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Bioremediation of triphenyl phosphate in river water microcosms: Proteome alteration of *Brevibacillus brevis* and cytotoxicity assessments

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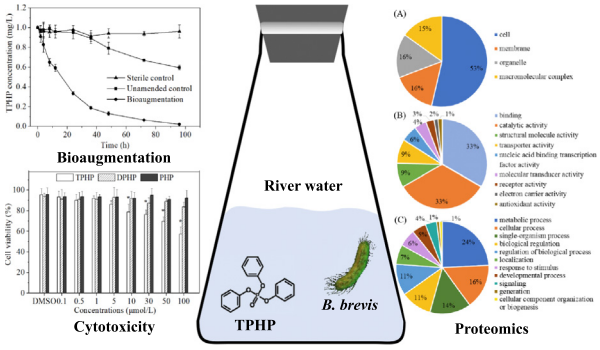
HIGHLIGHTS

- High concentration of TPHP was detected in river water around e-waste recycling area.
- Bioaugmentation with *B. brevis* improved TPHP degradation in river water microcosms.
- Proteomic analysis revealed 182 proteins significantly changed under TPHP stress.
- TPHP metabolites showed a lower cytotoxicity to HepG2 cells than their precursor.

GRAPHICAL ABSTRACT



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ABSTRACT

Triphenyl phosphate (TPHP), an organophosphate flame retardant, was detected in river water samples collected from an electronic waste recycling area in Guiyu, Southern China. The concentrations of TPHP ranged from not detected to 347.2 ng/L, with an average of 138.8 ng/L. The bioaugmentation potential of *Brevibacillus brevis* on TPHP biodegradation by aerobic microcosms contained in river water from Guiyu was assessed. The results showed that TPHP degradation efficiency was significantly improved to 97.9% by bioaugmentation with *B. brevis* after 96 h incubation. A total of 182 significantly changed proteins in *B. brevis* were identified and quantified by isobaric tags for relative and absolute quantification (iTRAQ) in response to TPHP stress. The differentially expressed proteins were mainly associated with energy metabolism, lipid metabolism, cell wall biosynthesis, amino acid transport, and metabolism. The identification that proteins of *B. brevis* respond to TPHP existence provides novel insights into biodegradation mechanisms of bacteria under environmental stress. Additionally, cytotoxicity assays indicated that the degrading intermediates of TPHP, namely diphenyl phosphate and phenyl phosphate, were less cytotoxic to human HepG2 cells compared with TPHP. Collectively, these findings suggest that aerobic bioaugmentation with degrading microorganisms is a potential strategy for *in situ* treatment of TPHP-contaminated sites.

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1. Introduction

Electronic waste (e-waste), a complex mixture of metals, plastics, glasses, and ceramics, originates from discarded electrical and electronic equipment (Wong et al., 2007). However, improper dismantling of e-waste can severely pollute the regional environment and seriously threaten the health of local residents *via* some direct exposure routes such as dietary ingestion, dust ingestion, and inhalation (Zheng et al., 2016). Flame retardants (FRs) are usually employed as plasticizers, antifoaming agents, and additives in electronic products. High concentrations of FRs have been found in various environmental media in the e-waste recycling area (Zhou et al., 2017; Zheng et al., 2015).

In recent years, the use of phosphate flame retardants (PFRs) has increased markedly with the phase-out of brominated flame retardants (BFRs) (Wei et al., 2015; van der Veen and de Boer, 2012). Triphenyl phosphate (TPHP), an organophosphate flame retardant (OPFR), has been used in a variety of consumer products, including electronic devices, plastics, building materials, and polyurethane foams (van der Veen and de Boer, 2012; Hou et al., 2016). As an additive flame retardant forming no chemical bonds with polymeric materials, TPHP can diffuse into the surrounding environments easily (Marklund et al., 2003). In consequence, it is frequently identified with high abundance among the OPFRs detected in indoor air and dust, river water, soil, and even in human placenta and blood (He et al., 2018; Ding et al., 2016; Zhao et al., 2016; Cristale et al., 2013a). There is increasing evidence that TPHP may pose a risk to human health. Previous studies found that TPHP could increase the concentration of thyroid hormone in zebrafish by disrupting the hormone synthesis and central regulation pathways (Kim et al., 2015). Also, TPHP could affect the progesterone biosynthesis in the placenta, thus affecting female reproduction and fetal development (Hu et al., 2017). Therefore, specific attention must be paid to TPHP in the OPFRs pollution investigations of e-waste processing sites.

Bioaugmentation is considered a promising strategy for the *in situ* remediation of media contaminated with recalcitrant chemicals due to its environmental-friendly and cost-effective characteristics (Cea et al., 2010; Takahashi et al., 2008). To date, bioaugmentation processes have been designed to remediate the environments polluted with phenanthrene, pyrene, benzo(a)pyrene, thiabendazole, and 2,4,6-tribromophenol (Zafra et al., 2017; Papadopoulou et al., 2018; Xiong et al., 2017). However, little is known about the treatment of TPHP-contaminated river water stimulated by bioaugmentation using degrading microorganisms. Also, necessary information about toxicity and biological effects of the main metabolites of TPHP for organisms is very limited. As the primary degrading intermediates, diphenyl phosphate (DPHP) and phenyl phosphate (PHP) have been identified during the TPHP biodegradation (Jurgens et al., 2014; Wei et al., 2018). Kojima et al. (2016) reported that the diester TPHP-metabolites may have limited nuclear receptor activity compared to their parent compound. In addition, as an *in vitro* model, human hepatoma cell line HepG2 have been widely used in toxicological studies to analyze the cytotoxicity of OPFRs (Krivoshiev et al., 2018; Zhang et al., 2017a).

Proteins are the major determinants of biological functions in the actual phenotype of an organism. Isobaric tags for relative and absolute quantification (iTRAQ) labeling quantitative proteomic technology has been used to obtain the information on functional proteins to better understand the relationship between a specific organism and its environment (Lyu et al., 2016). By means of iTRAQ, Ye et al. (2017) revealed that the energy generation and transmembrane transport in *Escherichia coli* were significantly activated when the cells were exposed to degrading products of tris(2-chloroethyl) phosphate (TCEP).

In this study, the extent of TPHP contamination was investigated for the river water from Guiyu, Guangdong Province, an e-waste recycling region. TPHP biodegradation in river water bioaugmented with *Brevibacillus brevis* was conducted to explore the bioaugmentation potential. To reveal the response mechanism of microorganisms under environmental stress, the changes in the protein expression profile of *B. brevis* challenged by TPHP were analyzed. Furthermore, cytotoxicity experiments in HepG2 cells were performed to evaluate the cytotoxicity of TPHP and its two key metabolites, DPHP and PHP.

2. Materials and methods

2.1. Chemicals, medium and strain

TPHP (≥99%), DPHP (99%), dimethyl sulfoxide (DMSO), streptomycin, and penicillin were obtained from Sigma-Aldrich (USA). PHP (>99%) was purchased from Tokyo Chemical Industry (Japan). TPHP-d_{15} obtained from J&K Scientific Ltd. was used as a surrogate standard. Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were purchased from Gibco (USA). The beef extract peptone medium used for strain culture and mineral salt medium (MSM) for strain exposure to TPHP were prepared based on previous research (Wei et al., 2018). The pH value was adjusted to 7.0 ± 0.2. Sterile media were prepared and autoclaved at 121 °C for 30 min. Stock solution of 500 mg/L TPHP used for microcosm was prepared by dissolving TPHP in methanol. Stock solutions (500 mmol/L) of TPHP, DPHP, and PHP were prepared in DMSO, respectively, and were serially diluted to yield working solutions prior to experimental use. All other reagents of high-performance liquid chromatography grade were obtained from Sigma-Aldrich. *B. brevis* was an effective strain for TPHP degradation (Wei et al., 2018), which was isolated from an e-waste dismantling area in Guiyu, Guangdong Province, China and preserved in our laboratory.

2.2. Sample collection

Field sampling was conducted in September 2016. The sampling region was seriously polluted by heavy metals, polycyclic aromatic hydrocarbons (PAHs), BFRs, and PFRs due to the primitive dismantling and extraction of e-wastes (Shi et al., 2016; Leung et al., 2011; Zheng et al., 2015; Quan et al., 2014). A total of 10 water samples (GY1-GY10) from 10 locations around Guiyu town, Guangdong Province, China were collected. The detailed sampling locations and characteristics are provided in Table S1. Water samples were collected 20 cm below the water surface using a 1-L brown glass bottle, which had been washed and rinsed with Milli-Q water and river water three times before use. Additionally, 3 L of water sample (GY6) was collected for microcosm experiments from Lianjiang River, a source of irrigation water for surrounding farmlands. All the sampling bottles were stored in the dark under cold condition, transported to the laboratory within 48 h, and stored at 4 °C until analysis.

2.3. Sample extraction and analysis

Extraction of water samples was conducted according to the literature (Li et al., 2014), with some modifications. Briefly, the water samples (500 mL) were filtered through a glass fiber filter (0.7 μm, Whatman, UK), spiked with 20 ng of TPHP-d_{15} as surrogate standard, and extracted using ENVI-18 solid phase extraction (SPE) cartridges (6 mL, Supelco, USA). The cartridges were conditioned by elution with 10 mL of ethyl acetate, 10 mL of acetonitrile, and 10 mL of Milli-Q water sequentially. The filtered water samples were percolated through the cartridges using a vacuum pump. After the cartridges were dried and eluted, analytes were then eluted twice with 6 mL of acetonitrile. The extracts were cleaned by 2.5 g of anhydrous sodium sulfate. The eluents were concentrated under a gentle nitrogen flow till almost dryness and then dissolved with 0.2 mL of acetonitrile for instrumental analysis.

Identification and quantification of TPHP and TPHP-d_{15} were performed using an ultra-performance liquid chromatographic (UPLC) system as described previously (Wei et al., 2018), the details of which are given in Text S1.