RESEARCH ARTICLE

Isolation and Identification of Hydrocarbon-Degrading Bacteria from Polychaete *Marphysa moribidii*

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Abstract

Marine contamination caused by anthropogenic activities has side effects and causes severe contamination to the environment. Polychaetes are benthic organisms that live in the sediment and can be a good indicator of sediment contamination by organic compounds. In this study, bacterial strains were isolated and identified from the gut of polychaete worm *Marphysa moribidii* and the potential of the bacteria was evaluated to degrade hydrocarbon compounds. The isolated bacteria were primary and secondary screened on Minimal Salt Media (MSM) agar supplemented with 1% v/v of diesel oil. Diesel degradation analysis was performed by inoculating potential bacterium into MSM broth with 1% v/v diesel oil and incubated at 37 °C for 20 days. Diesel degradation percentage was analyzed using the gravimetric method, while the bacteria cell densities were measured using the standard plate count method. Then, the selected isolates were identified based on their morphological characteristics and 16S rDNA sequences. As a result, two bacteria isolates coded as Isolate 6 and Isolate 8 were able to degrade diesel oil up to 52.29% and 39.24% after 20 days of incubation. The 16S rDNA sequence analysis revealed that it was identified as *Bacillus* sp. strain UMTFA1 (RB) and *Staphylococcus kloosii* strain UMTFA2 (RS). Our result showed that these strains have the potential in oil-degrading processes, which will provide new insight into bioremediation process and decrease environmental pollution in soil and water contaminated with hydrocarbons.

Keywords: biodegradation, bacteria, diesel oil, pollutant, polychaete

Introduction

Worldwide, the consumption of Polycyclic Aromatic Hydrocarbon (PAH) sources as energy has risen from year to year. According to the World Watch Institute as cited in Cusick (2013), PAHs are present in products made from fossil fuels. It has continuously been the dominator in the 2012 global energy sector despite all the efforts, such as using renewable energy sources to decrease carbon emissions. It is expected that fossil fuels will continue to provide up to 80% of world energy through 2040 (Ghasemian et al., 2020). PAH also can be found in diesel oil. Diesel oil comprises aliphatic hydrocarbons approximately 75%; ($C_{10}H_{20}$–$C_{15}H_{28}$) and aromatic hydrocarbons (25%). It is used for many purposes, such as in diesel engines in trucks, trains, boats and nearly all consumable products. It serves as a principal source of energy. However, diesel oil is considered the most ubiquitous organic pollutants (Xu et al., 2018). Hydrocarbons such as petroleum and diesel have been the ultimate source of energy. Thus, incidents such as accidental spills during transportation, accidental leakage, and disposal of gasoline and diesel fuel into the environment can cause severe contamination to our environment and affect human health (Kuppusamy, Maddela, Megharaj & Venkateswarlu, 2020). The burning of PAH can cause air pollution as it feeds more carbon into our atmosphere and can evaporate into the air straight from soil or from water bodies. It can cause danger to the respiratory systems of living organisms when exposed to a high toxicity level.

Diesel oil is a complex and significant source of PAH that usually contributes to human health. Some of the effects include jaundice, cataracts, which can cause toxicity to genetic, immune, and endocrine systems (Kuppusamy et al., 2020). PAH has been recognized as a carcinogenic and a potential cause of

mutagenic effects (de Souza & Corrêa, 2016). The EPA has classified seven PAH compounds as probable human carcinogens: benz[*a*]anthracene, benzo[*a*]pyrene, benzo[*b*]fluoranthene, benzo[*b*] fluoranthene, chrysene, dibenz[*a,h*]anthracene, and indeno[*1,2,3-cd*]pyrene (Rengarajan et al., 2015). Thus, it is crucial to eliminate PAH from our environment. PAH is classified as one of the Persistent Organic Pollutants (POPs). Pozo et al. (2012) concluded that the concentration of PAH is higher up to eight-fold at industrial sites compared to that in rural areas.

Since diesel is composed of toxic compounds such as PAHs, it represents a constant environmental threat. Various techniques have been employed to decrease the pollution caused by hydrocarbon to overcome this crisis. To reduce the hydrocarbon contamination in the coastal area, physical, chemical and thermal methods treat the oil-contaminated sites (Pranowo & Titah, 2016). However, bioremediation is introduced to treat the oil-contaminated sites. This method is much safer than the other methods mentioned as it does not feed any chemicals to the surrounding environment (Gomathi et al., 2020). In bioremediation methods, microorganisms, such as bacteria are used to mediate, reduce, remove or even to convert the contaminants in the air, soil and even in aquatic environments and transform the organic pollutants into their fewer toxic metabolites or mineralize them into CO_2 and water (Yadu, Satapathy, Sahariah, & Anandkumar, 2020). According to Tomei and Daugulis (2013), bioremediation is much easier to sustain. It can ensure complete elimination of the contaminants and an attractive solution for soil decontamination no matter how big the contaminated area is.

In this study, the bacteria degrading hydrocarbon are isolated from a marine worm called polychaetes. Polychaetes are one of the benthic communities that live in sediment, which is utilized as a good indicator of sediment contamination by organic compounds. Some species of polychaetes, such as *Marphysa moribidii* can live in sediments relatively resistant to organic contaminants and pesticides. Hence, microbial communities associated with the polychaetes may indirectly be sensitive to contaminants. They can also be resistant to environmental changes (Allison & Martiny, 2008). According to Blaise, Gagné, Gillis and Eullaffroy (2013), the gut microbial community of polychaetes can tolerate and metabolize toxic compounds encountered by the host gut. They may produce specific enzymes and genetic regulations which guide their metabolic network. These survival mechanisms include resilience to environmental stressors, efficient reproduction, and stringently regulated carbon utilization. Their diverse metabolic capabilities enable them to survive toxic exposure making these degradation mechanisms important to understand (Ostrem & Yu, 2018).

The effectiveness of biodegradation is affected by a few factors such as temperature, pH, and availability of nutrients such as nitrogen, phosphorus and iron. These factors can limit or enhance biodegradation activity. According to Mehetre, Dastager and Dharne (2019), the biodegradation of PAH is more effective at elevated temperatures. The progressively increasing temperature from 20 °C to 60 °C will increase the mass transfer rates of PAHs in the aqueous solutions enhancing the biodegradation rates of PAH compounds (Annweiler et al., 2000).

Biodegradative enzymes play a significant role in the biodegradation of hydrocarbons. Usually, the degradation of hydrocarbons by microorganisms is mediated by a particular enzymatic system and other implementations such as biosurfactants production by the microorganisms themself (Kubicki et al., 2019). Many bacterial strains such as *Acinetobacter* sp. P3d, *Bacillus* sp. *P4a and Pseudomonas* sp. P6 (Fazilah, Darah & Ismail, 2018), *P. aeruginosa* PSA5, *Rhodococcus* sp. NJ2 and *Ochrobactrum* intermedium (Mishra & Singh, 2012), were reported to produce degradative enzymes during biodegradation of hydrocarbons. Hence in the present study, the objectives are to isolate and identify polychaete *M. moribidii* associated bacteria and to determine the diesel oil degradation activity by these isolated bacteria. This finding could contribute to the bioremediation of used diesel oil-polluted environment.

Materials and Methods

Sampling Site

In this study, polychaetes were collected from Pantai Kelanang. It is located six kilometers from Morib shoreline at Banting in Selangor, Malaysia. The sampling site took place at mangrove forests which are distributed near the beach at 2°45'40.559" N and 101°26'6.157" E.

Sampling Method

The polychaete sample of *M. moribidii* is usually found in the sediments at the mangrove area. Five samples of *M. moribidii* were collected during spring at low tide in the sediment. The sediments were shoveled and the worms were pulled out slowly so a whole *M. moribidii* shape will be obtained, otherwise the polychaete would be cut off if pulled aggressively. The morphological characteristic of *M. moribidii* was confirmed by referring to the study done by Idris (2014).

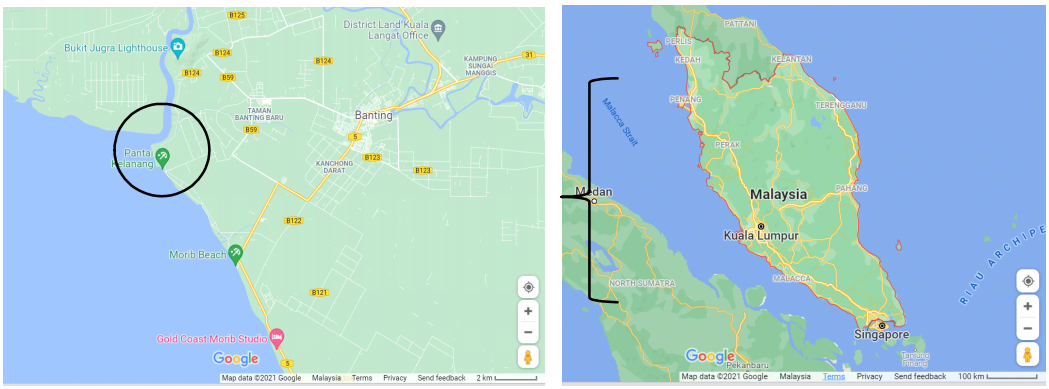

Figure 1. The study area is in Pantai Kelanang, Selangor, Malaysia (Source: Google Map).

Next, the polychaetes were washed with seawater to wash away the leftover sediments and were placed into a zip lock bag with a damp paper towel to mimic its environment's humidity. The polychaetes were placed in an icebox and transported to the laboratory. The polychaetes were kept in a -20 °C to preserve it until further analysis (Perumal et al., 2020)

Isolation of Bacteria from *M. moribidii*

The frozen polychaetes were defrosted first by rinsing them with distilled water and dried using tissue paper. The workbench was sterilized using 70% alcohol. Next, all five polychaete samples were dissected along its body (midsection). Sterile cotton swabs were used to swab the polychaete's gut fluid, and the gut fluid sample was streaked on the nutrient agar (NA) plates (Steffi & Anburaj, 2020). The plates were incubated at 37 °C for 24 h. After 24 h, the mixed colonies were obtained. Next, a sub-culture is performed to get a single colony of bacteria. The pure isolated bacteria were used for subsequent analysis.

Primary Screening of Hydrocarbon Degrading Bacteria

The mineral salts medium (MSM) containing diesel oil as the sole nutrient source was used to screen hydrocarbon-degrading bacteria. MSM was prepared in an Erlenmeyer flask consisting of as follows: 1.8 g K_2HPO_4, 4.0 g NH_4Cl, 0.2 g $MgSO_4.7H_2O$, 0.1 g NaCl, 0.01 g $FeSO_4.7H_2O$ dissolved in 1 L of distilled water, pH at 7.0. It was then sterilized at 121°C for 15-20 min. The primary screening of potential hydrocarbon-degrading bacteria was conducted using the spread plate method. Each pure isolated bacterium was spread onto the surface of a Mineral Salt Medium (MSM) agar supplemented with 1% diesel oil. The MSM was incubated at 37 °C for seven days. The medium was used to screen the bacteria that can utilize diesel oil as the sole carbon and energy source (Ariffin, Min, Ze, Yussof & Ismail, 2020). The bacteria isolate that was able to grow on the diesel-oil supplied MSM was identified as potential diesel oil degraders.

Identification of Bacteria

Identification and characterization of selected bacterial isolates were based on macroscopic and microscopic observations. The morphology of the bacteria isolates was observed using the Gram staining technique and further identified using 16S rRNA sequencing.

Gram Staining Method

The staining procedures started with a uniform smearing bacterial film made on a clean glass slide and heat-fixed. Then, one drop of crystal violet solution was dropped approximately in a minute on the entire bacterial smear. Then the slide was washed under a slow flow of water and later, a few drops of iodine solution were flooded onto the colored stain and left for a minute. Alcohol 95% was then used to decolorize the stain. After that, the slide was flooded with safranin counterstain for about 30 to 60 s and then rinsed with running tap water. The glass slide was air-dried, and then it was further examined under a light microscope with oil immersion. Gram-positive bacteria showed a purple cell, while Gram-negative bacteria showed a red cell (Isaac & Jennings, 1995).

16S rRNA Sequencing Method

The 16S rRNA sequencing was performed to further identification of the bacteria isolates. The bacteria were grown on nutrient agar for 24-48 h and then the extraction of the bacterial DNA was conducted using Vivantis Nucleic acid Extraction Kit manufactured by Vivantis Technologies Sdn. Bhd. The extracted DNA was stored in -20 °C to further amplify by Polymerase Chain Reaction (PCR).

The 16S rRNA gene sequence was determined by direct sequencing of PCR-amplified 16S rDNA. The bacterial 16S rDNA, full-length 1.5 kb, was amplified using universal primers 27F and 1492R. The total reaction volume of 25 µl containing gDNA was purified using the in-house extraction method, with 0.3 pmol of each primer and deoxynucleotides triphosphates (dNTPs, 400 µM each), 0.5 U DNA polymerase, supplied PCR buffer and water.

The PCR was performed as follows: one cycle (94 °C for 2 min) for initial denaturation; 25 cycles (98 °C for 10 sec; 53 °C for 30 sec; 68 °C for 1 min) for annealing and extension of the amplified DNA. The PCR products were purified by standard method and directly sequenced with primers 785F and 907R using Big Dye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems).

A phylogenetic tree was constructed using the Neighbor-Joining method. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. Evolutionary analyses were conducted in MEGA X.

Secondary Screening for Hydrocarbon Degradation Analysis

The secondary screening was conducted to determine the diesel oil degradation by isolated bacteria using gravimetric analysis. Biodegradation analysis was conducted using 100 mL Mineral Salt Media (MSM) in a 250 mL erlenmeyer flask with a mixture of 1% v/v of sterilized diesel oil and 1 mL of the isolated bacterial culture at O.D 600 nm (0.5). The control contained MSM and sterilized diesel oil 1% but without bacteria. The MSM was incubated under shaking condition at 150 rpm for 20 days at 37 °C (Fosso-Kankeu, Marx, & Brink, 2017). The extraction was done on day 0 and every 5 consecutive days.The diesel oil was extracted using liquid-liquid extraction of n-hexane with the equivalent volume of the mixture 1:1 (Latha & Kalaivani, 2012). The extraction was done twice in a separating funnel to ensure the complete extraction of the diesel oil. The extraction was evaporated until dryness in rotary evaporator under reduced pressure (Singh, Gautam & Vaishya, 2016). The volume of extracted oil was deducted from the previously weighed beaker.

The percentage of degradation can be calculated using the formula below (Latha & Kalaivani, 2012):

$$\text{W of Residual diesel oil} = \text{W of diesel oil added in the media} - \text{W of residual crude oil}$$

$$\text{Amount of diesel oil degraded} = \text{W of beaker containing extracted diesel oil} - \text{W of empty beaker}$$

$$\%\ \text{degradation} = \frac{\text{Amount of diesel oil degraded}}{\text{Amount of diesel oil added in the media}} \times 100$$

Growth Profile of Bacteria

The bacteria colonies were enumerated by serial dilution with a dilution factor of 10-fold. Then, 100 µL of the aliquot was spread onto the Nutrient Agar followed by incubation at 37 °C for 24 h. The colony-forming unit/ml (CFU/ml) was calculated using the formula as follows (Moll, 2017):

$$\text{CFU/ml} = \frac{\text{Number of colonies x Dilution factor}}{\text{Volume of culture plated}}$$

Statistical Analysis

In this study, the mean values of diesel oil degradation percentage were calculated using Microsoft Excel 2010. T-test was used for the analysis to determine if there is a significant difference between the two types of bacteria tested.

Results and Discussion

Screening of Hydrocarbon Degrading Bacteria

The screening step was done to distinguish whether the bacteria isolated from the gut fluid of *M. moribidii* are able to degrade hydrocarbon (degrader) or unable to degrade hydrocarbon (non-degrader). The media used for this purpose was Mineral Salt Media (MSM) agar supplemented with diesel oil as a carbon source. The result showed that two isolated bacteria (Isolate 6 and Isolate 8) grew on the surface of MSM agar supplemented with 1% diesel oil after seven days of incubation at 37 °C (Figure 2). The growth of bacterial colonies on the MSM plate indicates their ability to grow in the presence of diesel oil contaminants. Hence both bacteria isolates were identified as potential hydrocarbon degraders that can utilize components in diesel oil as its carbon source. The present results are agreed with the finding of Ho, Li, McDowell, MacDonald and Yuan (2020), who observed the growth of bacteria on the minimal medium supplemented with 0.2% diesel as the only carbon source to assess their diesel-degrading properties. Carbon source is essential for building cells and important in various metabolic processes for bacteria to grow and multiply. These potent isolates were further tested for their ability to degrade diesel oil in MSM broth culture.

Identification of the Hydrocarbon-Degrading Bacteria Isolated from Polychaete

The isolates were well characterized based on cell morphology and colony features such as color, shape

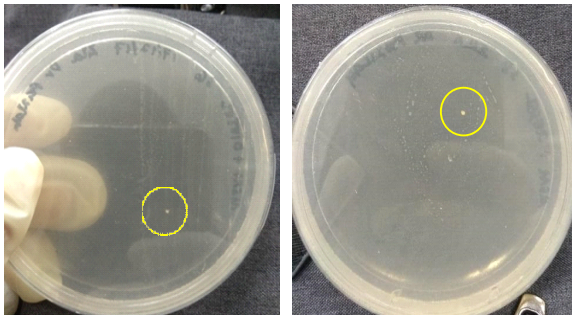

Figure 2. Isolate 6 (a) and Isolate 8 (b) grow on MSM supplemented with 1% diesel oil after seven days of incubation at 37 °C.

and size (Table 1). The isolates were identified as Gram-positive bacteria and had a circular colony shape. Isolate 6 has a moderate size, while Isolate 8 was punctiform. The margin for Isolate 6 was undulate and Isolate 8 had the entire margin. Isolate 6 was non-pigmented but the color was milky–white, while Isolate 8 was pigmented and yellow. The elevation for Isolate 6 was flat to rise, while Isolate 8 has convex elevation. Both have a smooth texture, Isolate 6 appearance was dull, while glistening in Isolate 8. Lastly, the optical property of both isolates was opaque. The colony morphology characteristics for Isolate 6 and Isolate 8 resembled the genera Bacillus and Staphylococcus morphology characteristics, respectively.

To confirm the results of morphological identification, the 16S rDNA gene sequences of the two isolates were determined. The phylogenetic comparison of Isolate 6 was *Bacillus* sp. UMTFA1 (RB) and Isolate 8 was *Staphylococcus kloosii* strain UMTFA2 (RS) is shown in Figure 3.

Diesel Oil Degradation using Gravimetric Analysis

Based on the degradation of diesel oil using gravimetric analysis, it was found that both bacteria, *Bacillus* sp. UMTFA1(RB) and *S. kloosii* strain UMTFA2 (RS) can degrade diesel oil as the percentage of degraded diesel oil (Figure 4). Based on this figure, *Bacillus* sp. UMTFA1 (RB) degraded about 52.29% of diesel oil in 20 days compared to *S. kloosii* strain UMTFA2 (RS) 39.24% at the same incubation period. Control 1 and Control 2 were MSM supplied with 1% of sterilized diesel oil in the absence of *Bacillus* sp. UMTFA1 (RB) and *S. kloosii* strain UMTFA2 (RS).

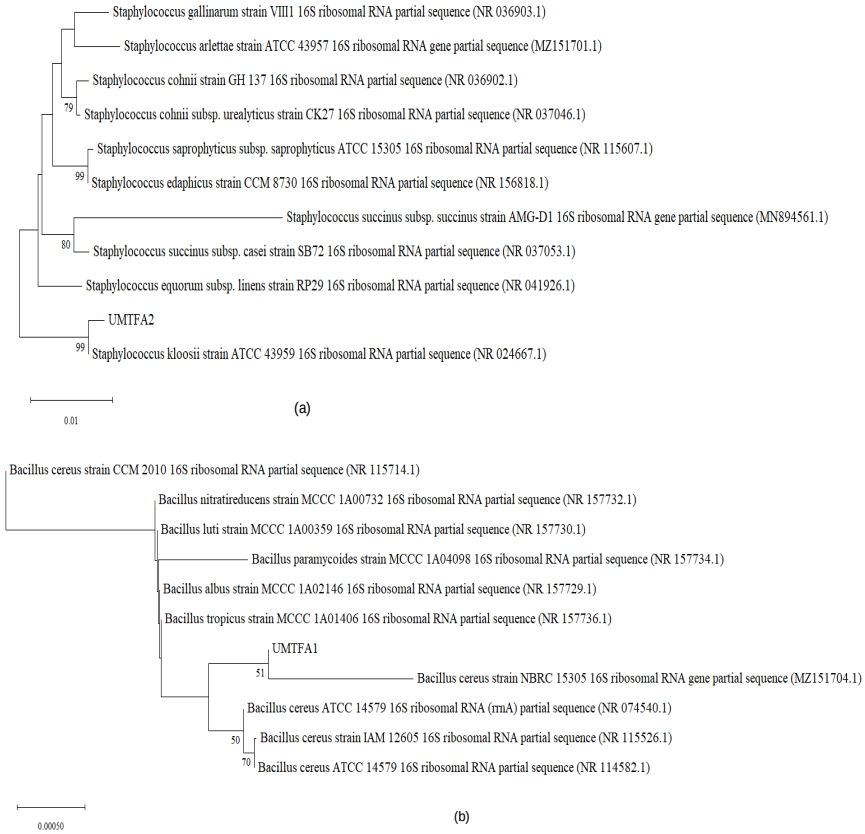

Figure 3. Neighbor-joining phylogenetic tree of a) *Staphylococcus kloosii* strain UMTFA2 (RS) and b) *Bacillus* sp. UMTFA1 (RB) as well as related bacteria based on 16S rRNA sequence comparisons. Accession numbers are given.

Table 1. Morphology characteristics of the isolated bacteria

Isolates	Cell Morphology	Colony Morphology
Isolate 6	Gram-positive, Rod shape	Shape: Circular Size: Moderate Margin: Undulate Elevation: Flat Colour/Pigmentation: Non-pigmented, milky-white Texture: Smooth Appearance: Dull Optical Property: Opaque
Isolate 8	Gram-positive, Cocci shape	Shape: Circular Size: Punctiform Margin: Entire Elevation: Convex Colour/Pigmentation: Pigmented, yellow Texture: Smooth Appearance: Glistening Optical Property: Opaque

After 20 days of incubation, the degradation did not occur in the absence of bacteria. Hydrocarbons will not decompose on their own, but there must be a substance that can use these hydrocarbons as the source of energy and food. Hydrocarbons in the environment are biodegraded primarily by bacteria, yeast, and fungi (Das & Chandran, 2011)

Our study is the study conducted by Abdulrasheed et al. (2020), which showed that the diesel degradation potential assessed using 0.5% (v/v) diesel-supplemented medium as the sole carbon source and determined using gravimetric analysis. It was found that strain AQ5-06 achieved 37.5% diesel degradation, while strain AQ5-05 achieved 34.5%. In comparison, Sun et al. (2019) showed about 42% to 66% degradation within two weeks. These results were quite similar to this study.

One of the major components found in diesel oil is naphthalene the simplest polycyclic aromatic hydrocarbon (Majewski & Jääskeläinen, 2018). According to Fazilah, Ismail, and Darah (2020), *Bacillus* sp. can utilize PAH such as naphthalene, phenanthrene and other hydrocarbons with high molecular weight such as pyrene and benzo. Ijah and Antai (2003) reported that *Bacillus* sp. as the predominant isolate of all the crude oil-utilizing bacteria was characterized from highly polluted soil samples. It was postulated that *Bacillus* sp. is more tolerant of high hydrocarbons in soil due to their resistant endospores. However, degradation by *Bacillus* sp. was also reported by earlier studies (Abdelhaleem, Zein, Azeiz, Sharaf & Abdelhadi, 2019).

Previous studies have also shown that *Staphylococcus* sp. can utilize naphthalene as well as benzene as their carbon sources (Zhuang, Tay, Maszenan, Krumholz & Tay, 2003). *Staphylococcus* sp. and *Acinetobacter* sp. are also able to transform phenanthrene to catechol (Fazilah et al., 2018; Ubani, Atagana, Thantsha & Rasheed, 2016). While for *S. kloosii*, there is no evidence supporting whether this species can utilize carbon from hydrocarbon sources. However, our finding showed that *S. kloosii* strain UMTFA2 (RS) was able to degrade diesel oil even though the percentage of degradation is lower than *Bacillus* sp. UMTFA1 (RB). It is considered that *S. kloosii* strain UMTFA2 (RS) was first found to be the potential diesel oil degrader. These two isolated bacteria diesel oils utilizing bacteria transformed hydrocarbon into simpler compounds via metabolic pathways. The result also signified that *Bacillus* sp. could be best exploited for bioremediation of oil-contaminated soil since it had the highest degradation potential among the isolates.

In addition, these bacteria could degrade diesel oil because they are associated with the polychaete that lives in sediment that has been contaminated with oil pollution. The degradation activity by these bacteria is due to the presence of enzymes and genes that gives them an edge over other bacteria that could not survive in the seemingly harsh environment due to diesel pollution. For example, enzymes including dioxygenases and dehydrogenases are usually involved during the degradation process under aerobic conditions. Abdelhaleem et al. (2019), reported that *Bacillus subtilis* showed the highest catechol 1, 2-dioxygenase activities in MSM supplemented with a hydrocarbon such as anthracene. Several species of bacteria can completely degrade diesel and convert it into non-toxic compounds such as CO_2 and H_2O (Das & Chandran, 2011).

Several other factors also affect the biodegradation of diesel oil. In this study, the temperature used for incubation was 37 °C. Usually, the degradation process was maximum at an incubation temperature of 30 and 37 °C; nevertheless, the most favorable temperature for biodegradation was 37 °C (Ibrahim, 2016). Temperature is one of the factors that give effectiveness in the biodegradation of hydrocarbon. Similarly, environmental factors such as agitation speed and pH directly affect bacterial survival and growth, consequently affecting biodegradation in the shake flasks or the bioreactor. The availability of oxygen is also vital in aerobic biodegradation. The variation in the agitation speed affects the degree of mixing and the nutrient availability to the microorganism (Abusham, Rahman, Salleh & Basri, 2009). In this study, the agitation speed was 150 rpm following the method of

Ibrahim (2016). The degradation activity of bacteria strains increased and reached the maximum value at rotation speed 150 rpm, at which they exhibited a degradation percentage of 61 to 77%. It maintains a sufficient supply of dissolved oxygen in the medium for the bacterial strains to grow and degrade the diesel oil. In agreement, Deng et al. (2014) found that the degradation rate of diesel oil using *Achromobacter* sp. HZ01 strain was gradually increased by increasing the agitation speed and reached the maximum at 150 rpm. Knowledge of the factors that influence bioremediation is thus valuable in developing cost-effective bioremediation strategies (Varjani & Upasani, 2017).

The biodegradation efficiency of diesel oil by *Bacillus* sp. UMTFA1 (RB) and *S. kloosii* strain UMTFA2 (RS) were analysed using T-test analysis to compare between these two isolates of bacteria. The T-test result concluded that there is no significant difference between these two bacteria species regarding the capacity in degrading diesel oil (p value > 0.05). The result means that the capability for these two isolates to degrade diesel oil was almost the same even though *Bacillus* sp. UMTFA1(RB) degraded 52.29% of diesel oil and *S. kloosii* strain UMTFA2 (RS) degraded 39.24% of diesel oil as shown in previous Figure 4. The potential of *Bacillus* sp. and *Staphylococcous* sp. of degrading diesel have been stated that these bacteria were found to show the degradation of diesel oil and crude oil to the extent of 80% after 60 days of incubation (Darma, Mansir & Riko, 2019; Sunkar, Vani, Barret & Nachiyar, 2020).

Enumeration of Bacteria using Colony Forming Unit per ml (CFU/mL)

Bacteria growth was evaluated during the biodegradation experiment by quantifying the viable cells expressed by CFU/mL. The average log value of bacteria counts by CFU/mL by *Bacillus* sp. UMTFA1(RB) and *S. kloosii* strain UMTFA2 (RS) showed that the bacteria count increased during the incubation period (Figure 5). Both species showed that they could grow and multiply in Mineral Salt Media broth supplied with 1% diesel oil as the carbon source. Both bacteria can degrade hydrocarbon components present in the diesel oil. Bacteria count for both species increased from the day 0 to day 20 of incubation. However, *Bacillus* sp. UMTFA1 (RB) count was much higher (1.54 x 10^8 CFU/mL) on day 20 when compared to *S. kloosii* strain UMTFA2 (RS) count (8.73 x 10^7 CFU/mL). The microbial growth increased while degradation of hydrocarbons occurred, considering that the cells were in an exponential phase and maintaining themselves on an alternative carbon and energy source. In this phase, the cells divide by binary fission and double in numbers after each generation time. Metabolic activity is high as the bacteria use diesel oil's carbon and energy source for growth and generate for cell division. The previous study has also proved that *Bacillus* sp. can utilize naphthalene, one of the major components of diesel oil (Das, Bhattacharya, Banu & Kotok, 2017). The results in this study indicated that the growth rates of the two strains reflected diesel oil degradation trends. These results were in good agreement with those of previous studies conducted by Sun et al. (2019). Besides that, the growth of the bacteria during the incubation time because of the accumulate of many compounds from the diesel oil itself for storage as a strategy to survive in variable environments. In this study, the bacteria growth shows an increase until the end of the incubation period due to the presence of diesel oil, which provides a source of energy to the bacteria. It is recommended, in further study, the incubation period should be increased until the bacterial growth reaches the stationary phase and subsequently faces the death phase. Normally, when the bacteria growth is increased, the nutrient available in the medium will be decreased because bacteria have been utilized it (Sathishkumar, Binupriya, Baik & Yun, 2008).

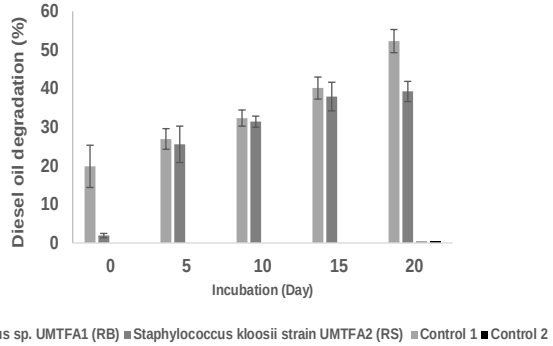

Figure 4. Biodegradation efficiency by *Bacillus* sp. UMTFA1(RB) and *Staphylococcus kloosii* strain UMTFA2 (RS) respectively against days of incubation. The data presented as mean ± SD.

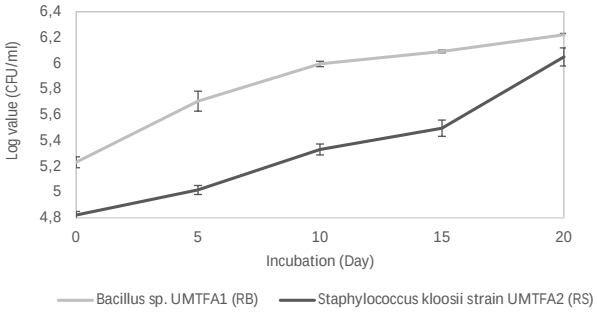

Figure 5. Bacteria count CFU/ml by *Bacillus* sp. UMTFA1 (RB) and *Staphylococcus kloosii* strain UMTFA2 (RS) against days of incubation. The data presented as mean ± SD.

Conclusion

In conclusion, the bacteria isolated from the gut fluid of *M. moribidii* which are *Bacillus* sp. UMTFA1(RB) and *S. kloosii* strain UMTFA2 (RS) have the capability to degrade hydrocarbons. The isolation of culturable bacteria from polychaete enabled us to understand the hydrocarbon-degrading ability of the indigenous bacteria. The ability of *Bacillus* sp. UMTFA1 (RB) to degrade hydrocarbons has been supported by previous studies. It was revealed that *Bacillus* sp. demonstrated the highest degradation potential and therefore could be exploited for bioremediation of used diesel oil-polluted environment.

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