the complete biodegradation process. Findings are useful for future investigations on biodegradation processes by native UAE microbes with a gall in mind to implement *in situ* bioremediation programs to clean up oil-contaminated sites.

7. Acknowledgement

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