Science of the Total Environment 616–617 (2018) 117–127

Contents lists available at ScienceDirect

Science of the Total Environment

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

Biodegradation of di-n-butyl phthalate (DBP) by a novel endophytic *Bacillus megaterium* strain YJB3

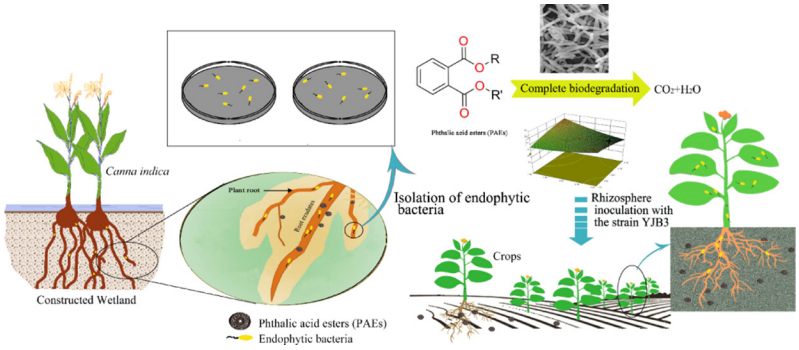
Nai-Xian Feng [a], Jiao Yu [a], Ce-Hui Mo [a,*], Hai-Ming Zhao [a], Yan-Wen Li [a], Bing-Xiao Wu [a], Quan-Ying Cai [a], Hui Li [a], Dong-Mei Zhou [a,b], Ming-Hung Wong [a,c]

[a] Guangdong Provincial Research Center for Environment Pollution Control and Remediation Materials, School of Environment, Jinan University, Guangzhou 510632, PR China
[b] Key Laboratory of Soil Environment and Pollution Remediation, Institute of Soil Science, Chinese Academy of Sciences, Nanjing 210008, PR China
[c] Consortium on Health, Environment, Education and Research (CHEER), Department of Science and Environmental Studies, The Education University of Hong Kong, Tai Po, Hong Kong, PR China

HIGHLIGHTS

- This is the first study on the biodegradation of PAEs using endophyte.
- A novel endophytic *Bacillus megaterium* YJB3 capable of degrading PAEs was isolated.
- PAEs catabolic genes in the stain YJB3 were excavated by whole genome analysis.
- The strain YJB3 is equipped with a complete degradation pathway of DBP.
- The strain YJB3 is an ideal candidate for *in situ* removal of PAEs in soil and crop.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 6 September 2017
Received in revised form 27 October 2017
Accepted 29 October 2017
Available online xxxx

Editor: Jay Gan

Keywords:
Endophyte
Di-n-butyl phthalate (DBP)
Bacillus megaterium
Whole genome sequencing
Degradation pathway

ABSTRACT

Phthalic acid esters (PAEs) are a group of recalcitrant and hazardous organic compounds that pose a great threat to both ecosystem and human beings. A novel endophytic strain YJB3 that could utilize a wide range of PAEs as the sole carbon and energy sources for cell growth was isolated from *Canna indica* root tissue. It was identified as *Bacillus megaterium* based on morphological characteristics and 16S rDNA sequence homology analysis. The degradation capability of the strain YJB3 was investigated by incubation in mineral salt medium containing di-n-butyl-phthalate (DBP), one of important PAEs under different environmental conditions, showing 82.5% of the DBP removal in 5 days of incubation under the optimum conditions (acetate 1.2 $g \cdot L^{-1}$, inocula 1.8%, and temperature 34.2 °C) achieved by two-step sequential optimization technologies. The DBP metabolites including monobutyl phthalate (MBP), phthalic acid (PA), protocatechuic acid (PCA), *etc.* were determined by GC–MS. The PCA catabolic genes responsible for the aromatic ring cleavage of PCA in the strain YJB3 were excavated by whole-genome sequencing. Thus, a degradation pathway of DBP by the strain YJB3 was proposed that MBP was formed, followed by PA, and then the intermediates were further utilized till complete degradation. To our knowledge, this is the first study to show the biodegradation of PAEs using endophyte. The results in the present study suggest that the strain YJB3 is greatly promising to act as a competent inoculum in removal of PAEs in both soils and crops.

* Corresponding author.
E-mail address: tchmo@jnu.edu.cn (C.-H. Mo).

https://doi.org/10.1016/j.scitotenv.2017.10.298
0048-9697/

1. Introduction

DBP is one of important phthalate acid esters (PAEs) that are primarily used to improve the flexibility and workability of the products, such as polyvinyl chloride resins, lubricants, plastic packing films, adhesives, cosmetics, cellulose materials, and insecticides (Gao and Wen, 2016). PAEs are widely investigated as hazardous organic pollutants and their occurrence in soils can be of great threat to human beings and wildlife by different paths. Some of them are suspected to be mutagens, hepatotoxic agents, and carcinogens. As environmental hormone, PAEs can affect reproduction, impair development, and induce genetic aberrations in humans even at low concentrations, thereby resulting in an increasing environmental concerns (Heudorf et al., 2007). Like other PAEs, DBP is not chemically bonded to the plastic polymer and can be easily released into the environment during the manufacture, use, and disposal of plastics, leading to ubiquitous occurrence in various environmental matrices (Net et al., 2015). Thus, DBP has been listed as one of the priority pollutants by the US Environmental Protection Agency (US EPA, 2013).

PAEs in soils are dominated by DBP, as well as di-2-ethylhexyl phthalate (DEHP), which originate mainly from wide use of agricultural plastic film in facility agricultures (Li et al., 2016, Wang et al., 2015). Moreover, various fertilizers are supposed to be another main source of PAEs for contamination in agricultural soils, and the application of fertilizers contained PAEs can lead to accumulation of PAEs in both soils and crops (Mo et al., 2008). Rhizospheric bacteria can survival in rhizosphere and are more versatile for bioremediation of organic pollutants contaminated soils. However, phytoremediation is often insufficient because plants do not completely degrade these compounds through their rhizospheric microorganisms. Mobility of PAEs in the soil-plant system make themselves enter agricultural crops easily (Li et al., 2016; Zhao et al., 2015), posing a potential risk for human health owning to direct and indirect human exposure *via* the food chain (Guo et al., 2012; Mo et al., 2009). Therefore, it is necessary to find an effective way to minimize the risk of PAEs contamination in crops, ensuring the safety of agricultural products and human health.

Endophytic bacteria, a kind of microorganisms inhabit the interior of plant tissues, can be used to solve the problem mentioned above. The most important benefit of using endophytic bacteria is that toxic organic contaminants accumulated in plant tissues may be mineralized in *planta*. In particular, where use of molecular biology techniques is required to express specific pollutant-degrading genes in *planta*, endophytic bacteria are easier to manipulate than plant and have been used alternatively and preferably to improve the phytoremediation efficiency without requiring integration of foreign DNA into the plant genomes to produce transgenic plants (Barac et al., 2004; Doty, 2008). Most of the endophytes including Bacillaceae, Pseudomonaceae, Burkholderiaceae, and Enterobacteriaceae, *etc.* are facultative endophytes that can thrive inside a wide range of plant species including both monocots and dicots (Bacon and Hinton, 2006). However, all these studies are restricted to an extremely limited number of organic pollutants such as polycyclic aromatic hydrocarbons (PAHs), petroleum hydrocarbons (PHs), trichloroethylele (TCE), and BTEX (benzene, toluene, ethyl-benzene, and xylene) compounds (Feng et al., 2017), while degradation of PAEs using endophytic bacteria is scarcely documented.

Municipal wastewater usually contains high concentrations of phthalate esters (Gao and Wen, 2016), and constructed wetland plants are considered to be one of the major factors in PAEs removal from aquatic environments (Tang et al., 2015). Many water-borne microorganisms can colonize on the root or rhizome surface, some of which penetrate through the surface and colonize within plant tissues that constitute the reaction surfaces for endophytic microbial degradation of organic pollutants (Ijaz et al., 2015). Endophytic bacteria in plants inhabiting constructed wetlands have scarcely been reported. Shehzadi et al. (2016) isolated endophytes from the roots and shoots of *Typha domingensis*, *Pistia stratiotes*, and *Eichhornia crassipes* inhabiting a constructed wetland that treated textile effluent, in order to search for endophytic bacteria with textile effluent-degrading and plant growth promoting abilities. *Canna indica* L. is a dominant ornamental plant of tropical origin. This plant can be easily obtained and grown in many soil types including wet soils such as wetlands or river banks, allowing it to be used not only for soil remediation but also for wastewater treatment in wetland areas (Zhang et al., 2008). Boonsaner et al. (2011) found that the removal of BTEX in canna planted soil was 80% higher than that of the control soil. Moreover, the presence of *C. indica* significantly accelerated the dissipation of the pesticide triazophos (Cheng et al., 2007). However, there is scarcely study of endophytic bacteria colonizing *C. indica*. It is valuable to explore the culturable bacterial endophytes colonizing *C. indica* inhabiting a wastewater treatment constructed wetland for enhancing remediation of pollutants (Calheiros et al., 2017).

The aims of this study are: 1) to isolate and identify endophytic DBP-degrading bacteria from *C. indica*; 2) to evaluate the significant factors responsible for DBP degradation by endophyte; and 3) to provide evidences that the stain YJB3 can completely degrade DBP and to deduce the biodegradation pathway. This study is helpful to the development of endophytic resources for bioremediation of PAE-contaminated soil and water environments.

2. Materials and methods

2.1. Samples and reagents

Root samples of *C. indica* were collected from a constructed wetland for the treatment of municipal wastewater in Guangzhou, southern China.

DBP (98.7%), Dimethyl phthalate (DMP, 99.0%), Diethyl phthalate (DEP, 99.6%), di-n-octyl-phthalate (DnOP, 98.0%), Di(2-ethylhexyl) phthalate (DEHP, 99.0%), di-isononyl phthalate (DINP, 99.0%), monobutyl phthalate (MBP, 98.0%), PA, 99.5%, and PCA (97.0%) were purchased from Aladdin Chemistry Co., Ltd. (Shanghai, China). DBP standard (99.8%) was obtained from Sigma-Aldrich Co. (St. Louis, USA). All other chemical reagents were of analytical grade and all solvents were of HPLC grade. Ultrapure water (18.2 MΩ cm^{-1}) was prepared with a Milli-Q water purification system (Millipore, Billerica, MA, USA).

Luria-Bertani medium (LB, pH 7.0) containing (L^{-1}) trytone 10 g, yeast extract 5 g, and NaCl 10 g and a mineral salt medium (MSM, pH 7.0) containing (L^{-1}) K_2HPO_4 5.8 g, KH_2PO_4 4.5 g, $(NH_4)_2SO_4$ 2.0 g, $MgCl_2$ 0.16 g, and 1.0 mL trace element solution were used in this study. The trace element solution contained (L^{-1}) $CaCl_2$ 20 mg, $Na_2MoO_4 \cdot 2H_2O$ 2.4 mg, $FeSO_4 \cdot 7H_2O$ 1.8 mg, and $MnCl_2 \cdot 4H_2O$ 1.5 mg.

Solid medium plates were prepared by adding 18–20 $g \cdot L^{-1}$ agar into the above mentioned liquid media.

2.2. Enrichment and separation of DBP-degrading endophytic bacteria

Healthy *C. indica* plants were uprooted from the constructed wetland mentioned above and thoroughly washed with tap water and sterilized distilled water orderly. Roots samples that were sliced into 1 cm × 1 cm pieces were sterilized by sequential immersion in 3% H_2O_2 for 3 min, 70% (v/v) ethanol for 3 min, and 5% sodium hypochlorite for 2 min, and then rinsed with sterilized distilled water to eliminate the surface sterilization agents. To verify the effectiveness of surface sterilization process, uncut root tissue and the final rinse water were spread onto LB agar plates. The absence of growth after incubation on the plates confirmed sterilization.

The sterilized root material (1.0 g fresh weight) was homogenized in a mortar and pestle containing 10 mL sterile phosphate-buffered saline (PBS, pH 7.2) to obtain a root suspension. 1 mL of the suspension was inoculated into a 250 mL sterile Erlenmeyer flask containing 100 mL MSM and 100 $mg \cdot L^{-1}$ DBP that were sterilized in an autoclave at 121 °C for 20 min and through a 0.22 μm Millipore filter, respectively.