# Heavy oil degrading *Burkholderia* and *Pseudomonas* strains: insights on the degradation potential of isolates and microbial consortia

**Hernando P. Bacosa[1,2,*] and Chihiro Inoue[1]**
[1]Graduate School of Environmental Studies, Tohoku University, Aoba 6-6-20, Aramaki, Aoba-ku, Sendai, Miyagi 980-8579, Japan
[2]School of Engineering and Computing Sciences, College of Science and Engineering, Texas A&M University-Corpus Christi, Corpus Christi, Texas 78412, USA
*Correspondence: herbacs3001@gmail.com
https://doi.org/10.69721/TPS.J.2020.12.1.06

**ABSTRACT**

Bacterial strains were isolated from heavy oil degrading microbial consortia enriched from mangrove sediment. Among the 60 distinct isolates that formed colonies on heavy oil as a sole carbon and energy source, *Pseudomonas* sp. T2B and *Burkholderia* sp. T2C showed the best growth in heavy oil and in various aliphatic and aromatic hydrocarbons. The two isolates were tested in their abilities to degrade heavy oil. At 1% oil concentration, T2B and T2C degraded 19.6% and 16.7% of heavy oil within 21 days, respectively. These values were significantly lower than that of the source consortium T2, which degraded 24.2% of the oil. This indicates that the consortium is more superior in degrading heavy oil than any of the isolates. The isolates can be used for biodegradation studies and can be utilized in producing an effective microbial consortium for bioremediation applications.

**Keywords:** *Pseudomonas, Burkholderia*, heavy oil, microbial consortium, hydrocarbons, biodegradation

## INTRODUCTION

The increasing industrial production leads to significant damage to the environment by numerous contaminants. Due to human activities, organic pollutants that affect the quality of the environment and threaten public health are spilled often as a contaminant of soil, groundwater, and marine environments (Kaufmann et al. 2004; Murphy et al. 2016; Bacosa et al. 2017). Crude oil and petroleum hydrocarbons are among these contaminants. It is estimated that worldwide, about 800 million liters of petroleum enter the marine environment each year from the extraction, transportation, and consumption of crude oil and products from it (National Research Council USA 2003). An additional 700 million gallons is contributed by natural seepage. Past analysis of reported oil spills indicated that most of the oil comes from tankers, barges and other vessels as well from coastal oil refineries and land pipeline spills. Major oil spills in history such as the Exxon Valdez (1989), the Nakhodka oil spill (1997), the Erica spill (1999), the Prestige spill (2002)

The Palawan Scientist, 12: 74-89

and Deepwater Horizon (2010), had brought extensive changes in marine ecosystem (Bacosa et al. 2015a; Gemmell et al. 2016; Murphy et al. 2016; Liu et al. 2017).

Heavy oil is one of the major products from crude oil and is defined by the U.S. Department of Energy as having API (American Petroleum Institute) gravities that fall between 10.0° and 22.3°. Like the conventional petroleum oils, it is a complex mixture of hydrocarbons and other compounds. Petroleum constituents represent saturates or aliphatic, aromatics, resins and asphaltenes (Bacosa et al. 2010; Evans et al. 2016, 2018). Heavy oil is deficient in simple alkanes and aromatics but enriched in long chain alkanes, high molecular weight aromatic hydrocarbons and polar components relative to conventional crude oil (Jack 1998; Bacosa et al. 2013). Polycyclic aromatic hydrocarbons (PAHs) are among the aromatic components of heavy oils that pose a serious threat to the environment (Bacosa et al. 2010). The United States Environmental Protection Agency named 16 PAHs in its priority list according to their toxicity and threat to the ecosystem. PAHs substances are known for their persistence, toxicity, carcinogenicity and mutagenicity (Bacosa and Inoue 2015). PAH-rich heavy oil is considered as resistant to biodegradation, recalcitrant and persistent in the environment. Thus, it is imperative to remove heavy oil from polluted environment to minimize its adverse impacts.

Oil spill is deleterious to a wide array of marine plants, animals and microbial communities through oxygen stress and direct toxic effects (Severin et al. 2016; Gemmell et al. 2017; Williams et al. 2017). In tropical and subtropical regions, the ultimate recipient of marine oil spills is the mangrove ecosystems which is one of the important components of the coastal environment. Oil spills in mangrove areas impart potential damage to their physical and ecological integrity. Removal of spilled oil in the environment can be done in various ways. It could be achieved by either physicochemical or biological methods (Bacosa et al. 2015b, 2016). However, the negative consequences as a result of implementing physicochemical approaches are currently directing greater attention to explore for more sustainable and environmentally-amiable biological alternatives. Among these popular options is bioremediation using a community of bacteria or isolates (Okoh 2006). Mangroves are sensitive ecosystem, which implies that less intrusive, biodegradation-based remedial alternatives are the suitable option since they present minimum harm to these ecosystems. These methods are less expensive and do not introduce additional chemicals to the environment. Compared to physiochemical methods, bioremediation offers a very feasible alternative for an oil spill response.

In an attempt to characterize the community composition and evaluate the oil and hydrocarbon-degrading abilities of bacteria in mangrove

areas in Okinawa, Japan, six bacterial consortia were obtained by enrichment method and reported in our previous study (Bacosa et al. 2013). These enriched bacterial consortia showed different abilities to degrade heavy oil. Although bacteria in nature behave as a community similar to an enriched consortium, studies on the abilities of each strain to grow or degrade specific hydrocarbon compound could provide insights on the importance of the strains in bioremediation applications. This study aims to isolate bacteria from the enriched consortia, investigate the abilities of the isolates to grow in pure hydrocarbon substrates, and determine the abilities of select strains to degrade heavy oil in comparison with the source consortium.

## METHODS

### Bacterial Isolation

The bacteria were isolated from the microbial consortia capable of degrading heavy oil as a sole carbon and energy source. These six consortia namely, T1, T2, T3, O, K1 and K2, were enriched from sediment samples that were collected from the mangroves of Okinawa, Japan (Bacosa et al. 2013). To obtain heavy oil-degrading isolates, a serial dilution of the bacterial suspension from each enrichment culture was plated in Petri plates containing mineral salt medium (MSM), 1.5% agarose, and 20 µl of heavy oil. The composition of the MSM is indicated in Bacosa et al. (2013). The plates were incubated for two weeks at 30°C. The colonies formed were preliminary screened using morphological characteristics as criteria which include, color, elevation, margin, and surface of the colony. A portion of the colony was inoculated into a test tube containing 10 ml MSM and 1% (v/v) of heavy oil. Subsequently, incubation was done for two weeks at 30°C with shaking.

### Growth in Heavy Oil and Hydrocarbons

The isolates were then screened for growth in heavy oil and pure hydrocarbon compounds namely, decane (C10), hexadecane (C16), eiocosane (C20), octacosane (C28), pristine (C19 branched), phenanthrene (3 rings) and pyrene (4 rings). Liquid hydrocarbons were filter sterilized and solid hydrocarbons were prepared in a stock solution with dichloromethane (10 mg $ml^{-1}$). Each hydrocarbon was added in 30 ml test tube containing 10 ml of MSM medium and 1% (v/v) of heavy oil or 1000 mg $l^{-1}$ of each hydrocarbon substrate. After allowing dichloromethane to evaporate, solid hydrocarbons were applied with 10 cycles of ultrasonic wave (UP 200s Untraschall-prozessor). The isolates were grown at 30°C, with shaking (120 rpm) for 14 days. Growth was determined periodically using spectrophotometer at an optical density of 600 nm (Chaerun et al. 2004). Inoculated cultures were compared to uninoculated control. Growth criteria were as follows: No growth

(0)–absorbance difference of below 0.100; Low growth (+)–absorbance difference of 0.100-0.499; Medium growth (++)–absorbance difference of 0.500-0.999; High growth (+++)–absorbance difference of 1.000 above. These methods and criteria were adapted from the work of Chaerun et al. (2004). Isolates which showed high growth in heavy oil and/or hexadecane and at least two of the pure hydrocarbon substrates were selected for sequencing

**DNA Extraction**

Total DNA was extracted from the six isolates to determine their identities. The bacterial cells were gathered by centrifugation (TOMY-High speed refrigerated microcentrifuge) at 5,000 rpm for 10 min. The cells were resuspended in 40 µl of sterile distilled water followed by freezing and thawing two times. Proteinase K (10 µl) and TTNE buffer (50 µl) were added and vortexed. Enzyme reaction was allowed to take place at 20 min for 60°C and enzyme de-excitation at 95°C for 10 min. To precipitate the bacterial cells, the suspension was centrifuged at 4°C for 5 min at 14,000 rpm. Supernatant containing the extracted DNA was then collected.

**DNA amplification and purification**

Nearly full-length 16S rRNA gene was amplified by polymerase chain reaction (PCR) using primers Eu 10F (5'- AGAGTTTGATCCTGGCTCAG-3'), corresponding to *Escherichia coli* positions 8-27 as a forward primer, and Eu 1500R (5'- GGTTACCTTGTTACGACTT -3'), corresponding to *Escherichia coli* positions 1492-1510 as a reverse primer (Takami et al. 1999). These primers can amplify the entire 16S rRNA gene (~1500 bp). PCR was performed in a 50 µl reaction containing Promega PCR Master Mix, $MgCl_2$, DMSO, primers, DNA template and nuclease free water using a 9700 Thermal Cycle (Applied Biosystems) (Bacosa et al. 2013). The PCR conditions were as follows: 9 min at 95°C and 30 cycles of denaturation (30 s at 95°C), annealing (30 s at 50°C), and extension (1 min at 72°C), followed by a 7-min extension at 72°C (Bacosa et al. 2010, 2011). The PCR amplification products were verified by 1.5% agarose gel electrophoresis and visualized under UV transilluminator.

PCR products were purified using Gene Elute Mammalian Total RNA Miniprep Kit (Sigma Aldrich) according to the manufacturer's protocol. Briefly, Wash solution 2 was diluted with 99% ethanol. The volume of the PCR product was then adjusted to 100 µl using elution solution. PCR product was added with Lysis solution and 2-Me mixture. After that, the solution with DNA was applied to the binding column and cleaned-up twice with Wash solution 2. Finally, the purified DNA was eluted from the column with 50 µl of elution solution

## DNA Sequencing

Purified DNA was sequenced following the Big Dye Terminator v1.1/3.1 Cycle sequencing chemistry. Four universal primers (10F, 530F, 800R, and 1400R) were used to sequence the nearly full-length fragment of 16S rRNA gene (~1500 bp) (Kawasaki et al. 1992; Takami et al. 1999). The 20 µl sequencing reaction contains 4 µl BigDye R Terminator v3.1 Cycle Sequencing Kit, 2 µl V3.1 sequencing buffer, 0.5 µl Primer, 2 µl DNA template, and sterile deionized water (Bacosa et al. 2010, 2013; Bacosa and Inoue 2015). Prior to sequencing, the solution was purified by sodium dodecyl sulfate (SDS). To attain a concentration of 0.2%, 2 µl of 2.2% SDS was added to the samples, heated at 98°C for 5 min, and allowed to cool at room temperature. Samples were transferred to spin columns and centrifuged at 800 g for 2 min. The liquid that passed through the spin column was dried for about 30 min using an aspirator (KUBOTA CHRIST Alpha 1-2, TAITEC VC-96 N). Twenty microliters of Hi-Di Formamide was added to dried samples, heated for 2 min at 95°C and cooled for 5 min at room temperature. The samples were then transferred to a 96-well plate and sequenced in Applied Biosystems Genetic Analyzer 3130. DNA sequences were assembled and compared to the sequences in the GenBank.

## Degradation Experiment and Oil Analysis

Isolates T2B and T2C were evaluated in the degradation of heavy oil following established procedure (Bacosa et al. 2013). Briefly, the standard inoculum was cultured for 1 week in 1% (v/v) heavy oil in MSM. The cells were harvested by centrifugation, rinsed three times with phosphate saline buffer and resuspended in MSM. An aliquot (500 µl) was added to 125-ml culture bottles containing 9.5 ml of MSM and heavy oil at a final concentration of 1% v/v. The cells were added to attain an initial concentration of ~5 x $10^5$ cell $ml^{-1}$. In order to compare the degradation abilities of the bacterial isolates with the enriched consortia, T1, T2, T3, O, K1 and K2 consortia were prepared in the similar manner. Triplicate bottles were prepared for each isolate, consortium, and uninoculated controls. The bottles were sealed and placed in an orbital shaker and incubated for 21 days at 30°C with shaking (120 rpm).

The residual oil was extracted after 21 days of incubation. The cultures were acidified to pH 2-3 using hydrochloric acid before extracting twice with the same volume of carbon tetrachloride. The extract was diluted at acceptable range and analyzed by Horiba-Oil Content Analyzer (Model OCMA-350). Quantification was based on a five-point calibration curve. Biodegradation potential was expressed as biodegradation efficiency relative to the control (Bacosa et al. 2013).

## Statistical Analysis

To determine the significant differences among the means of biodegradation efficiency, one-way analysis of variance (ANOVA) was performed using PAST software package V2.17 (Hammer et al. 2001). The differences between treatment means were further tested using Tukey's honestly significance difference (HSD).

# RESULTS

## Growth on Heavy Oil and Hydrocarbon Substrates

The screening of the isolates using colony morphology resulted in 60 distinct isolates. These isolates were investigated in their abilities to grow on heavy oil resulting in 25 good degraders. Moreover, these 25 strains were grown in various hydrocarbon compounds as sole carbon and energy sources. The results of the growth experiment are summarized in Table 1. Isolates T2B, T2C, T2E, T2F, and T2G grew well in heavy oil as a carbon and energy source. When investigated in the presence of hydrocarbon compounds, different patterns of degradation as indicated by bacterial growth were observed. Most of these strains did not grow in the presence of decane. Surprisingly, T2F which showed good growth in heavy oil was not able to grow in hexadecane. Only T2B and T2C were able to utilize both phenanthrene and pyrene for growth. It suggests that T2B and T2C isolates have capabilities to degrade both the aromatic and aliphatic components of heavy oil. T2B and T2C cultures in test tube in the presence of different hydrocarbon substrates are presented in Figures 1a and b. Their respective colonies that formed on plates with heavy oil are shown in Figures 2a and b.

Table 1 also shows that isolates with moderate or low growth in heavy oil have different capabilities to utilize a wide range of pure hydrocarbon substrates. Some of these strains utilized decane as a carbon and energy source. Heavy oil is abundant in long-alkane chains, not in short chain alkanes like decane. OC and K1D isolates demonstrated low growth in several hydrocarbon substrates, including heavy oil. It is worthy to note that most of the isolates, which showed favorable growth in heavy oil and pure hydrocarbon compounds, were isolated from T2 consortium.

Table 1. Growth characteristics of isolates in heavy oil and hydrocarbon compounds (1% v/v) cultured in MSM for 14 days. Legend: 0 – no growth: + - low growth: ++ - medium growth: +++ - high growth.

| Isolates | Heavy oil | Decane | Hexadecane | Eiocosane | Octacosane | Pristane | Phenanthrene | Pyrene |
|---|---|---|---|---|---|---|---|---|
| T2B | +++ | 0 | +++ | ++ | ++ | 0 | + | + |
| T2C | +++ | 0 | +++ | ++ | 0 | + | + | + |
| T2E | +++ | 0 | +++ | + | ++ | 0 | 0 | 0 |
| T2F | +++ | 0 | 0 | + | + | + | 0 | 0 |
| T2G | +++ | + | +++ | + | 0 | + | 0 | 0 |
| T1A | ++ | 0 | +++ | ++ | + | + | 0 | 0 |
| T1B | + | + | + | + | + | 0 | + | 0 |
| T1C | ++ | ++ | ++ | ++ | 0 | ++ | 0 | 0 |
| T1F | ++ | + | ++ | + | + | + | 0 | 0 |
| T1G | + | + | ++ | 0 | 0 | + | 0 | 0 |
| T1H | ++ | 0 | ++ | ++ | + | ++ | 0 | 0 |
| T2A | ++ | + | ++ | ++ | + | ++ | 0 | 0 |
| T3A | ++ | 0 | +++ | + | 0 | ++ | 0 | 0 |
| T3B | + | 0 | ++ | ++ | 0 | + | + | 0 |
| T3C | + | + | + | + | 0 | + | 0 | 0 |
| T3D | + | + | ++ | 0 | 0 | + | 0 | 0 |
| T3E | + | ++ | ++ | + | 0 | + | 0 | + |
| T3G | + | 0 | ++ | + | 0 | + | 0 | 0 |
| OC | + | + | + | 0 | + | + | 0 | + |
| OD | + | + | + | + | + | + | 0 | 0 |
| K1B | ++ | + | + | 0 | + | 0 | 0 | + |
| K1D | + | 0 | ++ | + | ++ | + | 0 | + |
| K1F | + | + | + | 0 | + | + | 0 | 0 |
| K2B | ++ | + | + | ++ | 0 | + | 0 | + |
| K2C | ++ | + | + | + | + | 0 | + | 0 |
| K2D | ++ | 0 | ++ | + | + | + | 0 | 0 |

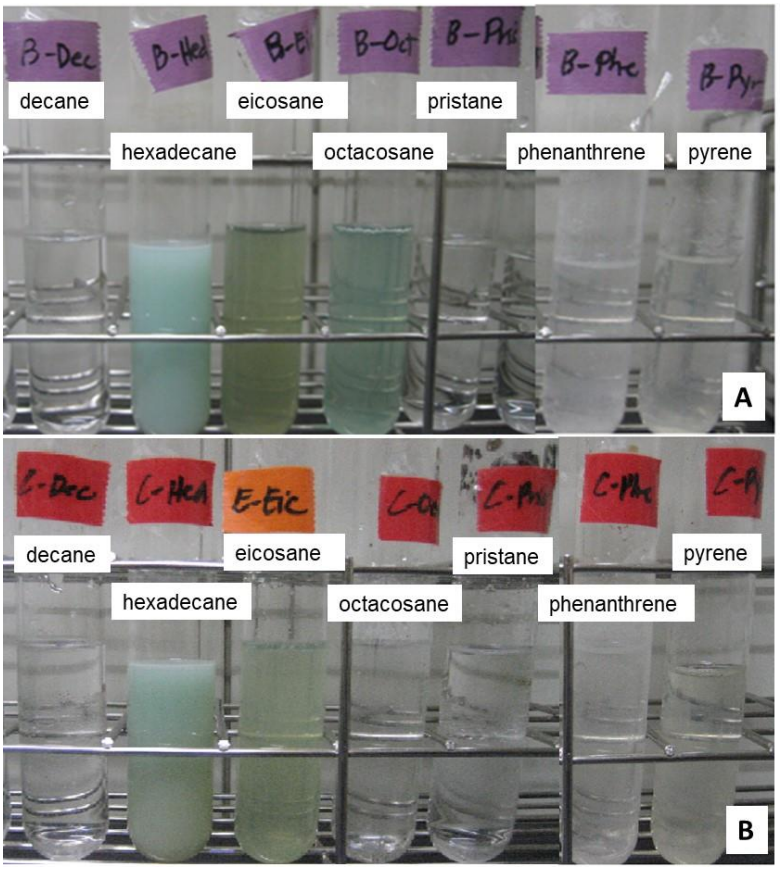


Figure 1. Growth of T2B (A) and T2C (B) isolates on different hydrocarbon substrates after 14 days of incubation. Increasing liquid turbidity indicates higher growth.

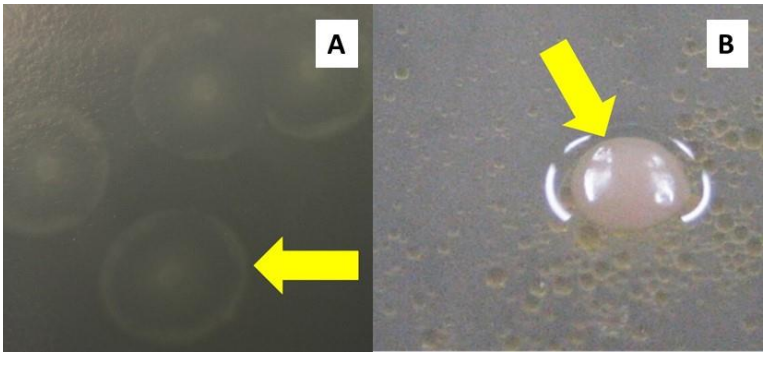


Figure 2. Colonies of T2B (A) and T2C (B) isolates on agar plate with mineral salt medium (MSM) and heavy oil.

## Identification of the Isolates

Six isolates were selected as superior heavy oil degraders and were identified to species level based on 16S rRNA gene. The identities of these isolates are presented in Table 2. Sequencing results revealed that the bacterial isolates were closely related to *Pseudomonas aeruginosa* and *Burkholderia cepacia*. T2B, T2G, and T1A were related to *Pseudomonas aeruginosa*, while T2C, T2E, and T2F were affiliated to *Burkholderia cepacia*.

Table 2. Identities of the bacterial isolates based on 16S rRNA gene sequencing.

| Strain | Closest relative sequence | Accession No. | Similarity |
|---|---|---|---|
| T2B | *Pseudomonas aeruginosa* K3 | EF064786 | 1319/1323 (99.7%) |
| T2C | *Burkholderia cepacia* RRE5 | AY946011 | 1322/1326 (99.7%) |
| T2E | *Burkholderia cepacia* WK | EF031062 | 1256/1257 (99.9%) |
| T2F | *Burkholderia cepacia* WK | EF031062 | 1346/1346 (100%) |
| T2G | *Pseudomonas aeruginosa* S25 | DQ095913 | 1287/1287 (100%) |
| T1A | *Pseudomonas aeruginosa* S25 | DQ095913 | 1285/1285 (100%) |

Results showed that even though they belong to the same species, these strains formed distinct colonies, and have different capabilities to degrade petroleum hydrocarbons. It is interesting that T2F, which belonged to genus *Burkholderia* was not able to utilize hexadecane, a medium-chain hydrocarbon. As previously reported, although the enriched bacterial consortia were dominated by bacteria closely related to *Pseudomonas* and *Burkholderia,* they have different oil degradation abilities (Bacosa et al. 2013). The remaining isolates were not sequenced because they have low degradation abilities and were likely closely related to *Pseudomonas* or *Burkholderia*.

## Heavy oil degradation by T2B and T2C isolates

Because T2B and T2C strains showed superior abilities to grow on heavy oil and utilized a wide range of aliphatic hydrocarbons and PAHs substrates, their potential to biodegrade heavy oil was further tested and compared to that of the bacterial consortia. After 21 days of incubation, *Pseudomonas* sp. T2B degraded 19.7% of the heavy oil, which was greater than *Burkholderia* sp. T2C (17.6%) (Figure 3). However, the heavy oil degradation abilities of both isolates were significantly lower than T2 consortium (24.2%, $p<0.05$). This shows that the consortium, which is a combination of both

strains and other bacteria that we were not able to isolate, was more effective in degrading hydrocarbons in heavy oil. The rest of the consortia degraded more heavy oil than any of the two isolates. K1, T1, O consortia utilized 31.6%, 30.2%, and 27.1% of the oil, respectively. T3 and K2 consumed about 23% of oil for growth within 21 days.

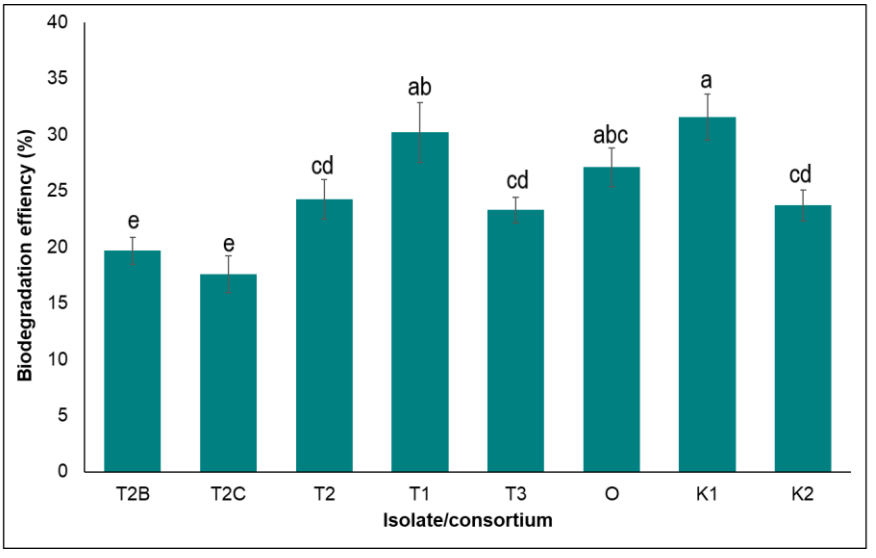


Figure 3. Heavy oil degradation efficiencies of T2B and T2C isolates compared to enriched bacterial consortia after 21 days of incubation. Error bars represent one standard deviation from the mean of three replicates. Different letters indicate significant difference based on Tukey's HSD test. P-value lesser than 0.05 was considered significant.

## DISCUSSION

*Pseudomonas* sp. T2B and *Burkholderia* sp. T2C were successfully isolated from T2 consortium that degraded heavy oil. Both isolates were able to utilize various aliphatic and aromatic hydrocarbons as sole carbon and energy sources. The genus *Pseudomonas* is arguably the most diverse and ecologically significant bacteria which is ubiquitous in many environments (Spiers et al. 2000). They are known to have simple metabolic systems, encode genes for catabolic enzymes and harbor degradative plasmids, which make them capable of using several organic compounds including hydrocarbons for growth (Mukherjee et al. 2010; Salazar et al. 2012). *Burkholderia* is a genus with remarkable nutritional and physiological versatility. Many *Burkholderia* possess plasmid that contributes to its versatility and produce oxygenases-the enzymes that are essential for the initial oxidation of hydrocarbon chain and

aromatic ring (Okoh et al. 2001; Coenye and Vandamme 2003; Suto et al. 2007).

*Pseudomonas* and *Burkholderia* in bacterial consortia had been implicated in the degradation of hydrocarbons and petroleum products. *Pseudomonas* dominated the bacterial consortium from leaf soil that preferably degraded alkanes (Bacosa et al. 2011). However, *Burkholderia* was the key player in the faster disappearance of aromatic hydrocarbon over aliphatic hydrocarbons in kerosene degrading consortium (Bacosa et al. 2010, 2011, 2012). Aromatic hydrocarbons, particularly polycylic aromatic hydrocarbons (PAHs), are more toxic that aliphatic hydrocarbons and known as mutagenic and carcinogenic (Bacosa and Inoue 2015). Crude oil is a complex mixture of hundreds to a thousand of aliphatic and aromatic hydrocarbon. Aliphatic hydrocarbons are more abundant but less toxic, while and aromatic hydrocarbons are less abundance but more toxic and are mutagenic and carcinogenic (Bacosa et al. 2015a, 2016; Evans et al. 2016; Evans et al. 2018). Risk-based bioremediation takes into account the relative toxicity of complex pollutants like oil and its products. If aromatic hydrocarbons were being degraded faster, the risk level will be reduced significantly. This will eventually result in an efficient and cost-effective remediation of contaminated environments.

T2 consortium is primarily composed of *Pseudomonas* and *Burkholderia* as shown in its profile using polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) (Bacosa et al. 2013). The current study revealed that both T2B and T2C did not surpass the degradation ability of T2 source consortium. Moreover, all bacterial consortia tested have greater degradation than both isolate suggesting that mixed bacterial consortia are more efficient in the degradation of heavy oil. Notably, T2 consortium has one of the lowest biodegradation efficiencies among the eight enriched consortia. This observation could be attributed to the competition of other bacteria in T2 consortium with T2B and T2C, or non-isolation of superior bacteria from other consortia. Biodegradation is a community and collective effort leading to greater results. Rahman et al. (2002) showed that individual bacterial cultures showed less growth and lower degradation of crude oil than the mixed bacterial consortium. The same pattern was also observed by Sathishkumar et al. (2008), in which a consortium composed of *Pseudomonas, Bacillus* and *Corynebacterium* degraded oil more effectively than any of the isolate. Another advantage of a bacterial consortium is its ability to metabolize completely the parent compound into carbon dioxide and water something that is not possible using chemical and physical remediation methods (Khehra et al. 2005; Bacosa et al. 2016). While a single strain can be simply incubated with a substrate, the relationship is quite complex in a microbial consortium. Some members of the consortium produce the oxygenases and dioxygenase to oxidize the hydrocarbon chain, while other

members produce the necessary micronutrients necessary for the main degraders (Dominguez et al. 2019). Other bacteria may produce biosurfactant to make the oil more soluble in water and biodegradable (Bacosa et al. 2015b, 2018a,b). Moreover, other bacteria consume the metabolic products from the degradation of parent compound. Some of the metabolites have been reported to be inhibitory and toxic to the degrader of parent compound (Kazunga and Aitken 2000). These toxicants have to be removed by other bacteria in the consortium to enhance the degradation of the parent compound. It is worth mentioning here that the isolates from the most effective consortia, T1 and K1, did not show remarkable growth in heavy oil and pure hydrocarbons substrates. The high degradation of these consortia was not due to superbacteria but can be attributed to the synergistic relationships of bacteria in the consortium.

Although the degradation potential was not as high as any of the consortia, isolating these strains from the mixed culture offers numerous advantages. The potential of the strains to degrade different hydrocarbons and their combinations, crude oil, and oil products can be ascertained. The full-length of the DNA sequence of these strains are already known, and the whole genome analysis to unravel the full potential of these strains can be carried out. Individual isolates can be also used in combination with other microorganisms to produce an effective and robust bacterial consortium for bioremediation applications.

Overall, *Pseudomonas* sp. T2B and *Burkholderia* sp. T2C were successfully isolated from T2 consortium. These strains were effective in the degradation of heavy oil and several aliphatic and aromatic hydrocarbons. Although their degradation was lower compared to the source consortia, these isolates could be useful in making a more effective synthetic consortium for the remediation of contaminated sites. The combination of these isolates with other consortium or strains towards applications in the degradation of petroleum hydrocarbons warrants further investigation.

## ACKNOWLEDGEMENTS

Hernando P. Bacosa was supported by the Japanese Government Scholarship program to pursue a Master's degree in Tohoku University. We would like to thank the two referees for the invaluable comments and suggestions, which substantially helped in improving the quality of the manuscript.

## REFERENCES

Bacosa HP, Erdner DL and Liu Z. 2015a. Differentiating the roles of photooxidation and biodegradation in the weathering of light Louisiana sweet crude oil in surface water from the Deepwater Horizon site. Marine Pollution Bulletin, 95: 265-272.

Bacosa HP, Erdner DL, Rosenheim B, Shetty P, Seitz K, Baker B and Liu Z. 2018a. Hydrocarbon degradation and response of seafloor sediment bacterial community in the northern Gulf of Mexico to light Louisiana sweet crude oil. The International Society for Marine Ecology Journal, 12: 2532-2543. DOI: 10.1038/s41396-018-0190-1.

Bacosa HP, Evans ME, Wang Q and Liu Z. 2017. Assessing the Role of Environmental Conditions on the Degradation of Oil following the Deepwater Horizon Oil Spill. In: Stout S and Wang Z (eds). Oil Spill Environmental Forensics Case Studies, 1st Edition. Elsevier. United Kingdom, pp. 617-637.

Bacosa HP and Inoue C. 2005. Polycyclic aromatic hydrocarbons (PAHS) biodegradation potential and diversity of microbial consortia enriched from tsunami sediments in Miyagi, Japan. Journal of Hazardous Materials, 283: 689-697.

Bacosa HP, Kamalanathan M, Chiu M, Sun L, Labonte J, Schwehr K, Hala D, Santschi P, Chin W and Quigg A. 2018b. Extracellular polymeric substances (EPS) producing and hydrocarbon degrading bacteria isolated from the northern Gulf of Mexico. PLoS One, 13(12): e0208406. DOI: 10.1371/journal.pone.0208406.

Bacosa HP, Liu Z and Erdner DL. 2015b. Natural sunlight shapes crude oil-degrading bacterial communities in northern Gulf of Mexico surface waters. Frontiers in Microbiology, 6: 13-25. DOI: 10.3389/fmicb.2015.01325.

Bacosa HP, Suto K and Inoue C. 2010. Preferential degradation of aromatic hydrocarbons in kerosene by a microbial consortium. International Biodeterioration and Biodegradation, 64: 702-710.

Bacosa HP, Suto K and Inoue C. 2011. Preferential utilization of petroleum oil hydrocarbon components by microbial consortia reflects degradation pattern in aliphatic–aromatic hydrocarbon binary mixtures. World Journal of Microbiology and Biotechnology, 27: 1109–1117.

Bacosa HP, Suto K and Inoue C. 2012. Bacterial community dynamics during the preferential degradation of aromatic hydrocarbons by a microbial consortium. International Biodeterioration and Biodegradation, 74: 109-115.

Bacosa HP, Suto K and Inoue C. 2013. Degradation potential and microbial community structure of heavy-oil enriched microbial consortia from mangrove sediments in Okinawa, Japan. Journal of Environmental Science and Health, Part A: Toxic/Hazardous Substances and Environmental Engineering, 48: 835-846.

Bacosa HP, Thyng KM, Plunkett S, Erdner DL and Liu Z. 2016. The tarballs on Texas beaches following the 2014 Texas City "Y" spill: modeling, chemical, and microbiological studies. Marine Pollution Bulletin, 109: 236-244. DOI: 10.1016/j.marpolbul.2016.05.076.

Chaerun KS, Tazaki K, Asada R and Kogure K. 2004. Bioremediation of coastal areas 5 years after the Nakhodka oil spill in the Sea of Japan: Isolation and characterization of hydrocarbon-degrading bacteria. Environment International, 30: 911-922.

Coenye T and Vandamme P. 2003. Diversity and significance of *Burkholderia* species occupying diverse ecological niches. Environmental Microbiology, 5: 719-729.

Dominguez JJA, Bacosa HP, Chien MF and Inoue C. 2019. Enhanced degradation of polycyclic aromatic hydrocarbons (PAHs) in the rhizosphere of sudangrass (*Sorghum* × *drummondii*). Chemosphere, 234: 789-795.

Evans M, Wang Q, Bacosa H, Rosenheim B and Liu Z. 2018. Environmental petroleum pollution analysis using ramped pyrolysis-gas chromatography-mass spectrometry. Organic Geochemistry, 124: 180-189.

Evans M, Liu J, Bacosa H, Rosenheim BE and Liu Z. 2016. Petroleum hydrocarbon persistence following the Deepwater Horizon oil spill as a function of shoreline energy. Marine Pollution Bulletin, 115: 47-56.

Gemmell B, Bacosa HP, Dickey B, Gemmell C, Alqasemi L and Buskey E. 2017. Assessing the community response of marine protists and microbes following the Texas City "Y" oil spill. Ecotoxicology, 27: 505-515.

Gemmell B, Bacosa HP, Liu Z and Buskey EJ. 2016. Can gelatinous zooplankton influence the fate of crude oil in marine environments? Marine Pollution Bulletin, 113: 483-487.

Hammer Ø, Harper DAT and Ryan PD. 2001. PAST: paleontological statistics software package for education and data analysis. Palaeontologia Electronica, 4(1): 1-9. http://palaeo-electronica.org/2001_1/past/issue1_01.htm.

Jack TR. 1998. Microbial Aspects of Heavy Oil. In: Meyer RF (ed). Exploration for Heavy Crude oil and Natural Bitumen. American Association of Petroleum Geologists. USA, pp. 249-256. DOI:10/1306/St25468.

Kaufmann K, Christophersen M, Buttler A, Harms H and Hohener P. 2004. Microbial community response to petroleum hydrocarbon contamination in the unsaturated zone at the experimental field Voerlose, Denmark. FEMS Microbiology Ecology, 48(3): 387-399.

Kawasaki H, Hoshino Y, Kuraishi H and Yamasato K. 1992. *Rhodocista centenaria* gen. nov., sp. nov., a cyst-forming anoxygenic photosynthetic bacterium and its phylogenetic position in the Proteobacteria Alpha group. The Journal of General and Applied Microbiology, 38: 541-551.

Kazunga C and Aitken MD. 2000. Products from the incomplete metabolism of pyrene by polycyclic aromatic hydrocarbon-degrading bacteria. Applied and Environmental Microbiology, 66: 1917-1922.

Khehra MS, Saini HS, Sharma DK, Chadha BS and Chimni SS. 2005. Comparative studies on potential of consortium and constituent pure bacterial isolates to decolorize azo dyes. Water Research, 39: 5135-5141.

Liu J, Bacosa HP and Liu Z. 2017. Potential environmental factors affecting oil-degrading bacterial populations in deep and surface waters of the northern Gulf of Mexico. Frontiers in Microbiology, 7: 21-31. DOI: 10.3389/fmicb.2016.02131.

Mukherjee S, Bardolui NK, Karim S, Patnaik VV, Nandy RK and Bag PK. 2010. Isolation and characterization of a monoaromatic hydrocarbon-degrading bacterium, *Pseudomonas aerugin*osa from crude oil. Journal of Environmental Science and Health, Part A: Toxic/Hazardous Substances and Environmental Engineering, 45: 1048-1053.

Murphy D, Gemmell B, Vaccarri L, Li C, Bacosa HP, Evans M, Gemmell C, Harvey T, Jalali M and Niepa T. 2016. An in-depth survey of the oil spill literature since 1968: Long term trends and changes since Deepwater Horizon. Marine Pollution Bulletin, 113: 371-379.

National Research Council USA. 2003. Oil in the Sea III: Inputs, Fates, and Effects. National Academy of Science, Washington DC, USA. 259pp.

Okoh A. 2006. Biodegradation alternative in the cleanup of petroleum hydrocarbon pollutants. Biotechnology and Molecular Biology Review, 1(2): 38-50.

Okoh A, Ajisebutu S, Babalola G and Hernandez MR. 2001. Potential of *Burkholderia cepacia* RQ1 in the biodegradation of heavy crude oil. International Microbiology, 4: 83-87.

Rahman KSM, Thahira-Rahman J, Lakshmanaperumalsamy P and Banat IM. 2002. Towards efficient crude oil degradation by mixed bacterial consortium. Bioresource Technology, 85: 257-261.

Salazar M, Morales M and Revah S. 2012. Biodegradation of methyl tertbutyl ether by cometabolism with hexane in biofilters inoculated with *Pseudomonas aeruginosa*. Journal of Environmental Science and Health, Part A: Toxic/Hazardous Substances and Environmental Engineering, 47, 1017-1026.

Sathishkumar M, Binupriya AR, Baik SH and Yun SE. 2008. Biodegradation of crude oil by individual bacterial strains and a mixed bacterial consortium isolated from hydrocarbon contaminated areas. Clean, 36: 92-96.

Severin T, Bacosa HP, Sato A and Erdner DL. 2016. Dynamics of *Heterocapsa* sp. and the associated attached and free-living bacteria under the influence of dispersed and undispersed crude oil. Letters in Applied Microbiology, 63: 419-425.

Spiers AJ, Buckling A and Rainey PB. 2000. The causes of *Pseudomonas* diversity. Microbiology, 146: 2345-2350.

Suto K, Bacosa H, Inoue C and Matsushima E. 2007. Microbial diversity in an iron oxidation tank of an AMD treatment plant at an abandoned sulphur mine. Advanced Materials Research, 20: 493-496.

Takami H, Kobata K, Nagahama T, Kobayashi H, Inoue A and Horikoshi K. 1999. Biodiversity in deep-sea sites located near the south part of Japan. Extremophiles, 3: 97-102.

Williams K, Bacosa HP and Quigg A. 2017. The impact of dissolved inorganic nitrogen and phosphorous on responses of microbial plankton to the Texas City "Y" oil spill in Galveston Bay, Texas (USA). Marine Pollution Bulletin, 121: 32-44.

***ARTICLE INFO***

*Received: 02 September 2019*
*Revised: 31 March 2020*
*Accepted: 03 April 2020*
*Available online:09 April 2020*

*Role of authors: HPB – conceptualization, investigation, methodology, formal analysis, writing; CI - Chihiro Inoue -funding acquisition, conceptualization and supervision.*