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Vertical distribution of dehalogenating bacteria in mangrove sediment and their potential to remove polybrominated diphenyl ether contamination

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ABSTRACT

The removal and degradation of polybrominated diphenyl ethers (PBDEs) in sediments are not clear. The vertical distribution of total and dehalogenating bacteria in sediment cores collected from a typical mangrove swamp in South China and their intrinsic degradation potential were investigated. These bacterial groups had the highest abundances in surface sediments (0–5 cm). A 5-months microcosm experiment also showed that surface sediments had the highest rate to remove BDE-47 than deeper sediments (5–30 cm) under anaerobic condition. The deeper sediments, being more anaerobic, had lower population of dehalogenating bacteria leading to a weaker BDE-47 removal potential than surface sediments. Stepwise multiple regression analysis indicated that *Dehalococcoides* spp. were the most important dehalogenating bacteria affecting the anaerobic removal of BDE-47 in mangrove sediments. This is the first study reporting that mangrove sediments harbored diverse groups of dehalogenating bacteria and had intrinsic potential to remove PBDE contamination.

1. Introduction

Polybrominated diphenyl ethers (PBDEs), commonly used brominated flame retardants (BFR), have been extensively added to a variety of manufactured materials over the past few decades. Because of their lipophilicity and high resistance to degradation, PBDEs are likely to accumulate in aquatic sediments when disposed to the environment (Mai et al., 2005; Wang et al., 2007). Mangroves, one of the typical coastal ecosystems in tropical and subtropical regions, often receive various man-made pollutants, such as heavy metals, polycyclic aromatic hydrocarbons and polychlorinated biphenyls (Li et al., 2009; Zhu et al., 2014a). In recent years, mangroves were also reported to be contaminated by PBDEs in many places, such as mangroves in Sundarban, Singapore, Hong Kong, *etc.*, but with different degrees of contamination (Bayen et al., 2005; Binelli et al., 2007; Zhu et al., 2014a). The extent of PBDE contamination also varied with sediment depths (Zegers et al., 2003). Zhu et al. (2014a) reported that surface mangrove sediments had higher contamination levels of PBDEs, ranging from 47.2 to 112 ng g^{-1} dry weight (dw), than that of lower layers of sediments with concentrations varying from 7.69 to 72 ng g^{-1} dw. These findings indicated that mangrove sediments encountered both spatial and vertical PBDE contamination. However, most previous work only focused on the distribution and levels of PBDE contamination in different environmental matrices. Questions such as how to remove PBDEs from contaminated sediments, what is the intrinsic ability of the sediment to self-remedy PBDEs, what are the functional bacterial groups involved and how about their distribution in different depths of the sediment, have seldom been answered. So far, only one study showed that aquatic sediments, including marine and mangrove sediments, possessed intrinsic potential to remove PBDEs but their abilities varied from sediments to sediments (Zhu et al., 2014b).

The intrinsic ability of sediments to remove toxic contaminants was reported to rely on the distribution and activity of the indigenous bacteria in the sediments, especially the functional bacterial groups with reductive dehalogenation activities (Ding and He, 2012). The functional bacteria that have been reported mainly belonged to the genera of *Dehalococcoides* (*Dhc*), *Dehalogenimonas* (*Dhg*), *Dehalobacter* (*Dhb*), *Desulfitobacterium* (*Dsb*) and *Geobacter* (*Geo*) (Maphosa et al., 2010). Under anaerobic condition, these bacteria could use halogenated compounds as electron acceptors and replace halogen atoms of these compounds with hydrogen atoms, generating less or none halogenated ones. Energy generated during this reductive dehalogenation process would be conserved or consumed for bacterial growth or proliferation (Ding and He, 2012). The organohalide-respiring bacteria (OHRB) had been proved to play a vital role in removing halogenated pollutants, like polychlorinated biphenyls (PCBs) and PBDEs, and might be

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promising candidates for the bioremediation of polluted environments (Liang et al., 2014).

In mangrove ecosystem, reduced conditions are prevailing because of frequent tidal flushing with tidal water periodically flooded onto sediment surface, forming a decreasing trend of redox potential from top to bottom layers of sediment (Li et al., 2009). The bacterial community composition in mangrove sediments is influenced by redox potential and concentrations of inorganic electron acceptors related to anaerobic respiration, such as nitrate (NO_3^-), Fe^{3+}, and sulfate (SO_4^{2-}) in the sediment (Li et al., 2009; Husson, 2013). Mangrove sediments are also known to have high organic matter content, which can serve as a nutrient pool for the survival and growth of bacteria (Thatoi et al., 2013). The distribution, population and activity of bacteria involved in the degradation of organic contaminants in sediment are affected by total organic matter (TOM), total Kjeldahl nitrogen (TKN) and total phosphorus (TP) (Neumann et al., 2014). Additionally, grain size cannot be neglected as bacterial community structure varies a lot among sediments having different particle sizes, resulting in differences in the mineralization potential of organic pollutants (Hemkemeyer et al., 2015). All these physico-chemical and biological properties of sediments change along sediment depth profiles. Li et al. (2009) reported that surface layer sediment was more aerobic and contained higher amounts of electron acceptors and nutrients, leading to more aerobic but less anaerobic bacteria than deeper sediments. So far, published reports on the vertical distribution of dehalogenating bacteria in mangrove sediments are very few. The intrinsic potential of sediments to remove PBDEs and their variations at different depths along sediment cores are still unclear.

The present study aims to investigate the vertical distribution of dehalogenating bacteria, and the potential functional bacteria involved in the anaerobic degradation of PBDEs, in sediment cores collected from Mai Po mangrove swamp in Hong Kong, South China. The study also attempts to evaluate the intrinsic potential of mangrove sediments at different depths to remove and debrominate PBDEs under anaerobic condition, and relate the removal with the abundance of dehalogenating bacteria. BDE-47 was selected as the model PBDE congener because it is known to be more toxic and more readily biomagnifies in food webs, thus exerting greater risk to the health of higher trophic level biota, like fish and mammals, than BDE-209. It could also be generated through the debromination of BDE 209 and get accumulated in sediment, thus inducing more serious environmental problems than BDE-209 (Ross et al., 2009). Mai Po mangrove swamp, a designated RAMSAR in Hong Kong SAR for residential and migratory birds and other wildlife, was chosen as the study site, because this is a typical mangrove swamp in South China and is the largest wetland in Hong Kong (Zhao et al., 2012). The swamp receives various discharges from local communities, as well as from Pearl River, one of the most polluted rivers in China because of intensive industrial manufacturing in adjacent area (Zhao et al., 2012). In terms of PBDE contamination, Mai Po mangrove swamp was reported to contain the highest level of PBDEs in surface sediments compared to other mangrove wetlands in Hong Kong (Zhu et al., 2014a).

2. Materials and methods

2.1. Sediment sampling and analyses

30-cm deep core sediments were randomly collected in triplicates from the mature mangrove forest of Mai Po mangrove swamp. The core was divided into six layers from top to bottom, each layer was 5 cm deep. The sediment redox potential (Eh) and pH of each layer were measured *in situ* using an Eh meter (TPS WP-81, Australia) and a battery operated pH meter (E.W. System Soil tester, Tokyo, Japan), respectively. Sediments were transferred to the laboratory using plastic bags and divided into three portions, fresh, air- and freeze-dried, for further analyses.

Particle size distribution (from 0.02 to 2800 μm) of fresh sediment sample was determined using the Microtrac S3500 particle size analyzer (Microtrac Inc., USA). The amount of total organic matter (TOM) of air-dried sediment was measured by loss on ignition at a temperature of 550 °C in a muffle furnace (Carbolite, UK). Total Kjeldahl nitrogen (TKN) and total phosphorus (TP) were measured by flow injection analysis (FIA) analyzer (Lachat QuikChem Method 8000, USA) after digestion of air-dried sediment with concentrated sulphuric acid (H_2SO_4) at 390 °C for 4 h. Nitrate (NO_3^-) in fresh sediment was extracted with 2 M potassium chloride solution and measured by the same FIA colorimetric analysis. Sulfate (SO_4^{2-}) in fresh sediment was extracted with deionized water and measured by ion chromatography (IC) (Dionex Ion Chromatograph Model LC20, Dionex Corp.). HCl-extractable Fe^{2+} in fresh sediment was extracted with hydrochloric acid (1 M) for 2 h, centrifuged and the extract was measured using the 1,10-phenanthroline method (Song et al., 2015). Total iron was extracted in the same way, except hydroxylamine hydrochloride (10%, w/v) and sodium acetate (10%, w/v) were added to the extract to reduce Fe^{3+} to Fe^{2+} before measurement (Song et al., 2015). The HCl-extractable Fe^{3+} was the difference between total iron and HCl-extractable Fe^{2+} levels.

2.2. Analysis of PBDEs

PBDEs in freeze-dried sediment were extracted using the accelerated solvent extraction system (ASE 200, Dionex, USA). PCB-209 (AccuStandard, USA) and ^{13}C-BDE-209 (Wellington laboratories, Canada) were added as surrogate standards before extraction. The extract was purified using chromatography columns filled with 5 g of florisil powder (Sigma Aldrich, 100–200 mesh) and 1 g of acid-activated copper powder. Columns were activated with 10 mL acetone and 10 mL n-hexane sequentially prior of use. The concentrated extract was loaded into the column and eluted with 20 mL n-hexane. The eluent was collected and reduced to 1 mL before GC analysis. Quantification of PBDEs was conducted on a 6890 gas chromatography (Agilent Technologies, USA) coupled with an Agilent 5975 mass spectrometer using negative-ion chemical ionization (NCI) in the selected ion monitoring mode. A 15 m HP-5 fused silica capillary column (0.25 mm i.d., 0.25 μm film thickness) was used for the determination of PBDE congeners. The oven temperature began from 150 °C (held for 2 min) and increased to 320 °C at 6 °C min^{-1} (held for 2 min). The characteristic ion fragment (m/z) monitored was: 487 and 489 for BDE-209, 496.7 and 498.7 for ^{13}C-BDE-209, 79 and 81 for other PBDE congeners (BDE-7, -8, -15, -17, -28, -47, -99, -100, -153, -154, -183) and 498 for PCB-209. Two kinds of mixed standards (AccuStandard, USA) containing 8 and 39 components, respectively, were used. The recovery rate for BDE-209 was 92.3%, and ranged from 91.5 to 105.4% for other targeted PBDE congeners.

2.3. DNA extraction and quantitative real-time PCR (qRT-PCR)

Genomic DNA was extracted from 0.5 g fresh sediment using the FastDNA SPIN kit for Soil (MP Biomedicals, CA, USA), following the manufacturer's instruction. The copy numbers of total bacteria, *Dhc*, *Dhg*, *Dhb*, *Dsb* and *Geo* 16S *rRNA* genes were determined using quantitative real-time PCR (qRT-PCR) technique with primers listed in Table S1 and conditions described by Chen et al. (2016). Standard curves were created with serial dilutions of plasmids containing the cloned target sequences according to Chen et al. (2016).

2.4. Removal of BDE-47 by mangrove sediment under anaerobic conditions

The potential of mangrove sediments at different depths to remove BDE-47 under anaerobic condition was evaluated by mixing 100 g fresh sediment from each layer with 100 mL mineral salt medium (MSM) spiked with BDE-47 at a concentration equivalent to 1 mg kg^{-1} dry weight (dw) in 250 mL quick-fit conical flasks. Triplicate

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