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Native soil bacterial isolate in Malaysia exhibit promising supplements on degrading organic pollutants

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ABSTRACT

A novel strain was isolated from an agricultural soil in Malaysia. After morphological and genetic characterization, the novel strain showed the highest similarity to *Bacillus* species. The ability of biostimulation (waste tealeaf) and bioaugmentation (*Bacillus salmalaya* 139SI) activities was investigated on soil polluted with 6% (w/w) waste crude oil for duration of 60 days. Strain 139SI was able to decrease the surface tension to 35 mN/m. Degradation of the initial petroleum hydrocarbon by 70% was achieved in the treatments involving *B. salmalaya* strain 139SI and tealeaf compared to the sterilized polluted soil (15%), which served as a control for this study. The aerobic utilizing bacteria counts and dehydrogenase activity were significantly increased during the period of study. Analysis of residual waste crude oil monitored by gas chromatography spectrophotometer indicated 80–95% degradation of n-C_8 to C_{12} followed by a 40% removal ratio of C_{22}. Kinetic model showed that treatment amended with both tealeaf and strain has indicated the highest level of biodegradation rate, with rate constant of 0.107 day^{-1}, while the biodegradation rate was 0.08 day^{-1} in treatments amended with only tealeaf. The finding showed the potentiality of tealeaf and *Bacillus salmalaya* 139SI toward the degradation/decomposition of crude oil in contaminated soil.

Abbreviations: TL, tea leaf; GC/MS, gas chromatography spectrophotometer; TPH, total petroleum hydrocarbon; BHI, brain-heart infusion broth; ST, surface tension; EI, emulisification index; INT, triphenyltetrazolium chloride; CFU, colony forming unit; DHA, dehydrogenase activity; GI, germination index; AHB, aerobic heterotrophic bacterial.

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1. Introduction

Environmental damage due to oil spills is a widespread environmental issue throughout the world. Oil spillage, leakage and other forms of petroleum release can lead to large oil spread pollution, causing a serious consequence that has been drawing public concerns globally (Qin et al., 2013). Global statistics indicated that in 2003, the world consumption of oil was over 63.5 million barrels per day and it is expected to reach up to 118 million barrels/day in 2030 (Jain et al., 2011). An estimated value of 600,000 metric tons of natural crude oil spill has been reported per annum (Sonawdekar, 2012). In such cases, the utilization of biological techniques toward remediation of contaminated sites has been recognized as an eco-friendly, economical and self-driven method and has received scientific attention. Due to the adaptation of microorganism to extreme environmental conditions, they were given more attention as per bioremediation of polluted environment as well as oil exploitation (Liu et al., 2014). With the help of suitable microorganisms, mineralization of highly hazardous oily to harmless products materials can be easily achieved (Kumari et al., 2012; Pacwa-Płociniczak et al., 2014; Ferreira et al., 2015). The concept of inoculating the soil with fast degrading microbes in order to increase the efficiency of the process is commonly known as bioaugmentation (Alisi et al., 2009; Chang et al., 2011). However, the bioavailability of contaminates and the potential of microorganisms in polluted areas are current limitation factors (Szulc et al., 2014). Indigenous microorganisms present in the environment can play a role in degrading a wide variety of organic compounds, however; the activity and population of microorganisms are affected by present at higher concentration of organic compounds. Furthermore, in normal circumstances the initial biodegradation potential is low; therefore, to overcome this problem selected microorganisms should be incorporated into polluted areas. Several studies have confirmed the increase in degradation rate with reducing time, by incorporating selected microorganisms in polluted areas (Dadrasnia and Salmah, 2014; Suja et al., 2014; Xu and Lu, 2010).

In contrast, it was also reported that this strategy did not improve the biodegradation rate (Chang et al., 2011). The reason might be due to poor adaptability of microorganisms and improper strain selection. Bioaugmentation is a promising strategy, but one of the important keys for effectiveness is the maintenance of high biomass of bacteria population in this process. Additionally, using the local microbial strains is preferred because these strains are suitable and well adapted compared to imported microbial populations which may not be effective in different application regions (Suja et al., 2014). Also, imported microbial populations are not cost effective, and the introduction of non-indigenous microbial species may impact the indigenous microbial population. On the other hand, solid waste management is going to be a serious challenging issue in the world. Many developing countries are failing to generate a large amount of biowastes annually. Since nutrient availability, especially N and P are important factors that can limit remediation process; therefore, organic waste provides required nutrients for enhancing the microbial activity and/or crude oil hydrocarbon degradation (biostimulation) (Dadrasnia et al., 2014; Margesin et al., 2007). In 2001, Obuekwe and Al-Muttawa reported (Obuekwe and Al-Muttawa, 2001) a good utilization of hydrocarbon degradation in aqueous medium by using sawdust and wheat bran as carriers during the incubation using self-immobilized cells. Therefore, biostimulation through biowaste addition when combined with bioaugmentation provided a good means for the hydrocarbons (Taccari et al., 2012). However, laboratory experiments needs to be established in order to assess the enhancement of petroleum degradation under controlled conditions; these experiments validate/re-affirm the scientific credibility of a scientific bioremediation. The aim of this study is to report the influence of bioaugmentation and biostimulation and a combined approach on the degradative efficiency of waste crude oil contaminated soil under laboratory condition. *Bacillus salmalaya* 139SI, a novel species isolated from local agriculture soil (Salmah et al., 2012), and used tea leaf were selected in this research work to investigate the feasibility of utilizing indigenous microbial population together with biowaste, as an alternative to enhance biodegradation of crude oil in waste contaminated soil.

2. Materials and methods

2.1. Isolation and identification of bacteria using 16S rRNA

Bacillus salmalaya strain 139SI was originally isolated and identified from indigenous agricultural soil obtained from Serdang Agricultural Center, Selangor, Malaysia (2.99917° N; 101.70778° E). The soil sample obtained was mixed with the sterile distilled water and the suspended mixture obtained was streaked on Brain-Heart Infusion (BHI) agar plates with 5% sheep blood as a supplement, then the plates were incubated at 37 °C for 16–24 h. For genomic DNA extraction, the bacterial isolate was cultured in 10 ml of BHI broth overnight at 150 rpm in a 37 °C shaking incubator. The bacteria cells were harvested and their genomic DNA extraction was carried out using NucleoSpin Tissue, based on the manufacturer's instructions. The selected 16S rRNA universal primers: 27Forward (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492Reverse (5′-GGTTACCTTGTTACGACTT-3′) were used to amplify the 16S rRNA region.

2.2. Inoculum preparation

In this study, biosurfactant production by *Bacillus salmalaya* 139SI was obtained in 1 L BHI medium contains: KCl 5 g/L, Dextrose 3 g/L, Na_2HPO_4 2.5 g/L, gelatin 14.5 g/L, brain heart infusion form 6 g/L and peptic digest of animal tissue 6 g/L for a period of 72 h at 35 °C in an incubator shaker at 150 rpm to obtain a standard inoculum (OD = 1) (Dadrasnia and Salmah, 2014).

2.3. Biosurfactant and emulsification activity (EI)

The emulsification index was measured at 25 °C by vertoxing 4 ml of biosurfactant and crude oil for 5 min. After 24 h EI was calculated as follows:

$$EI_{24}(\%) = \frac{\alpha}{\beta} \times 100$$

where α and β are the heights of the emulsified layer and the total height, respectively.

2.4. Determination of surface tension

In order to assess the surface activities of biosurfactant produced by strain 139SI, the surface tension (ST) was measured