Degradation of crude oil-degrading bacteria isolated from the coastal waters of Bengkalis Island, Riau

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Abstract. Bengkalis waters is relatively busy traffic area due to regional and international marine transportation which is very vulnerable to oil pollution. Cleaning of oil spill could be attempted by the use microorganisms (bioremediation). Bioremediation is an effective, economical and environmentally friendly treatment method in which microbes are used to degrade hydrocarbons. The objectives of this research were to isolate oil-degrading bacteria from seawaters in the North coast and in the South area of Bengkalis Island, to examine the bacterial isolates in degrading crude oil and to identify the isolates by phenotype (morphological observation, biochemical tests) and genotype characters (16S rDNA sequence). Bacteria was isolated on SMSS basal medium, oil content was measured by Gravimetry method, DNA sequence was analized by using polymerase chain reaction (PCR) followed by sequencing using genetic analyzer machine. Sixteen bacterial isolates from the seawaters had different morphology, physical and biochemical characters. Six isolates from the North coastal waters (BM 1a, BM 1c, BM 1d, BM 7a, BM 14a and BM 14b) and three isolates from Sungai Pakning waters (S1P26B, S2P35b and S3P35a) showed high ability in degrading crude oil at concentrations 1%, 2% and 3%). DNA sequence analysis indicated isolate BM 1a had similarity to Bacillus circulan W239 (99%), isolate BM 1c was similar to B. thungiriensis LDC507 (92%), isolate BM 1d was similar to B. flexus CORSS01 (96%), isolates BM 7a and S1P26B were similar to B. cereus MBGIPS 18 (94% and 95%, respectively), isolate BM 14a was similar to B. flexus MDLD1 (98%), isolate BM 14b was similar to B. thuringiensis LDC 507 (99%), isolate S2P3 5b was similar to B. cereus KJW1 (98%) and isolate S3P3 5a was similar to B. cereus MCCC1a06185 (99%). In conclusion, bacteria of genus Bacillus dominated the crude-oil degrading bacteria from Bengkalis Island waters.

1. Introduction

Bengkalis marine area is very vulnerable to oil pollution due to business of traffic line and human and industrial activities both in the North and in the South of Bengkalis Island. The existence of Bandar Sri Setia Raja (BSSR) International Port in the North which connects the island to Muar Malaysia and shipping activities could result in oil spill in the marine area. Meanwhile, the presence of Bandar Sri Laksamana (BSL) Domestic Porth in the South which connects Sungai Pakning in Sumatera Island to Bengkalis Island in addition to activities of national oil companies such as PT. Pertamina RU II Sungai Pakning) and PT. Kondur Petroleum contribute to oil pollution in the seawaters.

The effects of oil pollution in marine environment on living organisms directly and indirectly have been studied and documented in scientific and technical literatures over several decades. Direct effects of oil spills on wildlife can be through ingestion, absorption and inhalation, while indirectly effects cause
changes in behaviour, i.e. relocation for new sources of food, increase time must spend foraging and disruptions to natural life cycles [1]. Oil spill was harmful to seabirds and marine plants [2], and indicated a significant reduction in abundance and diversity of benthic, meiofauna and macrofauna as well as visual damage to deep-sea corals [3].

Cleaning of oil spill could be attempted by the use microorganisms (bioremediation). Bioremediation is an effective, economical and environmentally friendly treatment method in which microbes are used to degrade hydrocarbons [4]. The process is based on the use of nutrients or aeration to enhance the activity of indigenous organisms (biostimulation) and/or the addition of microbial inocula (bioaugmentation) to enhance the clean-up processes [5,6]. Our previous study found bacteria species of Providencia vermicular, Burkholderia cepacia and Myroides odoratimimus from Dumai seaways of Riau Province which had an ability to degrade the Sumatran crude oil [7].

Oil degrading bacteria play an important role for bioremediation. The bacteria can use petroleum hydrocarbon as sources of carbon and energy, and are often exploited for the bioremediation of petroleum oil contaminated environments [6]. A large number of hydrocarbon-degrading bacteria had been revealed from oil-rich environments, such as oil spill areas and oil reservoirs [8], and that their abundance and quantity are closely related to the types of petroleum hydrocarbons and the surrounding environmental factors [9,10]. Based on 16S rRNA gene sequences, three bacteria isolated from crude oil enrichments of natural seawater were identified as Alcanivorax borkumensis SK2, Rhodococcus erythropolis HS4 and Pseudomonas stutzeri SDM [11]. Genera Pseudomonas, Burkholderia, Enterobacter and Rahnella represented nearly 60% of the total population found from petroleum refinery waste [12]. Two potential oil-degrading isolates isolated from marine sediments collected from fuel oil–polluted coastal area and were identified as Chryseobacterium sp. strain AJ0 and Escherichia sp. strain UIWRF0110, respectively [13].

The objectives of this research were to isolate crude oil-degrading bacteria from the north coastal waters and from the marine area of Sungai Pakning in the south of Bengkalis Island; to examine the degradation of crude oil by selected isolates, and to identify the bacterial isolates from the phenotype and genetic characters.

2. Materials and Methods
The research was conducted from April until October 2018. Bacterial isolation and biodegradation test were performed in Marine Microbiology Laboratory of the Faculty of Fishery and Marine, and bacterial molecular analysis was performed in the Genetic Laboratory of Biology Laboratory of the Faculty of Math and Natural Sciences in Universitas Riau. Bacterial DNA isolates was sequenced by PT. Genetika Science Indonesia in Jakarta Barat.

2.1. Sample collection
Water samples were collected from two locations in Bengkalis Island. The first location was in the north coastal waters of Bengkalis Island which is located at coordinates 01°33’33.18”-1°33’46.35” N and 102°09’28.10”-102°14’34.93” E, and the second was in the south of the island which is located in Sungai Pakning marine area at coordinates 01°20’41.00” – 01°37’99.40” and 102°09’28.10” – 102°14’26.00” E. Three sampling points were determined in in the north, those were Selat Baru coastal waters, Bandar Sri Setia Raja (BSSR) International Port and mangrove area in the north of Selat Baru coast. Three sampling sites in the south were Bandar Sri Laksamana (BSL) domestic port, Sungai Pakning port and marine area around PT. Pertamina Sungai Pakning. All water samples were kept in cooling box during transportation to the Marine Microbiology and Chemical Oceanography Laboratories in the Faculty of Fishery and Marine Universitas Riau. Water quality parameters such as water temperature, salinity, pH and transparency were also measured during the sampling periodes.

2.2 Materials
Oil-degradation bacteria was isolated in Stone Mineral Salt Solution extract yeast (SMSSse) consisted of CaCO3, NH4NO3, Na2HPO4.7H2O, KH2PO4, MgSO4.7H2O, MnCl2.7 H2O, bacto agar (Oxoid), yeast
extract (Oxoid) and Zobell Marine Agar (Difco). Then, n-hexane and Duri crude oil were used for the oil degradation test.

2.3 Bacterial Isolation and Purification
Seawater bacteria was isolated and purified on basal medium: the stone mineral salt solution SMSS) and the Zobell Marine Agar. The SMSS medium consisted of 0.5 g CaCO$_3$; 0.25 g NH$_4$NO$_3$; 0.1 g Na$_2$HPO$_4$.7H$_2$O; 0.05 g KH$_2$PO$_4$; 0.05 g MgSO$_4$.7H$_2$O; and 0.02 g MnCl$_2$.7H$_2$O diluted in 200 mL of seawater. The basal medium was added with 0.01% yeast extract as nitrogen source, this medium was renamed as SMSSe medium. This medium was then added with Duri crude oil at different concentrations (1.0 %, 2.0 % and 3.0 %) as carbon source. The medium was set at pH 6.8-7 [14].

Three mL of each water sample was added and mixed well into the SMSSe medium. The suspension was incubated and for 14 days at room temperature on a modified shaker at 120 rpm. During the incubation sample was taken at day 1, 7 and 14 for bacterial isolation and enumeration. Enumeration was conducted on 3 mL of SMSS medium added with 3 gram of bacteriological agar by spread plate count. All inoculated plates were incubated at 30°C for 48 hours and the grown colonies were counted. Colonies indicated different characters were selected and streak on fresh agar medium and incubated for 48 hours. Colonies grown separately were observed the morphology including colony’s colour, shape, edge and elevation, type of cell and Gram staining.

2.4 Preparation of bacterial suspension for degradation test
Bacterial isolates used for the degradation test were selected based on the difference of morphology and Gram characters at the incubation day 1, 7 and 14. Each isolate was suspended in 0.85 % of NaCl solution of NaCl 0,85 %) at final density of $10^5$ cells/mL.

2.5 Oil degradation test
One mL of each bacterial suspension was put into a tube containing 150 mL of SMSSe broth medium added with 1.5 mL (1%), 2.0 mL (2%) and 3.0 mL (3%) crude oil separately. Control tube without crude oil was prepared as negative control. The cultures were then incubated at 30°C and 120 rpm for 24 hours.

2.6 Analysis of oil content
Oil content was analyzed by Gravimetry method following the procedure in APHA-AWWA-WEF [15]. Each of the treatment tubes was poured into separated funnel, added with 5 mL HCl 3 N and 60 mL n-hexane. The mixture was shaken for ± 15 minutes, and let until the n-hexane separated into three layers of oil, n-hexane and water. The water layer was removed, while the oil and n-hexane layers were filtered through a filter paper smeared with ± 0.5 g Na$_2$SO$_4$ into a 100 mL beaker glass which had been known the weight. The beaker glass was then heated at 60°C (same as the n-hexane melting point) until the n-hexane removed and the water evaporated and the rest part was oil. The glass was removed and let to cool, weighed and the weight was recorded. Oil content was calculated by using the following formula [15]:

\[ \text{Oil content (g)} = (W_2 - W_1) \]

Note: $W_1$ = dry weight of beaker glass (g); $W_2$ = weight of beaker glass with oil content (g)

2.7 Characterization of Bacterial Isolate Using Analysis of 16S rRNA Sequen

2.7.1 DNA extraction. DNA of each of oil-degradation bacterial isolates was extracted following the procedures of Geneaid® Gel/PCR DNA Fragment Extraction Kit. Each of the isolates was re-inoculated in 5 ml of NB and then was incubated at 30°C for 24 hours. 1.5 mL of the culture was transferred into a sterile microtube and was centrifuged at 13000 rpm for one minute, the supernatant was discarded, while
the pellet was re-suspended in 200 µL of Gram-buffer (GB) and was homogenized by pipetting. The suspension was heated in waterbath at 37°C for 10 minutes. Proteinase K (20 µL) was added to the suspension and heated at 60°C for 10 minutes. The suspension was added with 200 µL of GBand re-heated at 70°C for 10 minutes. After cooling, the suspension was centrifuged for three minutes. The supernatant was removed to a new tube, added with 200 µL of absolute ethanol and removed to a genomic DNA (GD) column and then was centrifuged for two minutes. The supernatant was removed to a new collection tube, added with 400 µL of wash buffer, and centrifuged for 30 seconds. The supernatant was discarded, and the pellet was added 600 µL of wash buffer and centrifuged for 30 seconds, then the supernatant was discarded. The tube was re-set to the CD column and was centrifuged for three minutes. The GD column was removed to a new tube and 60 µL of elution buffer was added, left for five minutes, then was centrifuged for one minute twice and stored until being used at PCR process.

2.7.2 Amplification of 16S rRNA. Genome of bacterial 16S rRNA was amplified with polymerase chain reaction (PCR) using thermal cycler (Eppendorf, Mastercycler, Personal). The PCR reaction consisted of 50 µL of final solution of 5.0 µL PCR buffer, 5.0 dNTP, 1.0 µL of universal primer (16th base) 24F (5'-AGAGTTTGATCCTGCT-3') and 1.0 µL primer 1541R (5'-AAGGAGGTGATCCAGCGCA-3'), 0.25 unit of DNA polymerase, and 36.75 µL of DNA template. Thermocycle program was run at the following condition: pre-denaturation at 94°C for 5 minutes; 35 cycles of denaturation at 94°C for 30 seconds; annealing at 50°C for 45 seconds; extension at 72°C for 90 seconds; final extension at 72°C for 10 minutes. The PCR product was detected by running electrophoresis in 1% of agarose gel, stained with loading dye (ethidium bromide, 1 µL/10 mL) and was visualized under UV transiluminator for 1500 base pairs of target product.

2.7.3 DNA sequence analysis. The PCR product was sent to PT. Genetika Science Indonesia for finally sequenced. Sequencing program was performed by ABI PRISM 3730xL GENETIC ANALYZER (Applied Biosystems, USA). Before sequencing, the PCR products were purified by using Geneaid® Gel/PCR Fragments Extraction Kit. The Sequenced products were analyzed by using the BLAST (Basic Local Alignment Search Tool) to know the bacterial species by treasuring at the NCBI GenBank. The results were presented as homology percentage of bacterial DNA sequence in comparison to database sequent [16]. Phylogenetic tree was constructed by using Mega 06 and Bioedit programs.

2.8 Data analysis
Data of water quality parameters of sampling sites, heterotrophic bacterial isolates, antagonism against pathogens and identified bacterial species were presented in tables and figures. The data were then analyzed descriptively and compared to previous related and similar researches.

3. Results and Discussion
3.1. Morphological and biochemical characters of oil-degradating bacterial isolates
Ten bacterial isolates were selected from the north coastal waters and six isolates from Sungai Pakning marine areas (in the south area of Bengkalis Island). Morphology and biochemical characters of each isolate were presented in Tables 1 and 2. In general, most of the colony shape was round, white, creamy and yellow in color and all had convex elevation. Thirteen isolates were motile. Biochemical characters showed that only one isolate was of Gram positive bacteria, fifty percents of the isolates produced enzyme catalase. Four isolates produced indole and only one isolate (BM 1a) produced H2S. Six isolates used citrate and three isolates used MrVp as carbon sources. One isolate (S1aP3aS5a) could not ferment TSIA and produced gas.

3.2. Degradation of crude-oil by bacterial isolates
Ten isolates from the north coastal waters of Bengkalis Island (Table 3) and six isolates from Sungai Pakning waters (Table 4) showed an ability to degrade crude oil. Table 3 shows that the highest
degradation at initial crude oil content of 1 mL (1%) was performed by isolate S1P5b, and the highest degradation at crude oil content of 2 mL and 3 mL was indicated by isolate S1P5a. While, Table 4 indicates the highest degradation at 1% crude oil was performed by isolat BM 7a, and the highest degradation of crude oil 2% and 3% was performed by isolat BM 14b.

Table 1. Morphological characters of oil-degrading bacteria isolated from the north coastal waters of Bengkalis Island and Sungai Pakning marine waters.

| No | Isolate code | Morphology of colony | Shape | Colour | Edge | Elevation | Cell Motility |
|----|--------------|----------------------|-------|--------|------|----------|--------------|
| 1  | S1P2 6b      | Irregular and spread | Irregular | White creamy | Smooth | Convex | Motile |
| 2  | S1P3 6a      | Round                | Round  | White creamy | Smooth | Convex | Non motile |
| 3  | S2P1 6b      | Round                | Round  | White creamy | Smooth | Convex | Motile |
| 4  | S2P2 5a      | Round                | Round  | White creamy | Smooth | Convex | Motile |
| 5  | S2P3 5b      | Round                | Round  | White creamy | Smooth | Convex | Motile |
| 6  | S2P2 6b      | Round                | Round  | White creamy | Smooth | Convex | Motile |
| 7  | S3P1 5a      | Round                | Round  | White creamy | Smooth | Convex | Motile |
| 8  | S3P1 5b      | Irregular and spread | Yellow | Smooth | Convex | Motile |
| 9  | S3P3 4a      | Irregular and spread | Yellow | Smooth | Convex | Non motile |
| 10 | S3P3 5a      | Irregular and spread | Yellow | Smooth | Convex | Non motile |
| 11 | BM 1a        | Round                | Yellow | Wavy | Convex | Motile |
| 12 | BM 1c        | Round                | White  | Smooth | Convex | Motile |
| 13 | BM 1d        | Round                | White  | Smooth | Convex | Motile |
| 14 | BM 7a        | Round                | Yellow | Wavy | Convex | Motile |
| 15 | BM 14a       | Round                | White  | Smooth | Convex | Motile |
| 16 | BM 14b       | Round                | White  | Smooth | Convex | Motile |

Note: Number 1 until 10 were bacterial isolates from the north coastal water; and number 11 until 16 were isolates from Sungai Pakning waters.

Table 2. Biochemical characters of oil-degrading bacteria isolated from the north coastal waters of Bengkalis Island and Sungai Pakning marine waters.

| No. | Isolate code | Gram staining | Catalase prod. | Indole prod. | Use of TSIA | Use of MrVp | Use of citrate | Use of H2S prod. | Use of Slant | Use of Butt | Elevation | Cell Motility |
|-----|--------------|---------------|----------------|--------------|-------------|------------|---------------|----------------|--------------|-------------|-----------|--------------|
| 1   | S1P2 6b      | +             | +              | -            | -           | -          | -             | -              | -            | -           | -         | Motile       |
| 2   | S1P3 6a      | -             | +              | -            | +           | +          | -             | -              | -            | -           | -         | Non motile   |
| 3   | S2P1 6b      | -             | -              | -            | +           | -          | -             | -              | -            | -           | -         | Motile       |
| 4   | S2P2 5a      | +             | -              | -            | +           | -          | -             | -              | -            | -           | -         | Motile       |
| 5   | S2P3 5b      | +             | -              | -            | +           | -          | -             | -              | -            | -           | -         | Motile       |
| 6   | S2P2 6b      | -             | -              | -            | +           | -          | -             | -              | -            | -           | -         | Motile       |
| 7   | S3P1 5a      | -             | -              | -            | +           | -          | -             | -              | -            | -           | -         | Motile       |
| 8   | S3P3 5b      | -             | -              | -            | +           | -          | -             | -              | -            | -           | -         | Motile       |
| 9   | S3P3 4a      | -             | -              | -            | +           | -          | -             | -              | -            | -           | -         | Motile       |
| 10  | S3P3 5a      | +             | -              | -            | -           | -          | -             | -              | -            | -           | -         | Motile       |
| 11  | BM 1a        | +             | +              | +            | +           | +          | +             | +              | -            | -           | -         | Motile       |
| 12  | BM 1c        | +             | +              | -            | +           | -          | -             | -              | -            | -           | -         | Motile       |
| 13  | BM 1d        | +             | +              | +            | +           | -          | +             | +              | -            | -           | -         | Motile       |
| 14  | BM 7a        | +             | +              | +            | +           | -          | -             | -              | -            | -           | -         | Motile       |
| 15  | BM 14a       | +             | +              | -            | +           | -          | -             | -              | -            | -           | -         | Motile       |
| 16  | BM 14b       | +             | +              | +            | +           | -          | +             | +              | -            | -           | -         | Motile       |

Note: Motility: + motile - non motile; Growth on TSIA: - not produced gas and acid; Citrate and MrVp are used as carbon sources; Catalase: +, enzyme is used for cell respiration.
Overall data indicates that bacteria species has different ability in degradating of crude oil. In this study the highest degradation of crude oil at the addition of 1% in culture broth was performed by isolate BM 7a which was isolated from Sungai Pakning waters, but at the addition of 2% and 3% crude oil was performed by isolate S3P35a isolated from the north coastal waters of Bengkalis Island. This also informs that bacteria species from the north waters of Bengkalis Island has higher ability than bacteria from Sungai Pakning seawaters in crude oil degradation.

The ability of bacteria to degrade crude oil is due to the bacteria produces enzymes which are able to degrade complex organic compound into simple compounds. Microbial oxidoreductases produced by various bacteria and fungi mediate detoxification of toxic organic compounds such as enzymes monooxygenases and dioxygenases [17]. Monooxygenases act as biocatalysts in bioremediation process, while enzyme dioxygenase produced by bacteria degrades polyaromatic hydrocarbon into cis-dihydrodiol [18]. The degradation ability of microorganism depends on the microorganism to adapt with the environment [19]. Factors impacting of petroleum hydrocarbon degradation were temperature, nutrients, oxygen, salinity, pH, microbial community, bioavailability, activity of waters and toxicity of end products [20].

### Table 3. Final crude oil content and degraded oil by bacterial isolates from the north coastal waters of Bengkalis Island

| No. | Isolate code | Treatment with 1% | Treatment with 2% | Treatment with 3% | Degraded oil content (mL) |
|-----|--------------|------------------|------------------|------------------|--------------------------|
| 1   | Control      | 1.00             | 2.00             | 3.00             | 0.00                     |
| 2   | S1P2 6b      | 0.36             | 0.96             | 1.81             | 0.64                     |
| 3   | S1P3 6a      | 0.60             | 1.44             | 2.05             | 0.40                     |
| 4   | S2P1 6b      | 0.78             | 1.84             | 2.68             | 0.22                     |
| 5   | S2P2 5a      | 0.38             | 0.98             | 1.79             | 0.62                     |
| 6   | S2P3 5b      | 0.18*            | 1.22             | 1.85             | 0.82                     |
| 7   | S2P2 6b      | 0.80             | 1.86             | 1.73             | 0.20                     |
| 8   | S3P1 5a      | 0.32             | 1.32             | 1.42             | 0.68                     |
| 9   | S3P1 5b      | 0.44             | 1.31             | 1.84             | 0.56                     |
| 10  | S3P3 4a      | 0.72             | 1.24             | 1.54             | 0.28                     |
| 11  | S3P3 5a      | 0.36             | 0.91*            | 1.24*            | 0.64                     |

Note: Degraded oil content is initial oil content (1.0, 2.0 and 3.0% of crude oil added) reduced with the final oil content

#### 3.3. Molecular characterization of oil-degradating bacteria

Genotype identification of crude oil-degradating bacteria was presented in Table 5. Nine isolates selected for the molecular characterization indicate that all are of genus *Bacillus*. Two isolates indicated the highest degradation at 1%, 2% and 3% of crude oil addition in broth cultures, those were isolates BM 7a and S3P35a, have 94% and 99% similarities with *B. cereus*.

*Bacillus cereus* is one of some bacteria which are effective in naturally hydrocarbon degradation. This bacteria species had the highest degradation ability in crude oil degradation among ten different bacteria species as it was reported before [21]. *B. cereus* can degrade hydrocarbon in non saline and environment but some strains are frequently in high salinity condition [22]. It was reported that the aromatic hydrocarbons were attacked more quickly during the crude oil degradation by marine microbial population [23]. *B. cereus* utilized hydrocarbon as sole carbon source for their growth and multiplication.
in the soil by degrading the hydrocarbon due to the production of biosurfactant so it maintained the soil fertility [20].

Table 4. Final oil content and degraded oil by bacterial isolates from Sungai Pakning waters

| No. | Isolate code | Final oil content (mL) | Degraded oil content (mL) |
|-----|--------------|------------------------|--------------------------|
|     | Treatment with 1 % | Treatment with 2 % | Treatment with 3 % | Treatment with 1 % | Treatment with 2 % | Treatment with 3 % |
| 1.  | Control       | 1                      | 2                        | 3                  | 1                      | 2                        | 3                  |
| 2.  | BM 1a         | 0.40                   | 1.33                     | 2.27               | 0.60                   | 0.67                     | 0.73               |
| 3.  | BM 1c         | 0.88                   | 1.37                     | 2.43               | 0.12                   | 0.63                     | 0.57               |
| 4.  | BM 1d         | 0.73                   | 1.97                     | 2.70               | 0.27                   | 0.03                     | 0.30               |
| 5.  | BM 7a         | 0.11                   | 1.61                     | 2.60               | 0.89                   | 0.39                     | 0.40               |
| 6.  | BM 14a        | 0.76                   | 1.98                     | 2.82               | 0.24                   | 0.02                     | 0.18               |
| 7.  | BM 14b        | 0.14                   | 1.21                     | 2.03               | 0.86                   | 0.79                     | 0.93               |

Note: Degraded oil content is initial oil content (1.0, 2.0 and 3.0% of crude oil added) reduced with the final oil content

Table 5. Genotype identification of crude oil-degrading bacteria from the north coastal waters of Bengkalis Island and Sungai Pakning waters.

| Isolate code | Bacterial Species | Strain | Query coverage | Homology | Accession Number |
|--------------|-------------------|--------|----------------|----------|-----------------|
| BM 1a        | *Bacillus circulans* | W239   | 100%           | 99%      | MF554628.1      |
| BM 1c        | *B. thuringiensis* | LDC507 | 100%           | 92%      | KF779471.1      |
| BM 1d        | *B. flexus*       | CORSS01| 100%           | 96%      | MF422682.1      |
| BM 7a        | *B. cereus*       | MBGIPS 18 | 100%        | 94%      | KX950682.1      |
| BM 14a       | *B. flexus*       | MDLD1  | 100%           | 92%      | FJ861081.1      |
| BM 14b       | *B. thuringiensis*| LDC507 | 100%           | 98%      | KF779471.1      |
| S1P2 6B      | *B. cereus*       | MBGIPS 18 | 100%        | 95%      | KX950682.1      |
| S2P3 5b      | *B. cereus*       | KJW1   | 100%           | 98%      | JQ799105.1      |
| S3P3 5a      | *B. cereus*       | MCCCIa06185 | 100%       | 99%      | KJ812453.1      |

4. Conclusion

Crude oil-degrading bacteria could be isolate from the marine water of Bengkalis Island. Two isolates (BM 7a and S3P3 5a) perfomed highest degradation activity at 1%, 2% and 3% of crude oil added in broth cultures. The two isolate bacteria was genetically identified as *Bacillus cereus*.

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