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Isolation and Identification of Native Bacteria from Total Petroleum Hydrocarbon Polluted Soil in Wonocolo Public Oilfields, Indonesia

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ABSTRACT

The presented study concerns on isolation and identification of indigenous bacteria in total petroleum hydrocarbon (TPH) polluted soil.. The composite TPH polluted soil was collected from Wonocolo public oilfields, Indonesia.. Pour plate and plate count techniques were used to bacterial population analysis and enumeration, respectively. Two dominant bacterial colonies were isolated from $4.06x10^7$ CFU/g population in polluted soil, then morphologically and biochemically were characterized using Microbact Identification Kits (Microbact™ GNB12A and 12B). The identification of isolated bacteria was performed using Bergey's Manual of Determinative Bacteriology. The results showed that the strains of bacteria are *Bacillus sp.* and *B. cereus* with probability of 72.00 and 77.00%, respectively. These strains potentially acted as biosurfactant producers and hydrocarbon degraders. Thus, biostimulation could be implemented to reduce the TPH levels in polluted soil at Wonocolo public oilfields.

Keywords: *Bacillus sp.*, *B. cereus*, TPH polluted soil, Wonocolo public oilfields.

INTRODUCTION

The crude oil exploration activities in Wonocolo public oilfields caused soil and surface water contamination of total petroleum hydrocarbons, TPH (Handrianto et al., 2012; Sari et al., 2018). Our previous study reported that topsoil in Wonocolo public oilfields was highly polluted by TPH in a range of 52,328.14–107,189.63 µg/g (Sari et al., 2018). TPH constitute hydrophobic compound that are toxic, mutagenic, and carcinogenic. The accumulation of TPH in soil may cause plant, animal, and human tissues to be damaged as they transport in biomagnification chain (Xu and Liu, 2010; Kumari et al., 2013). Therefore, the topsoil at Wonocolo public oilfields need bioremediation to avoid the impact on the people and environment in the local area (Sari et al., 2018). Bioremediation is an effective method to reduce the TPH level in polluted soil with feasibility cost. Several studies reported that the bioremediation effectiveness for TPH degradation reaches up to 80% (Handrianto et al., 2012; Xu and Liu, 2010; Asquith et al., 2012; Darmayanti et al., 2017).

Bioremediation, biostimulation and/or bioaugmentation, is defined as the process that utilizes the metabolic capabilities of microorganisms for TPH uptake and degradation into less toxic substance, or their removal from polluted soil. Biostimulation and bioaugmentation aim at increasing the level of TPH biodegradation by microorganisms. The difference of both techniques is bacteria used, where biostimulation and bioaugmentation use indigenous and specific competent exogenous bacteria introduced to the process, respectively (Xu and Liu, 2010; Asquith et al., 2012). Both bacteria were stimulated through environmental factors (nutrient, oxygen, moisture, pH, and temperature) monitoring

to enhance the effectivity of TPH degradation (Xu and Liu, 2010; Das and Chandran, 2011).

The viability of bacteria has been found to be useful in both bioremediation process. This study aimed at isolating and identifying the bacteria in polluted soil from Wonocolo public oilfields. It was necessary to determine the required technique for better TPH biodegradation performance. In addition, the potential role of bacteria in bioremediation process was reviewed.

MATERIALS AND METHODS

TPH polluted soil collection

The TPH polluted soil was taken in April 2017 from 0 to 30 cm below the surface of three contaminated sites at Wonocolo public crude oilfields. The description of the sites, soil sampling procedures, and the preparation were reported in our previous study (Sari et al., 2018).

MICROBIAL ANALYSES

Determination of bacterial population

Pour plate and plate count methods were used for bacteria population analysis and enumeration. One gram of soil was mixed with 50 mL of NaCl (0.8%). 1 mL of the mixture was diluted from 10^{-1} to 10^{-10} or more. 1 mL of dilution liquid was subculture into nutrient agar (NA) and incubated for 24 hours at a room temperature. After 24 hours of incubation, bacteria colonies forms were counted (Cappucino and Sherman, 1983).

Isolation, characterization, and identification of bacterial strain

The dominant bacteria colonies were subcultured on the NA using a streak method following Olukunle (2013). Morphological of selected colony such as color, shape, edge, and size were observed using microscope. The selected colony was picked for cellular morphological characterization through Gram staining analysis (Balows, 1992). Meanwhile, the biochemical characteristics of selected colony were performed using Microbact Identification Kits (Microbact™ GNB12A and 12B). Furthermore, individual bacterial strain was identified according to Bergey's Manual of Determinative Bacteriology (Holt et al., 1994).

RESULTS AND DISCUSSION

Total population and characterization of bacteria isolated from TPH polluted soil

The population of bacteria in TPH polluted soil amounted to 4.06×10^7 CFU/g with two dominant colonies. This indicates that bacteria are able to adapt with the presence of TPH in the polluted soil (Sari et al., 2016). The morphological characteristics of the colonies are presented in Table 1, and classified as gram-positive bacteria with basic forms of bacilli (Fig. 1).

The biochemical characteristics of bacteria (Table 2) show that colony 1 exhibited motility (using flagella to move). Furthermore, it also produces many enzymes such as oxydase, catalase, nitrate reductase, gelatinase, and voges proskauer. Barely same characteristics showed by Colony 2 are motility and enzyme producers (oxydase, amylase, catalase, nitrate reductase, and voges proskauer). The capabilities show that colony 1 and 2 may play a role in organic matter decomposition. However, both colonies are unable to ferment lactose and sucrose into glucose.

Identification of bacterial strain

On the basis of morphological and biochemical characteristics, two isolate colonies may belong to Bacillus genera. Furthermore, the prediction was confirmed that Bergey's Manual of Determinative Bacteriology which identified colony 1 and 2 belong to *Bacillus* sp. (72.00%), and *B. cereus* (77.00%) strains, respectively. Prakash et al. (2014) confirmed that *Bacillus* sp.

Table 1. Morphological characteristics of bacteria isolated

Bacteria	Morphological characteristics				
	Color	Shape	Edge	Size (cm)	Gram Strain
Colony 1	White	Irregular	Undulate	0.30	+
Colony 2	White	Circular	Filiform	0.20	+

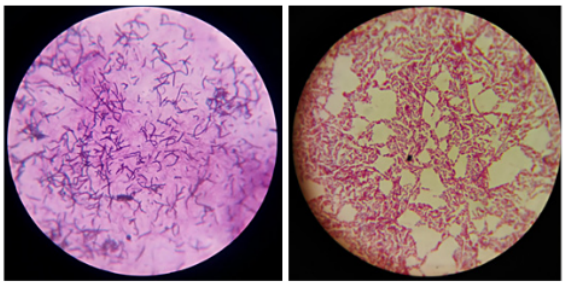

Figure 1. Gram strain colony 1 (left); colony 2 (right)

is one of indigenous bacteria in crude oil contaminated soil. Meanwhile, *B. cereus* is indigenous bacteria in diesel contaminated soil (Bento et al., 2005). Both conditions are in accordance to Wonocolo public oilfields that exploit crude oil and produce heavy diesel.

The presence of both indigenous strains in TPH polluted soil indicates that the bacteria might utilize the hydrocarbon as carbon and energy source to grow (Das and Chandran, 2011). Therefore, the indigenous bacteria in polluted soil could be responsible for TPH removal in bioremediation process (Kumari et al., 2013; Darmayanti et al., 2017; Balows, 1992). Olukunle (2013) stated that the capability of indigenous bacteria showed a possibility of in situ bioremediation which is an economical technique.

Role of *Bacillus* sp. and *B. cereus* in Bioremediation of TPH-Polluted Soil

Several researchers reported that *Bacillus* sp. and *B. cereus* are capable of acting as hydrocarbon degraders (Das and Chandran, 2011; Prakash et al., 2014; Cerqueira et al., 2011). *Bacillus* sp. and *B. cereus* can reduce the concentration of benzene up to 54.80% (Prakash et al., 2014) and 92.59–95.37% (Ole, 2017), respectively. It is due to *Bacillus* strain being able to produce surface active compounds, also known as biosurfactants, which could reduce surface tension and emulsify hydrocarbon (Cerqueira et al., 2011; Yangejeh et al., 2017).

Several studies reported that the surface tension of *Bacillus* sp. and *B. cereus* are lower than distilled water (73.05 mN/m) of 24.60 (Zhou et al., 2015) and 25.00 mN/m (Tuleva et al., 2005), respectively. Simultaneously, it facilitates hydrocarbon attack and diffusion into bacterial cells (Yangejeh et al., 2017) through affinity improvement in the change of the cell surface hydrophobicity (Kumar et al., 2007). The ability review of *Bacillus* sp. and *B. cereus* is presented in Table 3.

CONCLUSIONS

The current study confirms that *Bacillus* sp. and *B. cereus* are dominant indigenous bacteria at Wonocolo public oilfields polluted soil. These bacteria are hydrocarbon degraders and are able to produce biosurfactants. We recommend biostimulation such as composting and landfarming to be used as bioremediation techniques for TPH polluted soil at the site.

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Table 2. Biochemical characteristics of bacteria isolated

Bacteria	Biochemical characteristics														
	Motility	Oxidase	Nitrate reduction	Amylase	Catalase	H_2S production	Indole production	Gelatinase	Voges proskauer	Urease	Glucose	Mannitol	Xylose	Sucrose	Lactose
Colony 1	+	+	+	-	+	-	-	+	+	-	-	-	-	-	-
Colony 2	+	+	+	+	+	-	-	-	+	-	-	-	-	-	-

Table 3. The ability of *Bacillus* sp., and *B. cereus*

Bacteria	Source of Oil-Contaminated Soil	Degradation of Hydrocarbon	Biosurfactant	Reference
Bacillus sp.	Meerut Region	54.80% of benzene	-	Prakash et al., 2014
	Guanoco Lake, Sucre State	67.00±3.00 up to 69.00±4.00 of Phenantherene	Surface tension up to 40 mN/m	Kumar et al., 2007
	Oilfield in northwest China	-	Lipopeptide, surface tension of 24.60 mN/m	Zhou et al., 2015
	Automobile workshop in Manipal, India	-	Lipopeptide, surface tension reduction up to 36.10 mN/m	Varadavenkatesan and Murty, 2012
	Not explained	-	Lipopeptide, surface tension up to 27.80 mN/m	Hesty and Putra, 2017
B. cereus	Hongkong, China	-	Surface tension of 50.00 mN/m	Bento et al., 2005
	Petrochemical industry in the city of Triunfo, Rio Grande do Sul State, Brazil	• 88.40±0.60% of aliphatic hydrocarbon • 40.30±6.70% of aromatic hydrocarbon	• Surface tension of 41.40±0.30 mN/m • Emulsifying activity of 33.30±0.10%	Cerqueira et al., 2011
	Artificial	92.59–95.37% of BTX	-	Ole, 2017
	Not explained	72.00±4.00% of Naphthalene	Rhamnolipid, surface tension of 25.00 mN/m	Tuleva et al., 2005
	Cepu, East Java, Indonesia	54.00% of TPH	-	Darmayanti et al., 2017

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PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5