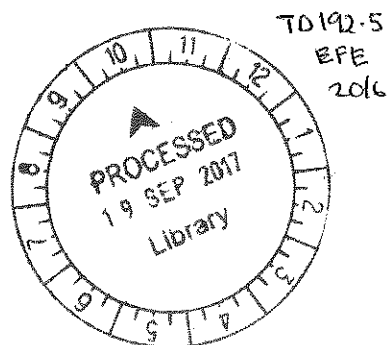


ISOLATION AND MOLECULAR PHYLOGENETIC  
CHARACTERIZATION OF CADMIUM-TOLERANT  
HYDROCARBON DEGRADING BACTERIA

EFENDY CHEW

DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE  
REQUIREMENTS FOR THE DEGREE OF BACHELOR OF BIOTECHNOLOGY  
(HONOURS)



FACULTY OF HEALTH AND LIFE SCIENCES  
INTI INTERNATIONAL UNIVERSITY  
PUTRA NILAI, MALAYSIA

MAY 2016

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## ABSTRACT

Three bacteria isolates (A, B and C) previously shown to biodegrade hydrocarbons were subjected to cadmium (Cd) toxicity test at 0.1 mg/L and 1.0 mg/L. Of the three isolates, only two (isolates A and C) were observed to survive Cd at the two tested concentration. Increasing the Cd concentration 10 times higher from 0.1 mg/L to 1.0 mg/L did not inhibit the growth of both isolates ( $p>0.05$ ). However isolate C showed the highest growth ( $p<0.05$ ) at the highest concentration of Cd tested at 1.0 mg/L, which is 100 times higher than the permissible limit for Cd in industrial effluent according to the Malaysian Environmental Quality Regulations. Gram staining results showed that isolate C to be Gram negative. DNA was successfully extracted from isolate C, visualized as a single band on 1.2% agarose gel. Amplification of the DNA extracted using primers targeting the 16s rDNA region yielded a band size of 1500 bp. The PCR product was sequenced and the sequence obtained was used successfully to construct a phylogenetic tree, identifying the Cd resistant bacteria to be *Pseudomonas aeruginosa*.

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## LIST OF ABBREVIATION

|                 |  |
|-----------------|--|
| $^1\text{O}_2$  | Singlet oxygen   |
| ATP             | Adenosine triphosphate                                   |
| BLAST           | Basic Local Alignment Search Tool                        |
| CBA             | CBA protein transporters                                 |
| Cd              | Cadmium  |
| $\text{CdCl}_2$ | Cadmium chloride   |
| CDF             | Cation Diffusion Facilitator                             |
| CFU/mL          | Colony-forming units per milliliter                      |
| CzcCBA          | CBA protein complex                                      |
| DNA             | Deoxyribonucleic acid                                    |
| EBI             | European Bioinformatics Institute                        |
| EPA             | Environmental Protection Agency                          |
| EPS             | Extracellular Polysaccharides                            |
| L               | Litre  |
| LPS             | Lipopolysaccharides                                      |
| MEGA            | Molecular Evolutionary Genetic Analysis                  |
| mg/L            | Milligrams per liter                                     |
| min             | minutes  |
| mL              | Millilitre   |
| NCBI            | National Center for Biotechnology Information            |
| nm              | nanometers   |
| OD              | Optical density  |
| PAHs            | Polycyclic aromatic hydrocarbon                          |
| PAUP            | Phylogenetic Analysis Using Parsimony                    |
| Pb              | Lead   |
| PCR             | Polymerase chain reaction                                |
| P-type ATPases  | Large family of membrane proteins in biological membrane |
| ROS             | Reactive Oxygen Species                                  |
| rpm             | Rounds per minute  |
| TAE             | Tris-acetate-EDTA  |

TEM

Transmission Electron Microscope

## CHAPTER 1

### INTRODUCTION

Major sources of energy come from crude oil which can be used as fuel for transportation and also generation of electricity. Because of this, frequent exportation and importation of this commodity leads to accidental spills. The seriousness of crude oil pollution can be seen whereby an estimated volume of 41,000 to 119,000 m<sup>3</sup> of crude oil spill from Exxon Valdez polluted the sea in Prince William Sound, Alaska in 1989 (Atlas & Bartha, 1997). Crude oil constituted of various hydrocarbon that are known carcinogens and neurotoxins that have deleterious effects on the biota (Das & Chandran, 2011), which is why removing hydrocarbon as a pollutant from the environment is important. Malaysia, a major crude oil producer is also susceptible to oil spills during extraction and refinery processes.

Bioremediation has currently contributed greatly to solve problems involving pollution. According to the United States Environmental Protection Agency (EPA), bioremediation is a process of breaking down pollutants into less toxic or to a non-toxic substance using organisms (Epa.gov, 1998). In the case of crude oil hydrocarbons, many bacteria have been reported to degrade hydrocarbons into smaller and less harmful constituents such as carbon dioxide, water and also minerals (Salleh et al., 2003). Some of the most common bacteria with hydrocarbon degradative abilities are *Pseudomonas sp*, *Bacillus sp*, and *Micrococcus sp*. (Wong et al, 2015; Paramanik & Rajalakshmi, 2013). However, the effectiveness of bacteria to degrade hydrocarbon can be compromised when there are other pollutants present in the contaminated area. Among the major co-contaminants found in the hydrocarbon-associated environment are heavy metals (Wong et al., 2013).

Heavy metals are commonly reported to co-contaminate industrial effluent and oil rigs (Abioye, 2011). The concentration and type of heavy metals determine the level of bacterial inhibition in the environment (Amor, Kennes & Veiga, 2001). Of the heavy metals, cadmium (Cd) is reported to inhibit hydrocarbon-degradative enzymes

of bacteria, this will impede the biodegradation process (Wong et al., 2013). Thus, the objective of this experiment is to select bacteria that can biodegrade hydrocarbons in the presence of Cd and identify it via phylogenetic analysis.

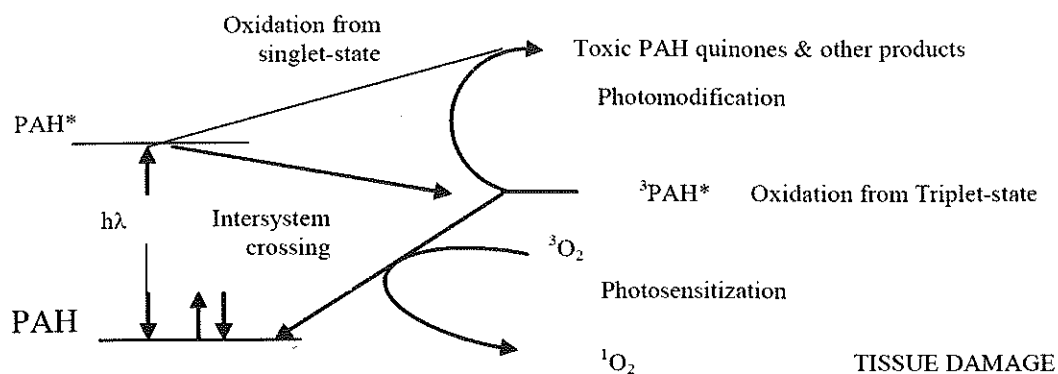
## CHAPTER 2

### LITERATURE REVIEW

#### 2.1 CRUDE OIL

Crude oil is fractionated into petroleum products such as fuel, raw materials to make plastics and industrial lubricants (Mahvi & Bazrafshan, 2007). Despite the numerous benefit of crude oil petroleum to ease human activities, it also has tremendous negative effect on the environment and biota. Crude oil mostly consists of hydrocarbons (95%) and the remaining five percent are made up of small amounts of oxygen, sulphur and traces of heavy metals (Salleh et al., 2003). Crude oil also consists of some polycyclic aromatic hydrocarbons (PAHs) compound (Domask, 1984). Substances that contains PAHs are dangerous to humans as they increase the risk of getting cancer (Boström et al., 2002).

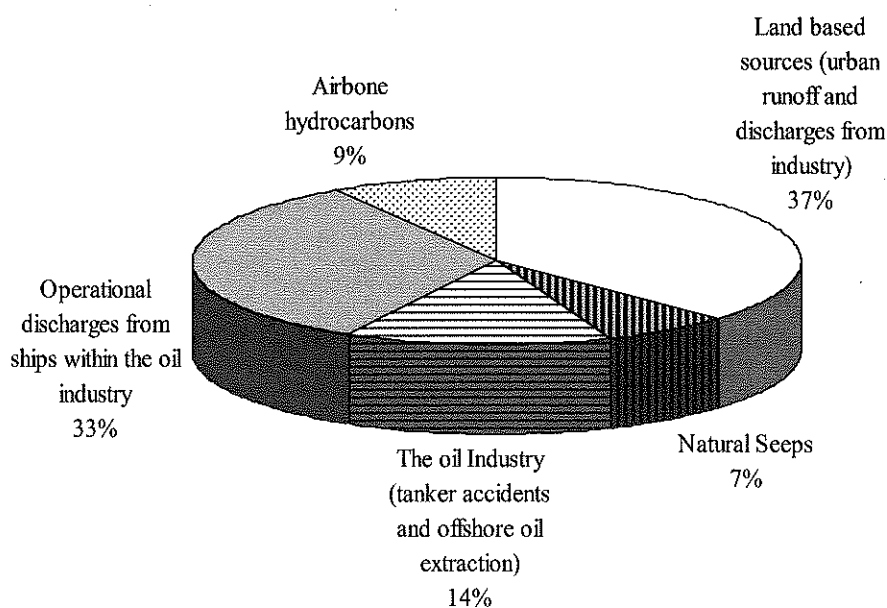
Oil spills in water is damaging as it affects the aquatic ecosystem when it starts to bioaccumulate in the food chain, affecting the biochemical and also physiological activity of organism (Cairns and Buikema, 1984; Onwurah et al., 2007). In marine animals, bioaccumulation of crude oil containing chemical residues such as PAHs can lead to fatality to the organism via generation of radical oxygen (Calfee et al., 1999; Heintz et al., 1999). Oil spill containing PAHs exposed to solar UV radiation will generate singlet oxygen,  $^1\text{O}_2$  via photosensitization (Babu et al., 2002). The direct reactions of  $^1\text{O}_2$  and the PAH will form unstable endoperoxides, and further rearrangement and oxidation of the endoperoxides will produce toxic quinones (Yu, 2002). Quinones are reported to have deleterious effect on the growth and survival rate of aquatic organism (Barbee et al., 1996).



**Figure 2.1** Production of  $^1\text{O}_2$  during photosensitization of PAHs (Babu et al, 2002).

## 2.2 CRUDE OIL CONTAMINATION IN THE ENVIRONMENT

**Figure 2.2** shows the global input of oil contamination to the marine environment (ITOPF 2008). Accidental releases due to tanker accidents, offshore oil drilling, operational discharges and industrial discharges contribute 84% of the total estimated global input of crude oil contamination to the marine environment. The rest in much smaller amounts are contributed by natural seepage and through airborne.



**Figure 2.2** Estimated global input of oil contamination to the marine environment from various sources. Adapted from ITOPF 2008