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Potential microbial consortium involved in the biodegradation of diesel, hexadecane and phenanthrene in mangrove sediment explored by metagenomics analysis

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ABSTRACT

Hydrocarbon contamination is a serious problem that degrades the quality of mangrove ecosystems, and bioremediation using autochthonous bacteria is a promising technology to recover an impacted environment. This research investigates the biodegradation rates of diesel, hexadecane and phenanthrene, by conducting a microcosm study and survey of the autochthonous microbial community in contaminated mangrove sediment, using an Illumina MiSeq platform. The biodegradation rates of diesel, hexadecane and phenanthrene were 82, 86 and 8 mg kg^{-1} sediment day^{-1}, respectively. The removal efficiencies of hexadecane and phenanthrene were > 99%, whereas the removal efficiency of diesel was 88%. A 16S rRNA gene amplicon sequence analysis revealed that the major bacterial assemblages detected were *Gammaproteobacteria*, *Deltaproteobacteria*, *Alphaproteobacteria*. The bacterial compositions were relatively constant, while reductions of the supplemented hydrocarbons were observed. The results imply that the autochthonous microorganisms in the mangrove sediment were responsible for the degradation of the respective hydrocarbons. Diesel-, hexadecane- and phenanthrene-degrading bacteria, namely *Bacillus* sp., *Pseudomonas* sp., *Acinetobacter* sp. and *Staphylococcus* sp., were also isolated from the mangrove sediment. The mangrove sediment provides a potential resource of effective hydrocarbon-degrading bacteria that can be used as an inoculum or further developed as a ready-to-use microbial consortium for the purpose of bioremediation.

1. Introduction

A mangrove ecosystem is a transitional area, connecting marine, freshwater and terrestrial environments. This ecosystem provides a variety of natural services, including the reduction of coastal erosion, nursery habitat for aquatic organisms, and the degradation of contaminants (Alongi, 2002; Duke et al., 2007). A mangrove ecosystem is a unique habitat because it is impacted by salinity, organic matter, and tidal variation, leading to a high diversity of living organisms. Mangrove ecosystems are thus considered biodiversity hotspots for microorganisms (Thatoi et al., 2013). Microorganisms play key roles in biogeochemical cycles and maintaining ecosystem health. One of the common threats to mangrove ecosystem is petroleum hydrocarbon contamination (Andreote et al., 2012; Brito et al., 2009; dos Santos et al., 2011). High levels of petroleum hydrocarbons and their derivatives directly affect living organisms in a contaminated environment. The toxicity of petroleum hydrocarbon can be either acute or chronic. Acute toxicity is generally associated with low molecular weight alkanes and aromatic compounds, whereas chronic toxicity mostly involves polycyclic aromatic hydrocarbons (PAHs) (Mukherjee and Chattopadhyay, 2017; NOAA, 2002).

Bioremediation is considered an attractive alternative approach for removing petroleum hydrocarbon from a contaminated area because it is environmental friendly and cost effective.

Bioremediation mainly relies on the ability of microorganisms to degrade toxic compounds. Consequently, the microbial community

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plays a key role in not only directly degrading contaminants, but also interacting with microbial species (Bento et al., 2005; Fuentes et al., 2016). The biodegradation of hydrocarbons has been investigated in hydrocarbon-contaminated environments, such as in soil (Sun et al., 2015; Sutton et al., 2013), the ocean (Sauret et al., 2014; Woolfenden et al., 2011) and mangroves (dos Santos et al., 2011; Jiang et al., 2013; Muangchinda et al., 2013). Although it has been reported that autochthonous bacteria in mangrove sediments were able to degrade petroleum hydrocarbons, including PAHs (Brito et al., 2009; Li et al., 2009; Tian et al., 2008), information on the degradation rates of hydrocarbon by autochthonous bacteria in mangrove sediment is still needed. Knowledge of biodegradation rates and removal efficiencies is required for the further development of sustainable bioremediation technology.

Culture dependent techniques have been used to study the capability of microorganisms in degrading hydrocarbon compounds. Hydrocarbon-degrading bacteria such as *Rhodococcus* sp., *Acinetobacter* sp., *Pseudomonas* sp., *Alcanivorax* sp., and *Sphingomonas* sp. have been isolated from contaminated mangrove sediments (Brito et al., 2006; Rocha et al., 2013; Yu et al., 2005). Recently, high throughput sequencing technologies (i.e., Illumina MiSeq, Illumina Hiseq and 454 GS FLX) have been used to explore in-depth microbial communities in contaminated environments, in soil (Abbasian et al., 2016; Sutton et al., 2013), arctic soil (Tan et al., 2013; Yergeau et al., 2012), marine sediment (Mason et al., 2014), and mangrove sediment (Andreote et al., 2012; dos Santos et al., 2011), for example. Although a mangrove ecosystem provides an important function and harbors high microbial diversity, a comprehensive dataset of microbial communities in the mangrove environment is limited. A microbial consortium in mangrove sediment collected from an intertidal mangrove wetland showed distribution into the inner and outer mangrove groups (Jiang et al., 2013). Illumina sequencing of the 16S rRNA gene showed that *Actinobacteria*, *Acidobacteria*, *Nitrospirae*, and *Verrucomicrobia* were dominant in the nutrient-rich inner mangrove sediment, whereas *Proteobacteria* and *Deferribacterias* were highly represented in the outer mangrove sediment (Jiang et al., 2013). The classes *Gammaproteobacteria* and *Deltaproteobacteria* were dominant in uncontaminated mangrove sediment from Restinga da Marambaia, Rio De Janeiro, Brazil; however, the orders *Oceanospirillales* and *Alteromonadales*, belonging to the class *Gammaproteobacteria*, significantly increased in hydrocarbon-contaminated mangrove sediment (dos Santos et al., 2011). The members of *Oceanospirillales* and *Alteromonadales* are known as hydrocarbon-degrading bacteria (Dombrowski et al., 2016).

The mangrove sediment analyzed in this study was likely exposed to chronic contamination, especially diesel, since the sampling location is located at a local petroleum station in Bangkhuntien, Bangkok, Thailand. Diesel has been extensively used for fueling boats at the sampling station. Diesel is mainly composed of saturated (i.e., *n*-alkenes and cyclo-alkanes) and aromatic (i.e., PAHs) hydrocarbons (Wu et al., 2016). Hexadecane and phenanthrene are frequently used as representatives *n*-alkene and PAH, respectively, in microcosm studies to elucidate the microbial response to hydrocarbon contamination (Sauret et al., 2014; Schurig et al., 2014; Schwarz et al., 2017; Towell et al., 2011). The main objectives of this study are to investigate the biodegradation rates of diesel, hexadecane and phenanthrene by an autochthonous bacterial community and to explore the in-depth microbial composition of the mangrove sediment supplemented with diesel, hexadecane and phenanthrene, using an Illumina MiSeq platform targeting the 16S rRNA gene. Diesel, hexadecane and phenanthrene-degrading bacteria were also isolated from the mangrove sediment. The knowledge gained from this study can be used to indicate whether bioremediation using autochthonous bacterial consortium is a promising strategy to remediate hydrocarbon-contaminated mangrove environments.

2. Materials and methods

2.1. Sampling site description and sample collection

The sampling station is a petroleum station located along the Sahakorn Canal in Bangkhuntien, Bangkok, Thailand (13°32′22.2″N 100°26′36.5″E). The canal is connected to the Gulf of Thailand. Consequently, seawater intrusion directly affects water chemistry in this area. There were only two petroleum stations along the canal in a length of area that spanned approximately 35 km. The locals mainly use water transportation. During the sample collection period of 4 h, an average of two boats an hour stopped by for fueling at the sampling station (Fig. S1). Oil film floating on the surface water around and nearby the sampling station was observed (Fig. S1). Consequently, the sediment samples were collected from that area and were likely impacted by chronic hydrocarbon contamination. The sediment samples were collected in March of 2016. They were randomly collected from five locations around the sampling station. The sediment samples were pooled on site and kept in a sterile bottle. Conductivity, pH, temperature, and dissolved oxygen (DO) were measured on site, using a portable pH meter (Hach Company, USA). Salinity was analyzed using a refractometer. Total phosphorus (TP), total nitrogen (TN), and total carbon (TC) were respectively analyzed by the ascorbic acid method, macro-Kjeldahl method, and a total organic carbon analyzer (APHA, 2012). The chemistry of the water is shown in Table 1.

2.2. Quantification of total bacteria and total diesel-, hexadecane-, and phenanthrene-degrading bacteria

Briefly, 1 g of the sediment sample was diluted with 9 ml of a Bushnell Haas mineral salt (BHMS) broth with the addition of 10 μL of nystatin. Then 20 μL of diluted sediment samples were transferred to a 96-well plate added with 180 μL of sterile Zobell Marine Broth 2216 (Himedia, India). The 96-well plate was incubated at room temperature for three to five days. Bacterial growth was measured by a victor ×3 2030 multilabel reader (PerkinElmer, USA) at a wavelength of 540 nm (Johnsen et al., 2002). The number of total bacteria was estimated using the most probable number method (MPN) method (Haines et al., 1996).

The numbers of total diesel-, hexadecane-, and phenanthrene-degrading bacteria were also measured using the MPN method. As with the quantification of total bacteria, the sediment samples were diluted with BHMS broth and were subsequently transferred to the 96-well plate added with 180 μL of sterile Zobell Marine Broth 2216 (Himedia, India). Then 2 μl of diesel, hexadecane, and phenanthrene in a stock solution (1000 mg l^{-1} hexane) were separately added to the 96-well plate. Seven serial ten-fold dilutions were performed for each sample and each diluted sample was conducted in triplicate. After the samples were incubated for one to two weeks, 50 μL of filtered sterile iodonitrotetrazolium chloride (INT dye) was added into each well. Then the samples were shaken for 30 min. Bacterial hydrocarbon degrading activity was measured by the victor ×3 2030 multilabel reader (PerkinElmer, USA) at the wavelength of 450 nm (Johnsen et al., 2002).

Table 1
Water chemistry at the sampling station.

Water chemistry	
Salinity (mg l^{-1})	33.00
Total N (mg l^{-1})	0.80
Total P (mg l^{-1})	1.18
Total C (mg $^{-1}$)	36.18
pH	7.33
Conductivity (mS cm^{-1})	46.60
DO (mg l^{-1})	6.38
Temperature (°C)	33.00