Journal of Hazardous Materials 357 (2018) 119–127

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

16S metagenomic analysis reveals adaptability of a mixed-PAH-degrading consortium isolated from crude oil-contaminated seawater to changing environmental conditions

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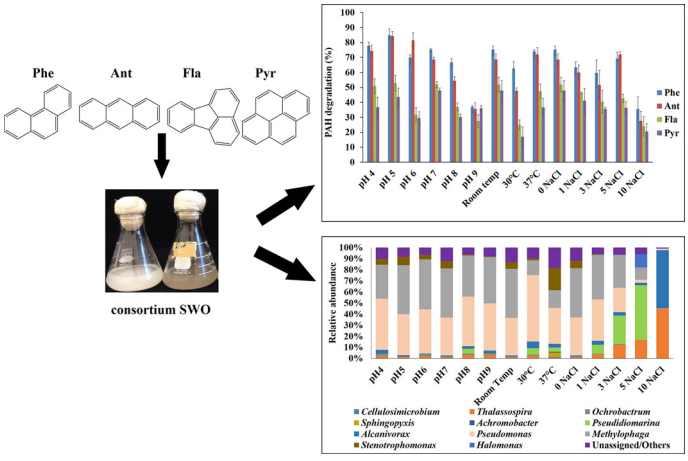
GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Oil spill
Polycyclic aromatic hydrocarbons
Biodegradation
Bacterial consortium
Next generation sequencing (NGS)

ABSTRACT

A bacterial consortium, named SWO, was enriched from crude oil-contaminated seawater from Phrao Bay in Rayong Province, Thailand, after a large oil spill in 2013. The bacterial consortium degraded a polycyclic aromatic hydrocarbon (PAH) mixture consisting of phenanthrene, anthracene, fluoranthene, and pyrene (50 mg L^{-1} each) by approximately 73%, 69%, 52%, and 48%, respectively, within 21 days. This consortium exhibited excellent adaptation to a wide range of environmental conditions. It could degrade a mixture of four PAHs under a range of pH values (4.0–9.0), temperatures (25 °C–37 °C), and salinities (0–10 g L^{-1} with NaCl). In addition, this consortium degraded 20–30% of benzo[*a*]pyrene and perylene (10 mg L^{-1} each), high molecular weight PAHs, in the presence of other PAHs within 35 days, and degraded 40% of 2% (v/v) crude oil within 20 days. The 16S rRNA gene amplicon sequencing analysis demonstrated that *Pseudomonas* and *Methylophaga* were the dominant genera of consortium SWO in almost all treatments, while *Pseudidiomarina*, *Thalassospira* and

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https://doi.org/10.1016/j.jhazmat.2018.05.062
Received 20 October 2017; Received in revised form 10 May 2018; Accepted 29 May 2018
Available online 29 May 2018
0304-3894/

Alcanivorax were predominant under higher salt concentrations. Moreover, *Pseudomonas* and *Alcanivorax* were dominant in the crude oil-degradation treatment. Our results suggest that the consortium SWO maintained its biodegradation ability by altering the bacterial community profile upon encountering changes in the environmental conditions.

1. Introduction

Oil spills in marine environments have become a serious problem throughout the world. They result in extensive damage to ecosystems and to the economy [1,2]. In July 2013, a serious crude oil spill occurred in Rayong Province, Thailand. Approximately 50,000 liters of crude oil were released into the sea and reached Phrao Bay (Ao Phrao), Samet Island. This oil spill disaster affected tourism and marine organisms around the region. In fact, the Pollution Control Department (Thailand) reported that petroleum hydrocarbons in seawater around Samet Island 1–3 months after the accident ranged from < 0.05-513.0 μg L^{-1} [3].

Crude oil is a complex mixture comprising saturated hydrocarbons, aromatic hydrocarbons, resins, and asphaltenes [4]. Polycyclic aromatic hydrocarbons (PAHs) are one of the crude oil components that are considered hazardous due to their toxic, mutagenic, and carcinogenic properties [5]. PAHs can be divided into two classes: low molecular weight (LMW) PAHs with two or three aromatic rings (naphthalene, acenaphthene, acenaphthylene, fluorene, anthracene, and phenanthrene) and high molecular weight (HMW) PAHs with four or more aromatic rings (fluoranthene, pyrene, benzo[*a*]pyrene, and perylene). Compared to LMW PAHs, the HMW PAHs are difficult to degrade and highly persistent in the environment [6]. The concentrations of PAHs detected in water and sediments from Thailand have been summarized by Wattayakorn et al. [7]. The total PAHs concentrations ranged from 0.003 to 10.7 μg g^{-1} dry weight, and the dominant PAHs were naphthalene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, chrysene, benzo[*a*]anthracene, and perylene.

Although PAHs in the environment can be removed by many processes, such as photooxidation, chemical oxidation, volatilization, bioaccumulation, and adsorption, microbial biodegradation is an effective and environmentally friendly process for the elimination of PAHs [8]. Nevertheless, PAH biodegradation efficiency is dependent on environmental conditions such as pH, temperature, and salinity [9]. These factors can affect microbial activity and PAH bioavailability [10]. Moreover, contamination of the environment by PAHs generally consists of multiple PAHs. Therefore, it is important to isolate microorganisms that have a stable degradation ability for a mixture of PAHs and that can survive in a wide range of environmental conditions.

Recently, the use of bacterial consortia has been suggested to improve biodegradation efficiency because the cooperative metabolic activities of several bacteria may enhance PAH utilization [11,12]. However, changes of bacterial populations in consortia during biodegradation processes are not well studied [13]. An understanding of the bacterial dynamics is a crucial aspect of the monitoring process for the success of bioremediation. Next-generation sequencing (NGS) of 16S rRNA genes has been increasingly used to determine complex bacterial diversity in numerous studies [14–16]. This technique can provide novel, accurate and reliable information about the taxonomic identities of microbes by high-throughput data generation [17,18].

Therefore, the purposes of this study were to obtain a bacterial consortium that exhibits high efficacy in the degradation of a mixture of LMW and HMW PAHs under different environmental conditions and to provide an understanding of bacterial community dynamics in the bacterial consortium during biodegradation, by high-throughput sequencing.

2. Materials and methods

2.1. Sampling and enrichment of a PAH-degrading bacterial consortium

Surface seawater was collected from Phrao Bay in Rayong Province, Thailand (12°34′12.2″N 101°26′57.7″E), which was the major site affected by the 2013 oil spill. The sampling was done approximately two weeks after oil spill. Total petroleum hydrocarbon (TPH) in the sample was quantified by an oil content analyzer (Horiba, Japan), and the concentration of TPH in seawater was 4433.33 ± 1401.19 μg L^{-1}.

Seawater sample (100 mL) was further enriched with Arabian light crude oil 0.5% (v/v) and then incubated at 200 rpm at room temperature for 10 days. The suspension (10 mL) was transferred to 40 mL Nutrient Seawater medium (NSM) with crude oil 0.5% (v/v). NSM contained 1 g of NH_4NO_3, 0.02 g of K_2HPO_4, 0.02 g of $C_6H_5FeO_7$, and 0.5 g of yeast extract, dissolved in 200 mL of filtered seawater and 800 mL of distilled water. After 10 days, 5 mL of suspension was transferred to 45 mL fresh medium supplemented with a mixture of four PAHs, namely, phenanthrene, anthracene, fluoranthene, and pyrene, at final concentrations of 50 mg L^{-1} each, and incubated as described above. After 15 transfers, a mixed PAH-degrading bacterial consortium was obtained and was designated consortium SWO. The consortium was stored at 4 °C for further experiments and stored in 50% glycerol at −80 °C for long-term preservation.

2.2. Biodegradation experiment

PAH degradation by consortium SWO was determined as follows: Five hundred microliters of inoculum were added to 4.5 mL of NSM containing a mixture of phenanthrene, anthracene, fluoranthene, and pyrene at 50 mg L^{-1} each to obtain an initial cell concentration of 10^5 CFU mL^{-1}. The cultures were shaken at 200 rpm at room temperature. Three replicate tubes were collected at days 0, 7, 14, and 21 for residual PAH analysis by a gas chromatograph equipped with a flame ionization detector (GC-FID) as described in a previous study [19], and another three replicate tubes were collected for DNA extraction. Uninoculated tubes containing only PAH-supplemented NSM served as the control. To calculate the PAH-degradation rate constant, pseudo-first-order kinetics were determined.

2.3. Isolation of pure cultures from consortium SWO and their PAH biodegradation potential

Serial dilutions of 10^{-1}–10^{-6} were prepared from consortium SWO. Bacterial suspensions in respective 10^{-5} and 10^{-6} dilutions were spread on Luria-Bertani (LB) agar plates to obtain the bacterial colonies between 30 to 300 colonies. Colonies with different morphologies were selected, purified, and tested for their ability to utilize PAHs. Isolated bacteria were identified according to their 16S rRNA gene sequences.

The utilization of PAHs by the isolated bacterial strains was performed in 5 mL of NSM supplemented with the individual PAHs (phenanthrene, anthracene, fluoranthene or pyrene) at final concentrations of 50 mg L^{-1}. The cultures were shaken at 200 rpm at room temperature for 21 days. Samples containing PAH-supplemented NSM without bacterial inoculation were set up as the controls. The residual PAH in the culture was quantified using HPLC as described by Klankeo et al. [20].