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Characterization of a phenanthrene-degrading microbial consortium enriched from petrochemical contaminated environment

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ABSTRACT

In this study, a novel aerobic microbial consortium for the complete degradation of phenanthrene was enriched from petrochemical contaminated soil in Puyang City, Henan Province, China. The consortium, named as ZY-PHE, could degrade more than 96% of 200 mg/L phenanthrene in a minimal salt medium in 3 days. Pyrosequencing analysis of the bacterial 16S rRNA gene suggested that *Proteobacteria* was the dominant phyla of ZY-PHE consortium representing 56.7% of the total number of detected sequences followed by *Actinobacteria* (21.7%), *Deinococcus-Thermus* (14.5%), *Bacteroidetes* (4.3%) and *Firmicutes* (0.8%). Several abundant genera, *Truepera* (14.47%), *Starkeya* (1.35%), *Nitratireductor* (1.35%), *Legionella* (0.71%), *Aeromicrobium* (0.47%), *Phyllobacterium* (0.38%) and *Cellulosimicrobium* (0.31%), have firstly been detected in PAHs contaminated environment. The consortium has the ability to degrade other PAHs such as pyrene and fluorene by utilizing them as sole carbon and energy source. Further, consortium ZY-PHE exhibited an excellent pH adaptation and extensive temperature flexibility, indicating this consortium could function well under a wide range of environmental conditions. Liquid chromatography −quadrupole-time-of-flight mass spectrometry assay revealed accumulation of few intermediate metabolites involved in phenanthrene degradation. The use of this bacterial consortium may be an attractive alternative for the bioremediation of polycyclic aromatic hydrocarbons in the environment.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a class of toxic environmental pollutants consisting of two or more fused benzene rings in various arrangements. Some PAHs have been classified by the US Environmental Protection Agency (USEPA) as priority pollutants owing to their known or suspected carcinogenicity, teratogenicity or acute toxicity (Kim et al., 2013). PAHs are released into the environment from both natural and anthropogenic processes, including forest fire, volcanic eruption, incomplete burning of fuels (e.g. coal, petroleum, wood) or garbage, oil spills, industrial discharge and petrochemical/oil refining industries (Zhang and Tao, 2009). The physicochemical properties of PAHs make them distribute widely across air, soil, and water bodies, which are usually difficult to be decomposed. Because of the ubiquitous presence of PAHs in the environment and the health risk associated with their exposure, they have been extensively studied in various environmental and biological compartments and gain great concern all over the world (Desalme et al., 2013; Kim et al., 2013).

Bioaugmentation is an environmental sustainable, cost-effective pollutant treating technique, involves the external microbial strains which have the ability to degrade PAHs (Li et al., 2009). A lot of microbial species have been enriched and isolated as efficient PAHs degrader, including bacteria, fungi, yeast and algae (Peng et al., 2008; Haritash and Kaushik, 2009; Fernandez-Luqueno et al., 2011). Much more efficient inoculums were microbial consortiums which contained different PAHs degraders (Li et al., 2009; Moscoso et al., 2012; Bacosa and Inoue, 2015; Chaudhary et al., 2015). Because in nature, bioremediation depends on cooperative metabolic activities of mixed microbial populations, while a pure strain does not function well in natural PAH-contaminated soils. Hernando et al. enriched several microbial consortia able to degrade phenanthrene and/or pyrene with a high rate (Bacosa and Inoue, 2015). Y.R. Luo et al. constructed a microbial consortium capable of degrading 44.07% of benzo(a)pyrene in 14 days (Luo et al., 2009). Generally, the synergistic interactions among members of the microbial populations could be benefit for PAHs degradation, since

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metabolic intermediates (that may inhibit microbial activities) produced by one group of microorganisms may serve as substrates for the growth of others (Ghazali et al., 2004). However, the accurate PAHs-degrading mechanism by a mix bacterial culture is still unknown.

There are two contrary approaches used to construct optimal microbial consortia for biodegradation, “top down” and “bottom up”. For a top-down approach, many diverse degraders with different degradation characteristics isolated from environmental samples are mixed and used as the inoculums to degrade PAHs. In this strategy, each strain used has been isolated and analyzed. During the consortium development, several strains adapting to PAHs degradation remain while others disappear(Rawlings and Johnson, 2007). Using this approach, many researchers have established efficient and robust cultures for PAHs degradation (Luo et al., 2009; Kumar et al., 2014). However, the metabolic diversity and consortium stability of the artificial bacterial community is dependent on the capacity of the bacterial degraders isolated and purified from the environment. In contrast, the “bottom up” approach is to enrich a highly efficient and stable consortium for bioremediation, which possesses enough bacterial diversity and metabolic plasticity. Because of high taxonomic and functional diversity, cultures prepared on the basis of the bottom-up approach were more efficient (Janbandhu and Fulekar, 2011; Wang et al., 2014; Chaudhary et al., 2015).

At present most studies of the microbial mediated PAH degradation have been confined to pure bacteria isolated from contaminated sites. The investigations of the microbial community that degrade organic contaminants will be helpful to clarify the mechanism of synergistic interactions among different bacteria in this process. The present study therefore aimed to construct a phenanthrene-degrading consortium as a further bioremediation resource, undercover the community structure and test its capability to degrade phenanthrene under different environmental conditions.

2. Material and methods

2.1. Chemicals and medium

Naphthalene (99%), phenanthrene(98%), fluorene(98%), fluoranthene (99%) and benzo[a]pyrene (96%) were purchased from Sigma-Aldrich (St. Louis, MO,USA). All other chemicals used in this study were of analytical grade. The minimal salt medium (MSM) contained (per liter) 8.5 g $Na_2HPO_4 \cdot 2H_2O$, 3.0 g KH_2PO_4, 0.5 g NaCl, 1.0 g NH_4Cl, 0.5 g $MgSO_4 \cdot 7H_2O$, 14.7 mg $CaCl_2$, 0.4 mg $CuSO_4$, 1.0 mg KI, 4.0 mg $MnSO_4 \cdot H_2O$, 4.0 mg $ZnSO_4 \cdot 7H_2O$, 0.5 mg H_3BO_3, 1.6 mg H_2MoO_4, 2.0 mg $FeCl_3 \cdot 6H_2O$, and solution pH was adjusted to 7.0–7.5.

2.2. Sampling and enrichment

Sampling was performed in September 2012. Three top layer (0–10 cm) soil samples were randomly collected from the sewage of Puyang refinery at Puyang city, Henan Province, China. The samples were mixed thoroughly as a composite sample, and then sieved through a 2 mm pore size sieve, stored at 4 °C in sterilized seal pack polythene bag for further assay.

Ten gram of mixed soil sample was added into 90 ml of MSM medium (containing 200 mg/L phenanthrene). After 7 days incubation at 28 °C, 180 rpm in the dark, 20 ml of the culture was transferred into 80 ml fresh MSM medium containing 200 mg/L phenanthrene for the second enrichment. After 10 consecutive transfers, the phenanthrene degradating microbial consortium was obtained and named as ZY-PHE consortium.

2.3. DNA extraction and community structure analysis

Total soil DNA was extracted from 0.5 g mixed soil using a FastDNA SPIN Kit for soil (MP Biomedicals, Irvine, CA). 50 ml of the ZY-PHE consortium culture was centrifuged to collect microbial cells and then community DNA was extracted using the same FastDNA SPIN Kit following the manufacturer's protocol. Triplicate DNA extraction was performed for both soil and consortium. Three soil DNA samples or consortium DNA samples were combined for further assays. The quantity and quality of DNA extracts were determined by NanoDrop ND-2000 UV–vis spectrophotometer (Thermo Scientific, Wilmington, USA). The extracted DNA was stored at −80 °C prior to further analyses.

To analyze the taxonomic composition of both original soil and ZY-PHE consortium bacterial community, the V4 region of 16S rRNA was amplified with the primer set 520f (5′- AYTGGGYDTAAAGNG-3′) and 802r (5′- TACNVGGGTATCTAATCC-3′) ((with Y = C/T, D = A/G/T, N = any base, V = A/C/G) (Claesson et al., 2009)Amplicon sequencing was conducted on an Illumina MiSeq platform at Personalbio Technology Co., Ltd., Shanghai, China. FLASH was used to merge reads pairs(Magoc and Salzberg, 2011). The reads were first filtered by QIIME quality filters. Default settings for Illumina processing in QIIME were used. Then operational taxonomic units (OTUs) were picked up at 97% similarity through UPARSE pipeline (Caporaso et al., 2010). For each OTU, a representative sequence was selected and used to assign taxonomic composition by using the RDP classifier(Wang et al., 2007).

2.4. PAHs degradation

The phenanthrene-degradation rate of the ZY-PHE consortium was determined in liquid culture in Erlenmeyer flasks containing 25 ml of MSM medium supplemented with 200 mg/L phenanthrene as a sole source of carbon and energy at 28 °C in a shaker (at 180 rpm) in dark. Briefly, 0.5 ml phenanthrene solution (1 g/L in acetone) was added into 100 ml Erlenmeyer flasks and allowed acetone to evaporate. Then, 24 ml of sterilized MSM medium and one milliliter consortium culture after 5-days of pre-incubation was added successively, to achieve a final concentration of phenanthrene at 200 mg/L. The same incubation conditions were used for the control culture, except that phenanthrene was not added. Each day, the culture flask was extracted three times with 25 ml hexane. Then the organic phase was combined and dehydrated with anhydrous sodium sulfate and then was concentrated to 10 ml.

1.0 μl of the organic phase was analyzed by gas chromatography (GC, Agilent 7890A, USA) with a capillary column (DB-5 model, 30 m long × 0.32 mm diameter × 0.25 mm thick). The GC condition were as follows: injector temperature was 280 °C; column temperature was ramped from 60 °C (5 min) to 180 °C at a rate of 3 °C/min, then programmed to 290 °C at a rate of 10 °C/min which was kept for 10 min; detector temperature was 300 °C. The carrier gas was nitrogen at a constant flow rate of 2 mL/min. The consortium microbial growth was monitored by measuring the absorbance at 600 nm.

Various factors were examined for their influences on the biodegradation of phenanthrene, including salinity, pH, temperature and different heavy metals. Microbial consortium culture was performed in accordance with the above. Various conditions are briefly described: incubation temperatures of 16, 28, 37 and 42 °C; medium pH of 4.0 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0 using 1 M HCl or 1 M NaOH; the salinity of 0, 1, 2, 3, 4, 5, 6 and 7 g/L with NaCl; 0.267 mM or 0.534 mM of $Cd(NO_3)_2$; 0.577 mM or 1.154 mM of K_2CrO_4. The ability of ZY-PHE consortium to degradate other PAHs as sole carbon, including pyrene, fluorene and fluoranthene, was checked. The phenanthrene concentration after 7-day incubation in various