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Isolation and characterization of oil-degrading bacteria from marine sediment environment

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ABSTRACT

The oil degrading bacterial strains were isolated from marine sediments collected from fuel oil–polluted coastal area in Penang, Malaysia. Bioremediation is an ideal tool to be applied as biological treatment of oil pollution due to it is cost-effective and eco-friendly. However the bacteria used in the bioremediation are highly important because they should achieve high efficient biodegradation rate and not pathogenic or virulence toward the environment. Two bacterial strains TZ1 and TZ2 were selected as potential oil-degrading isolates and were identified as *Chryseobacterium sp. strain AJ0 and Escherichia* sp. strain UIWRF0110, respectively. The emulsification index (E_{24}) and microbial adhesion to hydrocarbons (MATH) values of *Escherichia* sp. strain UIWRF0110 59.51 ± 5.56 and 28.40 ± 1.92 were slightly higher than *Chryseobacterium sp. strain AJ0* values 45.12 ± 10.86 and 19.11 ± 2.10, respectively. The degradation efficiency of *Escherichia* sp. strain UIWRF0110 was 90% as compared with *Chryseobacterium sp. strain AJ0 with 84%. Overall, these strains could be useful for the bioremediation of oil-polluted sediments.*

Keywords: Bioremediation; Emulsification index; Hydrocarbons; Protein profiling

1. Introduction

Saturated hydrocarbons composed of single bonds in molecular structure such as alkanes while unsaturated hydrocarbons have multiple bonds such as carbon–carbon double bond and carbon–carbon triple bond [1]. Oil compounds permeate in marine environment through anthropogenic and natural pathways. Sometimes, accidental oil spill also contribute small fraction of petroleum hydrocarbons in marine environment [2]. Although accidental spills do not happen frequently, the scale of contamination is huge enough to require plenty of resources and time to recover the spillage [3]. When the crude oil or its derived products are not carefully managed after use or before use, hydrocarbon pollution will happen as one of the worst consequences. The pollution might spread throughout the biosphere because the volatile hydrocarbons will evaporate to the atmosphere within a short period. While the non-volatile hydrocarbons will remain and being transport throughout the terrestrial and aquatic ecosystem due to the weathering process [4]. The automobile garage service is believed to be the main contributor of improper disposal of used engine oil. The contaminants will eventually end up in marine after long term surface runoff and infiltration [5]. The marine ecosystem will be largely affected as the consequences of marine pollution. Besides the improper disposal from public or automobile industries, the discharge from the production line of oil industry also contributes much in marine pollution. Produced water mainly consists of ionic constituents and organic compounds including aliphatic and aromatic hydrocarbons.

Furthermore, the oil will disperse slowly in some estuaries and increase the adsorption onto suspended particulate matter. When these particulate matters are bound with

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sediments, the benthic organisms are also exposed to hydrocarbons. Even though some aquatic organisms are resistant to oil or its byproducts, some of the oil residues are left inside their body tissues during food uptake [6]. In the end, humans consume the ultimate dose of hydrocarbons due to bio-magnification [7].

The coastal area of Penang, Malaysia, is polluted with oil spills which is dangerous for aquatic ecosystem and also for human health. The conventional methods hardly achieve complete removal of hydrocarbon residuals. For the long-term cheaper and eco-friendly clean-up, bioremediation is emerged as a reliable secondary treatment option. For oil spill scenario, normally the hydrocarbon degrading or oil-degrading bacteria will be introduced as the bioremediation strategy. The hydrocarbons uptake mechanism still rely on the species of microorganisms, the molecular weight of hydrocarbons and the environmental condition during the uptake process [8]. Different microorganisms have different capabilities to biodegrade the oil. Hence, the oil degradation profiles of different bacteria are very crucial for the technologist to select the ideal microbes in hydrocarbon bioremediation. It has generally been accepted that the susceptibility of hydrocarbons to microbial attack decreases in the following order: linear alkanes > branched alkanes > low-molecular weight aromatics > cyclic alkanes [9]. The objectives of the present study were to isolate and identify the oil-degrading bacterial strains from oil-polluted coastal area in Penang, Malaysia, and study their capacity in oil degradation. The physiological and biochemical features were used to characterize the strains. Phylogenetic analysis, based on 16S rDNA gene sequence data, was also used to reveal the genetic relationship between the strains and others. The oil degradation efficiency of the isolates was determined by gravimetric analysis by measuring the mass of residual oil extracted by *n*-hexane.

2. Material and methods

2.1. Sampling

The source of bacteria isolated throughout the study was the marine sediments collected by using manual sampling device from three different jetties in Penang Island which were Tan Jetty (5°24′43.3″N 100°20′19.6″E), Mixing Jetty (5°24′39.2″N 100°20′15.8″E), Yeoh Jetty (5°24′40.1″N 100°20′17.6″E). The sediments collected were combined as collected in a wide-mouth plastic bags in situ as single composite sample and stored in an ice box. The plastic bags were rinsed with seawater first before the sediments were transferred into it to remove any residuals or contaminants. After sampling was completed, the samples were immediately send back to laboratory and stored in the refrigerator at low temperature of 4°C.

2.2. Isolation of oil-degrading bacteria

Oil-degrading bacteria were isolated by two types of media, ONR7a and Bushnell hass mineral salts (BHMS), these medium were used for the isolation of hydrocarbon clastic and hydrocarbon-degrading bacteria, respectively. BHMS and ONR7a media were nourished with 1% (v/v) engine oil as the sole carbon source. Aliquots of sediments (1 g) were added to Erlenmeyer flasks containing 100 ml of medium, and the flasks were incubated for 10 d at 30°C on rotary shaker. Then 5-mL aliquots were removed and kept in fresh medium. After a series of four further subcultures, inoculums from the flask were streaked out, and phenotypically different colonies on BHMS agar and ONR7a agar were purified. Phenotypically different colonies attained from the plates were shifted to fresh medium with and without engine oil to eradicate agar-utilizing bacteria and autotrophs. The procedure was repeated, and only isolates exhibiting distinct growth on engine oil were stored in stock media with glycerol at –20°C for further characterization [10].

2.3. Identification of bacterial strains based on classical taxonomy and 16S rDNA

Oil-degrading bacteria was identified and characterized by following the standard protocols in Bergey's Manual of Systematic Bacteriology [11]. Morphological properties, growth character (temperature and pH) and enzyme activity (lipase and lactose) were practiced to identify this bacteria.

For molecular identification, bacterial DNA was extracted by using GenCheck® DNA Extraction Reagent (FASMAC) according to manufacturer's protocol. In amplification of bacterial 16S rDNA, the PCR reaction mixture was comprised of 12.5 μL of 2X KAPA HiFi HotStart Ready Mix, 0.75 μL of 10 μM universal forward primer (27F: 5′ TACGGYTACCTTGTTACGACTT 3′), 0.75 μL of 10 μM universal reverse primer (1492R: 5′ AGAGTTTGATCMTGGCTCAG 3 ′), 10.5 μL of PCR-grade water and 0.5 μL of extracted DNA sample. PCR profile for 5 min pre-denaturation (1 cycle) was at 95°C, denaturation for 30 s at 94°C, annealing for 1 min at 53°C and extension (35 cycles) for 2 min at 72°C, final cycle extension for 10 min at 72°C. PCR amplicons were noted by running 1.0% agarose gel electrophoresis [12].

2.4. Phylogenetic analysis

The GenBank database was used to compare the 16S rDNA sequences with the similar sequences. The phylogeny of bacterial strains was constructed by molecular evolutionary genetics analysis software.

2.5. Characterization of the bio-surfactant

2.5.1. Emulsification index (E_{24})

The cells in liquid culture were removed by centrifugation at 12,000 *g* for 5 min at room temperature in order to obtain supernatant layer. About 2 mL of the cell-free supernatant was mixed with 2 mL of fresh engine oil in a test tube. This mixture was vortexed for 2 min and left it to stand overnight for 24 h at room temperature [13]. The whole procedure was done in triplicates. The E_{24} index was measured by using the following equation:

$$E_{24} = \frac{\text{Height of emulsified layer}}{\text{Total height of the liquid column}} \times 100\% \quad (1)$$

2.5.2. Determination of cell surface hydrophobicity

The cell surface hydrophobicity of the isolated strains was determined by microbial adhesion to hydrocarbon (MATH) assay. The isolates were grown in 10 mL 1% (v/v) engine oil and incubated for 1 week in incubator shaker [3]. Then, the cell culture was centrifuged at 5,000 rpm for 15 min. The supernatant was decanted and the pellets were washed with buffer solution to remove the oil residue. The composition of buffer solution (per litre) was made of 22.2 g mono-potassium phosphate (KH_2PO_4) and di-potassium phosphate (K_2HPO_4). Same procedures were repeated twice. The cells were then re-suspended in 5 mL of buffer solution and the optical density (OD) was measured at wavelength 600 nm by using spectrophotometer, HACH DR2800. In a clean test tube, 500 μL of engine oil was added into 5 mL of bacterial suspension and vortexed for 2 min then set aside for 30 min for phase separation [2]. The OD was measured again and recorded after 30 min at the same wavelength. The whole procedure was done in duplicate. The MATH percentage was expressed as follows:

$$\mathrm{MATH}(\%) = 1 - \frac{A_1}{A_0} \mathrm{x}100 \tag{2}$$

A_1: absorbance value measured at wavelength 600 nm of aqueous phase; A_0: absorbance value measured at wavelength 600 nm of initial cell suspension.

2.6. Oil degradation study through gravimetric analysis

2.6.1. Liquid–liquid extraction

About 10 mL of fresh engine oil inoculated with 1 mL of bacterial liquid culture was taken in an Erlenmeyer flask. The flask was incubated for 1 week under the condition of 150 rpm and 37°C. One blank sample was prepared as negative control without bacterial inoculation and shaken under the same condition. After 1 week, all of the samples were ready for the extraction of residual oil through liquid–liquid extraction. The samples were acidified to pH < 2 to stop the microbial activity prior to extraction process, then the acidified samples were transferred into a separatory funnel [14]. About 30 mL of *n*-hexane was added to the Erlenmeyer flask to rinse the interior surface and the rinsate was transferred into separatory funnel. The separatory funnel was shook vigorously then immediately vented into the fume hood to release excess pressure. The shaking was continued for 2 min. After shaking, the organic layer was allowed to separate from aqueous layer within 10 min. The emulsion formed was broken by adding 99.7% of ethanol. The aqueous layer was drained into the original Erlenmeyer flask while the organic layer was drained into a new Erlenmeyer flask.

The extraction process was repeated twice more and then all the extracts were combined together. Anhydrous sodium sulphate was placed in a filter funnel with filter paper for draining the combined extracts until the milky extracts became clearer to ensure all moisture contents were removed. The dried extracts were transferred into a round bottom flask and placed on Bucchi rotary evaporator. The water bath temperature was set at 60°C and the needed vacuum for a solvent to boil at 40°C was adjusted accordingly. To remove *n*-hexane, ethanol and water contents from extracts, and the vacuum reading was adjusted to 335 mbar, 175 mbar and 72 mbar accordingly. The evaporation was stopped when no more condensates were produced and the extracts were concentrated into small volume of yellowish oily liquid [15].

2.6.2. Gravimetric analysis

The quantity of residual engine oil was measured after extracting the residual oil through liquid–liquid extraction and concentration. Before liquid–liquid extraction, the empty round bottom flask was weighed on an analytical balance and the mass was recorded. After the extracts were concentrated into yellowish oily liquid, the flask was measured again and the mass was recorded. The percentage of degradation was calculated as shown below:

$$\text{Percentage of oil degradation} = \frac{\text{weight of oil in negative control} - \text{weight of oil in culture sample}}{\text{weight of oil in negative control}} \times 100\% \tag{3}$$

2.7. Statistical analysis

One way analysis of variance has been conducted separately between oil degradation and E_{24} test and, between oil degradation and MATH assay. The statistical analysis was done through the Analysis ToolPak of Microsoft Excel 2010. *p*-value test of significance was carried out at 95% level of confidence.

2.8. Protein profiling

Both bacterial strains isolated were stressed with 1 μL of engine oil with control and cells were harvested through centrifugation after incubation for 16 h at 37°C in shaking incubator. About 100 μL of 1X loading dye was used to dissolve pellet and then for 5 min heat shock was given, for 2 min eppendorfs were moved on ice and then for 10 min were centrifuged at 12,000 rpm. Supernatant was shifted to new eppendorfs, then the final centrifugation was given at 12,000 rpm for 10 min, and the supernatant was transferred to new eppendorfs. The bacterial proteins were resolved by SDS-PAGE. Electrophoresis was practiced in vertical mini-slab gel (Mini Protean III; BioRad) with a gel size 8 × 7 cm and gel thickness of 0.75 mm. The gels were consisted of stacking gel (4%) and resolving gel (10%) and run for 50 min at constant voltage of 200 V.

Each well of the gel was loaded with about 10 μL of bacterial protein extracts. After electrophoretic separation, the gel was stained with 0.01% Coomassie blue solution, methanol (45% v/v) and glacial acetic acid (10% v/v) for 30 min at room temperature and consequently placed in the destaining solution methanol (50% (v/v) and acetic acid (2% v/v) for 1 h. The gel image was analyzed and captured by using VersaDoc Imaging System (BioRad) [16].

3. Results and discussion

3.1. Isolation and purification of oil-degrading bacteria

Based on color, two colonies were selected and isolated on fresh nutrient agar plates supplemented with oil.

The yellow pigmented colony was assigned as TZ1 while another white-colored colony was referred as TZ2.

3.2. Taxonomical and 16S rDNA identification

The morphological characterization test was done on TZ1 and TZ2 through gram staining, the result of which was observed under light microscope and magnifier. Morphological characterization is used as the preliminary step in studying the unknown microorganism. Biochemical test is used to identify the bacterial species based on their diverse biochemical reaction or profile as shown in Table 1. The results of the biochemical test will provide the physiological and ecological background of the particular microorganism. For lipase test, the positive result will be observed as clear zone of formation around the colonies and the microorganism able to synthesis lipolytic enzyme which can hydrolyze fats in food sources. However, both TZ1 and TZ2 were able to grow on the tributyrin agar but without clear zone formation, so they are not lipolytic microorganism. For lactose test, TZ2 had shown positive result with the presence of reddish pink colonies but no growth was shown by TZ1. The lactose-fermenting TZ2 has developed into reddish pink colonies as the neutral red pH indicator in MacConkey agar has changed due to acid production from lactose [17].

After completing the morphological and simple biochemical characterization, the identification of bacterial genera and species were accomplished through molecular characterization. Based on the analysis from the 16S rDNA, gene sequences of TZ1 and TZ2 were compared with the sequence databases of National Center for Biotechnology Information through Basic Local Alignment Search Tool (BLAST). The BLAST algorithm has listed out the best matches with specific scores. The 16S rDNA genes of strain TZ1 was 99% homologous to *Chryseobacterium* sp. strain AJ0 with accession number MF144183.1 while the strain TZ2 was 99% homologous to *Escherichia* sp. UIWRF0110 with accession number KR189862.1.

3.3. Blast phylogenetic tree

The blasting of nucleotides sequences of 16S rRNA gene is a very fast and accurate method to determine the position of bacterial strains in the phylogenetic tree. The full length phylogenetic trees of both TZ1 and TZ2 strains were constructed as shown in Fig. 1. We found that TZ1 was classified under *Chryseobacterium* sp. strain AJ0 with 99% similarity and closely placed with *Chryseobacterium indologenes*. The TZ2 was grouped with *Escherichia* sp. UIWRF0110 with 99% similarity index and far away placed with the reference of *Enterobacteria.*

Table 1
Morphological and biochemical characteristics of the isolates

Strains	TZ1	TZ2
Color	Yellow	White
Opacity	Opaque	Opaque
Colony shape	Round	Round
Colony margin	Smooth	Smooth
Colony elevation	Convex	Raised
Gram nature	Negative	Negative
Cell shape	Rod	Rod
Lipase test	Negative	Negative
Lactose test	Negative	Positive

3.4. Characterization of the bio-surfactant

3.4.1. Emulsification index (E_{24})

To characterize the bio-surfactant produced by both strains, the emulsification index against engine oil need to be determined as bio-surfactant plays important role in oil biodegradation. Emulsifying ability of TZ1 and TZ2 was quantified by involving agitation and observation. The E_{24} value is obtained through the formula as stated earlier. The results shown in Fig. 2 indicated that *Escherichia* sp. strain UIWRF0110 (TZ) has higher E_{24} value than *Chryseobacterium* sp. strain AJ0 (TZ1) after 24 h, which is 59.51% ± 5.56% compared with 45.12% ± 10.86%, respectively, while the blank has the lowest value with 34.43% ± 1.08%. These results tentatively infer that *Escherichia* sp. strain UIWRF0110 can achieve better biodegradation of oil since it has achieved higher emulsification activity. The presence of bio-surfactants is important in oil biodegradation process. Bio-surfactants are the surface active substances produced by microorganisms which consist of a polar and non-polar group that contribute to the amphipathic properties of bio-surfactant. Bio-surfactants help to increase the bioavailability of substrates for microbes by increasing the hydrophobicity or lipophilicity of cell surface and allow more hydrophobic substrates to associate onto it [18].

Although the E_{24} reading of blank sample was reasonably lower than the other two bacterial samples due to the absence of bacterial supernatant, the emulsification still occurred due to the mechanical forces applied when the sample was vortexed. High-speed vortex action has induced high shear stress to break up the oil layer into smaller droplets without biological and chemical agent. Traditionally, emulsification already can be achieved mechanically through high shear homogenization as determined by Mulligan and Gibbs [19]. Due to absence of bacterial cell-free supernatant, the emulsification scale was relatively lower compared with another two samples with supernatant.

3.4.2. MATH assay

The absorbance values of cell suspension before and after mixing with oil was measured by using spectrophotometer at a wavelength of 600 nm. In MATH assays, similar trend has occurred as shown in Fig. 3 as in determining the emulsification index previously. *Escherichia* sp. strain UIWRF0110 has achieved MATH value of 28.40% ± 1.92% which is higher than the *Chryseobacterium* sp. strain AJ0 with the reading of 19.11% ± 2.10%. The cell hydrophobicity promotes adherence of microbes onto substrates and more accessible to nutrient uptake. Adhesion is the initial stage of biodegradation of hydrocarbons followed by growth, desorption and surface renewal. Bacteria that attach at the oil–water interface is a good example of hydrophobic interaction because these bacteria can colonize the surface that cannot be reached by other microbes, which can be seen as a prior advantage in nutrient availability [20].

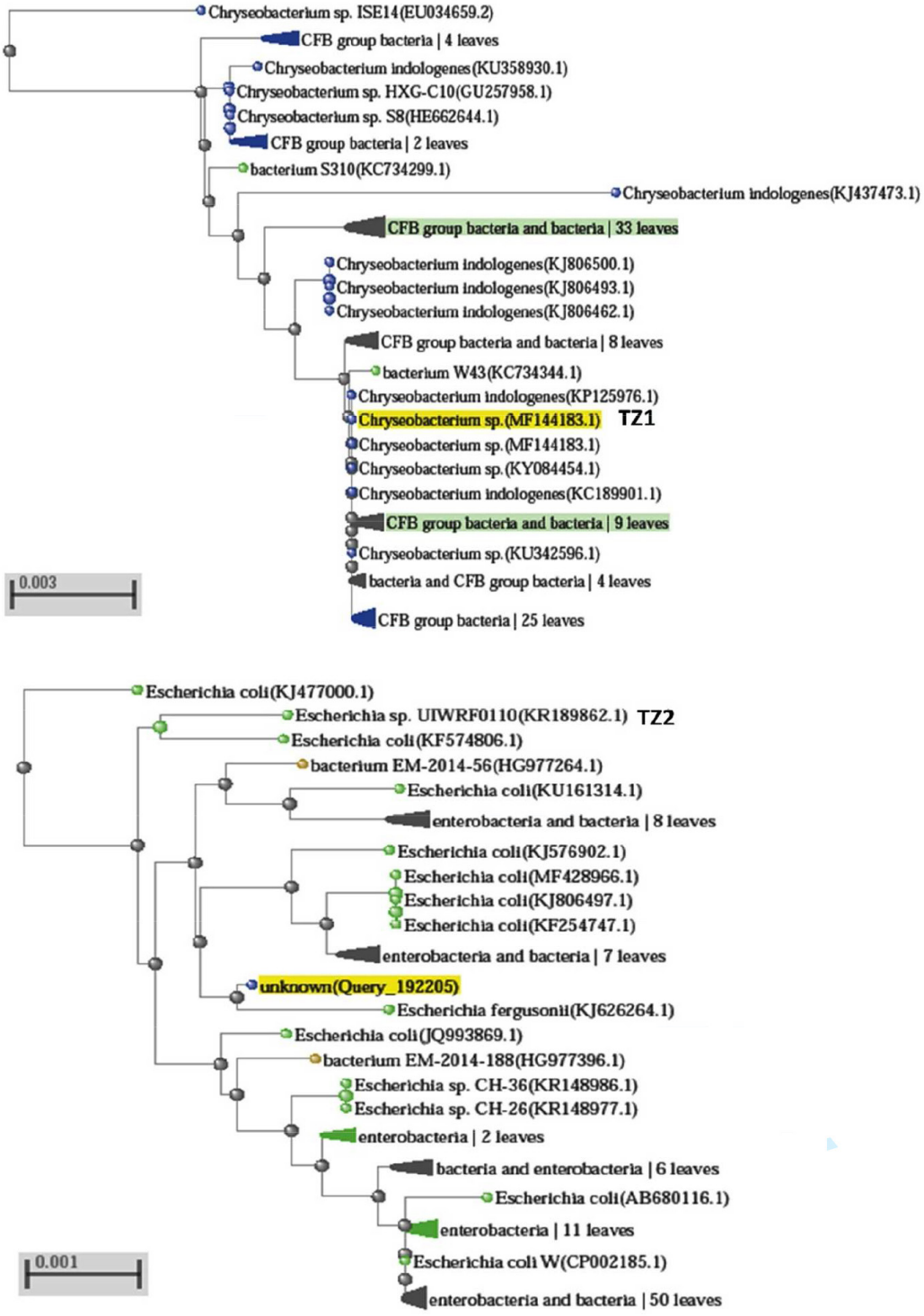

Fig. 1. Construction of phylogenetic trees based on 16S rDNA analysis: strain TZ1 (*Chryseobacterium* sp. strain AJ0) and strain TZ2 (*Escherichia* sp. strain UIWRF0110).

Furthermore, there are studies that have proved the presence of tiny oil hydrocarbon in aqueous bacteria suspension after vortex which contribute to the error in absorbance measurement [21]. The stable hydrocarbon droplets can be formed in aqueous phase after mechanical mixing even without surfactants. The adsorption of hydroxyl ions or other anions from aqueous phase and the weak Van Der Waals forces between oil droplets makes them stabilize in aqueous solution and hard to separate completely. This is why the blank sample also has minimal MATH reading although there are no bacterial cells in it due to the oil droplets stabilize in aqueous solution. A modified MATH assay was developed

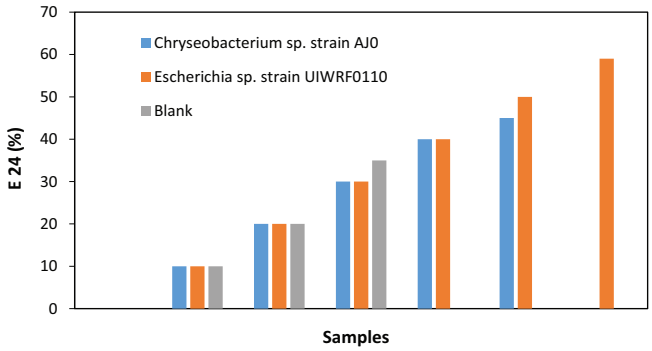

Fig. 2. E_{24} reading of *Chryseobacterium* sp. strain AJ0, *Escherichia* sp. strain UIWRF0110 and blank sample.

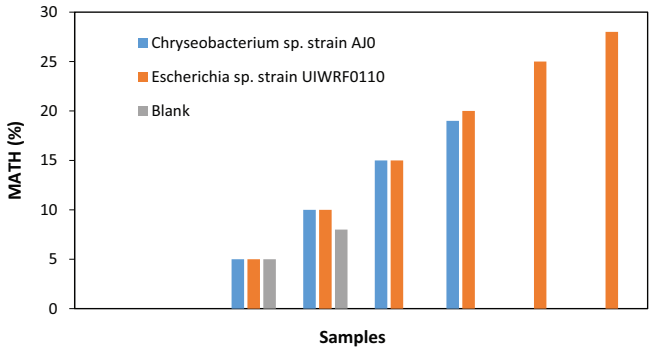

Fig. 3. MATH values *Chryseobacterium* sp. strain AJ0, *Escherichia* sp. strain UIWRF0110 and blank samples.

by Zoueki et al. [22] to reduce this type of absorbance error but it cannot be practiced throughout the study due to limitation of equipment and time.

3.5. Oil degradation studies through gravimetric analysis

There are many analytical methods available to measure oil concentration or total petroleum hydrocarbons in environmental samples. Gravimetric method is one of the most commonly used analytical methods which is quite simple and affordable to use. The gravimetric analysis was performed by measuring the weight of residual oil of bacterial samples and the weight of residual oil in blank sample after incubation for 1 week [23].

Based on results of Fig. 4, *Escherichia* sp. strain UIWRF0110 has achieved 90% of oil degradation which is slightly higher than *Chryseobacterium* sp. strain AJ0 with the ability to degrade 84% of engine oil, while the oil content in blank sample has decreased about 4% after 1 week of incubation. The remarkably low 1.5% loss of engine oil in the blank control is attributed to volatilization during incubation shaking at 37°C. However, the performance of *Escherichia* sp. UIWRF0110 and *Chryseobacterium* sp. strain AJ0 in biodegradation of oil are considered effective and ideal to be applied in oil bioremediation because they degrade more than 80% of oil after 1 week. There are several studies shown more efficient oil degrading bacterial isolates compare with the current study. Hassanshahian et al. [24] had isolated two bacterial strains *Acinetobacter calcoaceticus* and *Alcanivorax dieselolei* with rate of oil degradation of

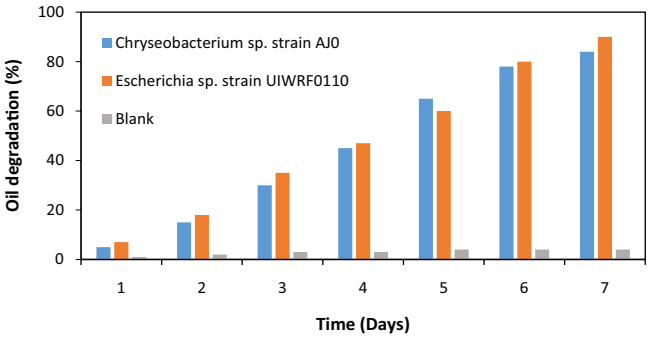

Fig. 4. Oil degradation level achieved by *Chryseobacterium* sp. strain AJ0 and *Escherichia* sp. strain UIWRF0110 after 1 week.

82% and 71%, respectively, after 1 week of cultivation. A novel *Acinetobacter* strain was isolated by Liu et al. [6] which was able to achieve high degradation around 70% in 7 d. Normally three types of oil-degrading ways are adapted by bacteria. They are as follows: (1) natural bioremediation: regular monitoring of oil-degradation by bacteria; (2) bio-stimulation: when the native bacterial population is not enough to degrade, then they are cultivated in the labs and again introduced into the polluted areas; (3) bio-augmentation: the use of genetic engineering tools to increase the enzyme contents in the cloned bacteria that are required to optimize the oil degradation [25,26]. Normally, contact zone between water and crude oil is center of biodegradation of crude oil. The resulting solution gradient causes influx of substances prone to degradation such as *n*-alkanes and isoprene alkanes into this zone and outflow of reaction products in the opposite direction. Mixing of biodegraded oil with inflowing unchanged oil takes place also in this region [27].

3.6. Statistical relationship between characterization of bio-surfactant and oil degradation

The data collected from the E_{24} test, MATH assay and oil degradation were subjected to analysis of variance [7] by using the Analysis ToolPak of Microsoft Excel 2010 with significant value of $p = 0.05$. Two separate one way ANOVA were performed which were in between E_{24} activity and oil degradation efficiency, and, in between MATH assay and oil degradation efficiency [28]. For the first statistical test, the null hypothesis (H_o) is the emulsification index of bacterial strains has the significant effect on oil degradation efficiency when $p \leq 0.05$. While for the second statistical test, the H_o is the cell hydrophobicity of bacterial strains has the significant effect on oil degradation efficiency when $p \leq 0.05$.

From the result of first statistical analysis as shown in Table 2, it shows that the emulsification index of bacterial strains has significant effect on oil biodegradation with *p*-value of 0.007 which is less than the significant value $p = 0.05$, thus the H_o is accepted. As variance analysis showed, the oil degradation efficiency in *Escherichia* sp. strain UIWRF0110 is slightly higher due to its higher emulsification index than the *Chryseobacterium* sp. strain AJ0. The bio-surfactant action of *Escherichia* sp. strain UIWRF0110 able to emulsify or break down the oil layer into micro-droplets and increase the uptake bioavailability of hydrocarbon to the bacterial cells.

Table 2
Variance of oil degradation with regards to emulsification index, E_{24}

Source of variation	SS	df	MS	*F*	*p*-value
Between groups	2,306.92	1	2,306.92	25.17762	0.007397
Within groups	366.5033	4	91.62583		
Total	2,673.424	5			

SS: Sum of squares; df: degree of freedom; MS: mean square; F: F ratio = MS of between groups/MS of within groups.

Table 3
Variance of oil degradation with regards to cell hydrophobicity based on MATH assay

Source of variation	SS	df	MS	*F*	*p*-value
Between Groups	207.2113	1	207.2113	3.528804228	0.13351075
Within Groups	234.8799	4	58.71997		
Total	442.0911	5			

SS: Sum of squares; df: degree of freedom; MS: mean square; F: F ratio = MS of between groups/MS of within groups.

The results of second analysis shown in Table 3, it can be clearly seen that the *p*-value of 0.134 is more than the significant value. Hence, the H_o is rejected which means no significant effect of cell surface hydrophobicity toward the oil degradation efficiency. This might be due to the methodological error when performing the MATH assay because the assay was carried out without knowing and adjust the ionic strength of cell suspension as the classical MATH assay need to perform at high ionic strength, otherwise it is simply an adhesion test and not indicating cell hydrophobicity of the bacterial strains. The positive correlation shall exist in between cell surface hydrophobicity and oil degradation efficiency as demonstrated by Obuekwe et al. [29], the oil-degrading bacteria should possess hydrophobic surface character as a necessity to utilize hydrophobic substrate such as petroleum hydrocarbons.

3.7. SDS-PAGE of oil-degrading bacteria

To study the protein bands of bacteria under non-stressed and stressed conditions, after 16 h of oil exposure, total cell proteins of bacteria were extracted. As the bacteria indicated their log phase at 0.3 nm, then oil-stress was given to bacteria. The induced protein bands were observed in bacterial strains under the stressed conditions at 10 and 120 kDa as shown in Fig. 5. It showed that the oil-degrading proteins in the bacteria are induced under only stressed condition. The 10 and 120 kDa were may be representing the phytase and S-adenosylhomocysteine hydrolase because they are normally involved in the hydrocarbons degradation. The phytase and S-adenosylhomocysteine hydrolase are extra-cytoplasmic proteins. Alternatively it may be new proteins bands which induced after adapting oil-stressed environment. These protein profiling results agree with the findings of Ennouri et al. [30]. They found that the cytoplasmic and matrix proteins are involved in the degradation of hydrocarbons.

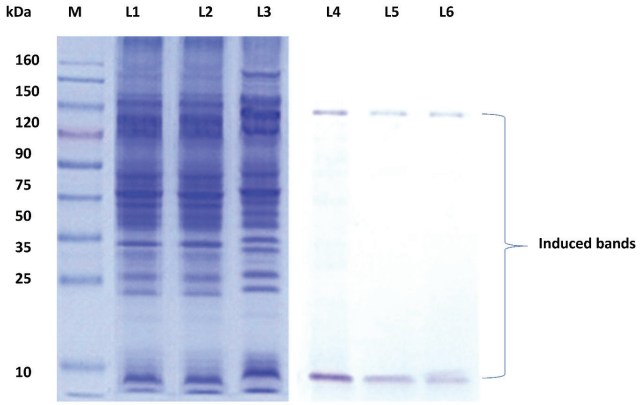

Fig. 5. "M" indicating the marker lane 1, 2 and 3 representing non-stress protein bands of strains TZ1 and TZ2, respectively, while lane 4, 5 indicating induced protein bands of strain TZ1, lane 6 signing induced protein bands of strain TZ2.

4. Conclusions

Two bacterial strains which were named as TZ1 and TZ2 were isolated from the marine sediments collected and were later identified as *Chryseobacterium* sp. strain AJ0 and *Escherichia* sp. strain UIWRF0110, respectively. Throughout this study, *Escherichia* sp. strain UIWRF0110 has shown slightly higher capability in engine oil biodegradation than *Chryseobacterium* sp. strain AJ0 after 1 week of incubation. These two bacterial strains have potential to be applied in bioremediation of marine oil pollution. The ability of these strains to degrade the hydrocarbons suggests that they can be applied for the bioremediation of other oil wastes. This can be achieved by generation of their bio-surfactants which can be applied on the broad-range of bioremediation fields.

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References

[1] G.D. Todd, R.L. Chessin, J. Colman, Toxicological Profile for Total Petroleum Hydrocarbons (TPH), 1999.
[2] D. Borah, R. Yadav, Biodegradation of petroleum oil by a novel *Bacillus cereus* strain DRDU1 from an automobile engine, Int. J. Environ. Res., 8 (2014) 1287–1294.
[3] R. Chandankere, J. Yao, M. Cai, K. Masakorala, A. Jain, M.M. Choi, Properties and characterization of bio-surfactant in crude oil biodegradation by Bacterium *Bacillus methylotrophicus* USTBa, Fuel, 122 (2014) 140–148.
[4] O. Hentati, R. Lachhab, M. Ayadi, M. Ksibi, Toxicity assessment for petroleum-contaminated soil using terrestrial invertebrates

and plant bioassays, Environ. Monit. Assess., 185 (2013) 2989–2998.
[5] I. Madanhire, C. Mbohwa, Mitigating Environmental Impact of Petroleum Lubricants, Springer International, Switzerland, 2016.
[6] H. Liu, J. Yao, Z. Yuan, Y. Shang, H. Chen, F. Wang, K. Masakorala, C. Yu, M. Cai, R.E. Blake, Isolation and characterization of crude-oil-degrading bacteria from oil-water mixture in Dagang oilfield, China, Int. Biodeterior. Biodegrad., 87 (2014) 52–59.
[7] A. Jernelöv, The threats from oil spills: now, then, and in the future, Ambio, 39 (2010) 353–366.
[8] S.T. Orszulik, Environmental Technology in the Oil Industry, Springer Cham Heidelberg New York Dordrecht London, 2008.
[9] G.A. Olah, G.S. Prakash, Hydrocarbon Chemistry, 2 Volume Set, Published by John Wiley & Sons, Inc., Hoboken, New Jersey, 2017.
[10] D.W. Lee, H. Lee, B.-O. Kwon, J.S. Khim, U.H. Yim, B.S. Kim, J.-J. Kim, Bio-surfactant-assisted bioremediation of crude oil by indigenous bacteria isolated from Taean beach sediment, Environ. Pollut., 241 (2018) 254–264.
[11] D.H. Bebgey, R.S. Breed, E.G.D. Murray, A.P. Kitchens, Bergey's Manual of Determinative Bacteriology, Baltimore, US, The Williams & Wilkins Company, 1939.
[12] S.Z. Abbas, M. Rafatullah, N. Ismail, F.R. Shakoori, Electrochemistry and microbiology of microbial fuel cells treating marine sediments polluted with heavy metals, RSC. Adv., 8 (2018) 18800–18813.
[13] H.P. Bacosa, D.L. Erdner, B.E. Rosenheim, P. Shetty, K.W. Seitz, B.J. Baker, Z. Liu, Hydrocarbon degradation and response of seafloor sediment bacterial community in the northern Gulf of Mexico to light Louisiana sweet crude oil, ISME J., 12 (2018) 2532–2543.
[14] E. Gontikaki, L. Potts, J.A. Anderson, U. Witte, Hydrocarbon-degrading bacteria in deep-water subarctic sediments (Faroe-Shetland Channel), J. Appl. Microbiol., 125 (2018) 1040–1053.
[15] C. Muangchinda, A. Yamazoe, D. Polrit, H. Thoetkiattikul, W. Mhuantong, V. Champreda, O. Pinyakong, Biodegradation of high concentrations of mixed polycyclic aromatic hydrocarbons by indigenous bacteria from a river sediment: a microcosm study and bacterial community analysis, Environ. Sci. Pollut. Res., 24 (2017) 4591–4602.
[16] S.Z. Abbas, M. Rafatullah, N. Ismail, J. Lalung, Isolation and characterization of Cd-resistant bacteria from industrial wastewater, Desal. Wat. Treat., 56 (2015) 1037–1046.
[17] E. Goldman, L.H. Green, Practical Handbook of Microbiology, 2nd ed., CRC Press, Boca Raton, London, New York, 2008.
[18] D. Francy, J. Thomas, R. Raymond, C. Ward, Emulsification of hydrocarbons by subsurface bacteria, J. Ind. Microbiol., 8 (1991) 237–245.
[19] C.N. Mulligan, B.F. Gibbs, Types, production and applications of bio-surfactants, Proc. Indian Nat. Sci. Acad. Part B, 70 (2004) 31–56.
[20] I. Tontul, A. Topuz, Chapter 13 - Microencapsulation of Plant Oils Rich in Alpha-Linolenic Acid: Effect of Processing Parameters A2-Sagis, Leonard M.C, Ed., Microencapsulation and Microspheres for Food Applications, Academic Press, San Diego, 2015, pp. 253–269.
[21] A.S. Nwankwegu, E.M. Ikpe, E.I. Chukwura, C. Irondi, E.I. Obika, Ex Situ biodegradation of crude oil using bacterial isolates from palm oil mill effluent, Am. J. Life Sci., 4 (2016) 71–75.
[22] C.W. Zoueki, N. Tufenkji, S. Ghoshal, A modified microbial adhesion to hydrocarbons assay to account for the presence of hydrocarbon droplets, J. Colloids Interface Sci., 344 (2010) 492–496.
[23] L.Y. Clasohm, I.U. Vakarelski, R.R. Dagastine, D.Y. Chan, G.W. Stevens, F. Grieser, Anomalous pH dependent stability behavior of surfactant-free nonpolar oil drops in aqueous electrolyte solutions, Langmuir, 23 (2007) 9335–9340.
[24] M. Hassanshahian, G. Emtiazi, S. Cappello, Isolation and characterization of crude-oil-degrading bacteria from the Persian Gulf and the Caspian Sea, Mar. Pollut. Bull., 64 (2012) 7–12.
[25] Q. Liu, J. Tang, K. Gao, R. Gurav, J.P. Giesy, Aerobic degradation of crude oil by microorganisms in soils from four geographic regions of China, Sci. Rep., 7 (2017) 14856.
[26] A. Priya, P. Manab Sarma, B. Lal, Isolation and characterization of *Candida viswanathii* strain TERI MS1 for degradation of petroleum hydrocarbons in marine environment, Desal. Wat. Treat., 57 (2016) 22099–22106.
[27] P. Parthipan, P. Rahman, A. Rajasekar, Characterization of hydrocarbon degrading bacteria isolated from Indian crude oil reservoir and their influence on biocorrosion of carbon steel API 5LX, Int. Biodeterior. Biodegrad., 129 (2018) 67–80.
[28] A. Wentzel, T.E. Ellingsen, H.-K. Kotlar, S.B. Zotchev, M. Throne-Holst, Bacterial metabolism of long-chain n-alkanes, Appl. Microbiol. Biotechnol., 76 (2007) 1209–1221.
[29] C.O. Obuekwe, Z.K. Al-Jadi, E.S. Al-Saleh, Hydrocarbon degradation in relation to cell-surface hydrophobicity among bacterial hydrocarbon degraders from petroleum-contaminated Kuwait desert environment, Int. Biodeterior. Biodegrad., 63 (2009) 273–279.
[30] H. Ennouri, P. d'Abzac, F. Hakil, P. Branchu, M. Naïtali, A.M. Lomenech, R. Oueslati, J. Desbrières, P. Sivadon, R. Grimaud, The extracellular matrix of the oleolytic biofilms of *Marinobacter hydrocarbonoclastic*us comprises cytoplasmic proteins and T2SS effectors that promote growth on hydrocarbons and lipids, Environ. Microbiol., 19 (2017) 159–173.