

Aerobic Biotransformation of Polybrominated Diphenyl Ethers (PBDEs) by Bacterial Isolates

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Polybrominated diphenyl ethers (PBDEs) are flame retardants that have been used in consumer products and furniture for three decades. Currently, very little is known about their fate in the environment and specifically about their susceptibility to aerobic biotransformation. Here, we investigated the ability of the polychlorinated biphenyl (PCB) degrading bacteria *Rhodococcus jostii* RHA1 and *Burkholderia xenovorans* LB400 to transform mono- through hexa-BDEs at ppb levels. We also tested the PBDE transforming abilities of the related strain *Rhodococcus sp.* RR1 and the ether-degrading *Pseudonocardia dioxanivorans* CB1190. The two PCB-degrading strains transformed all of the mono- through penta-BDEs and strain LB400 transformed one of the hexa-BDEs. The extent of transformation was inversely proportional to the degree of bromination. Strains RR1 and CB1190 were only able to transform the less brominated mono- and di-BDE congeners. RHA1 released stoichiometric quantities of bromide while transforming mono- and tetra-BDE congeners. LB400 instead converted most of a mono-BDE to a hydroxylated mono-BDE. This is the first report of aerobic transformation of tetra-, penta-, and hexa-BDEs as well as the first report of stoichiometric release of bromide during PBDE transformation.

Introduction

Polybrominated diphenyl ethers (PBDEs) are a class of flame retardants that have been used for more than three decades in a wide variety of manufactured materials such as foams, textiles, and plastics at up to 20% by weight (1). PBDEs were widely integrated into products as mixtures of penta-BDEs (mostly penta- and tetra-BDE congeners), octa-BDEs (mostly octa- and hepta-BDE congeners), and deca-BDE. As of 2006, the penta- and octa-BDE technical mixtures have been banned and removed from production in Europe and the United States. Some PBDEs are toxic and others cause endocrine disruption in rats and mice (2). As a consequence of their extensive long-term use in consumer products, PBDEs

have been detected in a wide variety of environmental samples, ranging from air, water, soil, and sediment to biota (1, 3–5). Concentrations of total PBDEs in the San Francisco Bay in 2002 ranged up to 0.5 ng/L in water and 212 µg/kg dry weight in aquatic sediments (5). In 2000, PBDE concentrations in human breast milk were reported to be doubling every five years, creating substantial concern about the fate and transport of these compounds and human exposure to them (6).

PBDEs have been shown to be anaerobically and aerobically transformed by microorganisms. Recent work on anaerobic biodegradation has shown that PBDEs are debrominated to less brominated congeners by a variety of anaerobic dehalogenating bacteria (7–9). However, currently very little is known about aerobic biotransformation of PBDEs. In the early 1990s, Schmidt and colleagues isolated two *Sphingomonas sp.* strains capable of breaking down mono- and di-BDE congeners. *Sphingomonas sp.* SS3 was capable of transforming and growing on 4-bromodiphenyl ether while strain SS33 transformed 4,4'-dibromodiphenyl ether but was not able to use it as a growth substrate (10, 11). Several transformation products were identified, including 4-bromophenol, 4-bromocatechol, and bromide. Subsequently, the white-rot fungi *Trametes versicolor* was shown to convert 4-bromodiphenyl ether to its hydroxylated analog (12). More recently, additional *Sphingomonas* species have been shown to aerobically break down a number of mono-, di-, and tri-BDEs. *Sphingomonas sp.* PH-07, which grows on diphenyl ether, was shown to transform but not grow on 4-bromo-, 2,4-dibromo-, 4,4'-dibromo, and 2,4,4'-tribromodiphenyl ethers (13). In that study, brominated phenols were identified as PBDE breakdown products and in the case of 4,4'-dibromodiphenyl ether, the metabolite 2'3'-dihydroxy-4,4'-dibromodiphenyl ether was also identified.

More highly brominated congeners such as tetra- and penta-BDEs—the most frequently detected congeners in the environment—have not yet been shown to be aerobically biotransformed. Ascertaining whether bacteria can aerobically transform these congeners is important for understanding the fate of these compounds in the environment. We hypothesized that the chemical similarity of polychlorinated biphenyls (PCBs) and PBDEs would result in similar bacterial transformation patterns. PCB congeners with three or fewer chlorines are susceptible to transformation by certain species of aerobic bacteria resulting in the accumulation of metabolites such as chlorobenzoates. Degradation of PCBs with four to six chlorines has also been observed (14–17). In this study, we followed the disappearance of PBDEs and formation of bromide and other products when two known PCB-degrading bacteria, *Rhodococcus jostii* RHA1 and *Burkholderia xenovorans* LB400 and two related strains were exposed to PBDE congeners ranging from mono- to hexa-BDEs. Although experiments in which pure cultures of bacteria are exposed to relatively high concentrations of PBDEs may not be representative of environmental conditions, they provide insight into whether aerobic bacteria are capable of transforming tetra-, penta-, and hexa-BDEs.

Materials and Methods

Bacterial Cultures. The strains used in this work are *Rhodococcus jostii* RHA1, provided by Dr. Bill Mohn at the University of British Columbia, Vancouver, Canada (18); *Rhodococcus sp.* RR1, which was previously isolated from a toluene-contaminated site (19); *Burkholderia xenovorans* LB400 (DSMZ no. 17367); and *Pseudonocardia dioxanivorans*

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CB1190, supplied by Dr. Rebecca Parales at the University of California, Davis. The *Rhodococcus* and *Pseudonocardia* cultures were grown in a mineral medium modified from that described in ref 20 and contains 10 g/L NaNO₃, 1.7 g/L K₂SO₄, 0.37 g/L MgSO₄·7H₂O, 0.086 g/L CaSO₄·2H₂O, 5.3 g/L KH₂PO₄, 10.6 g/L K₂HPO₄, 5.74 mg/L ZnSO₄·7H₂O, 3.38 mg/L MnSO₄·H₂O, 1.24 mg/L H₃BO₃, and 5 mg/L CuSO₄·5H₂O. The following ingredients were added after autoclaving: 0.94 mg/L CoMoO₄·H₂O, 0.22 mg/L FeSO₄·7H₂O, 0.1 mL/L H₂SO₄. LB400 was grown in Brunner mineral medium (DSMZ medium no. 457). Because the Brunner mineral medium contains chloride and consequently some bromide impurities, LB400 was grown in the same minimal medium as RHA1 for experiments quantifying bromide release. LB400 and RHA1 were provided with 10 mM biphenyl as carbon and energy source. RR1 and CB1190 are not able to grow on biphenyl and consequently RR1 was grown with 20 mM pyruvate while CB1190 was provided with 5 mM of 1,4-dioxane every 3 days for 12 days. Cultures were grown at 30 °C and shaken at 200 rpm in 160 mL bottles sealed with Teflon-lined Mininert caps (VICI Valco Instruments, Houston, TX) containing 50 mL of medium. Experiments were conducted when cells had reached midexponential growth phase with absorbance between 0.26 and 0.27 when measured at 600 nm. Due to the propensity of CB1190 to form cell clumps and to float on the surface of the medium, cell density could not be measured spectrophotometrically. Protein measurements were taken by extracting 1 mL of sample and using the Coomassie blue protocol provided with the reagents by Thermo Scientific (Waltham, MA). Protein values averaged 26 mg/L when the experiments were started. All experiments were repeated at least twice.

Experimental Design. Our experimental methods were modified from previous studies performed to analyze PBDE transformation under anaerobic conditions (7, 9). Neat mono-BDE 3 was purchased from Sigma Aldrich (St. Louis, MO); all other individual congeners were purchased from Accustandard (New Haven, CT). The mixture DE-71 was purchased from Wellington Laboratories (Guelph, Ontario, Canada). Vials for the PBDE transformation experiments were prepared by adding 1 μL of a stock containing 50 μg/mL PBDE congener dissolved in nonane to 8 mL culture vials that were subsequently left in a laminar hood for 5 min to evaporate the nonane. Three mL of bacterial culture at an optical density of 0.26–0.27 at 600 nm were added to the vials and sealed with Teflon-lined screw caps. Any observable biphenyl solids in the culture bottle were allowed to settle to the bottom before transfer of the bacteria since biphenyl solids affect extraction efficiency of the PBDEs. The final PBDE concentration in sample vials was 17 ng/mL. Controls consisted of autoclaved cells and PBDEs. All sample vials and controls were performed in triplicate. All samples were incubated in a shaking incubator at 30 °C. The experimental vials were extracted after three days using 3 mL of isooctane, and 0.6 ng of 2,2',4,5'-tetrabromobiphenyl dissolved in methanol was added as an internal standard. The vials were briefly vortexed and then placed on a shaker table for 16 h.

The bromide formation experiments were carried out by adding 4 μL of a nonane stock containing 50 mg/mL mono-BDE 3 or 1 mg/mL tetra-BDE 47 to 60 mL glass bottles with a glass syringe. The bottles were left uncapped in a laminar hood to evaporate the nonane for 5 min. Twelve mL of cells at an optical density in the range of 0.26–0.27 were added to the bottles and capped with a Teflon lid. The final concentrations of mono-BDE 3 were 17 μg/mL and for tetra-BDE 47 were 3.3 μg/mL. The bottles were placed in a shaking incubator at 30 °C and monitored for 24 h. Periodically, 200 μL of sample were removed with a plastic disposable syringe, filtered with a 0.2 μm filter, and placed in centrifuge tubes kept at 4 °C until the end of the experiment for bromide

analysis. After 24 h the remaining liquid in the bottle was extracted with a 1:1 volume of isooctane for PBDE analysis using 2,2',4,5'-tetrabromobiphenyl as an internal standard. The percent transformation was determined by comparing the average concentrations of PBDEs in the triplicate live samples with the triplicate autoclaved controls. To determine if the average concentrations of samples and controls were statistically different, a student *t* test was used with a *p*-value of 0.1. Total error was used to determine error bars.

$$\text{total error} = \frac{\text{mean}_{\text{samples}}}{\text{mean}_{\text{controls}}} \times \sqrt{\frac{\text{stderror}_{\text{samples}}^2}{\text{mean}_{\text{samples}}^2} + \frac{\text{stderror}_{\text{controls}}^2}{\text{mean}_{\text{controls}}^2}}$$

Analytical Techniques. One mL of isooctane extract was transferred into a 2 mL vial with a disposable glass pipet and analyzed by gas chromatography with electron capture detection (GC-ECD) model 3800 from Varian Inc. (Walnut Creek, CA). A 30 m DB-1 column with 0.25 mm I.D. and 0.25 μm film thickness was used. The GC temperature program was as follows: 110 °C for 2 min, ramp at 30 °C/min to 200 °C and then ramp at 1.5 °C/min until 300 °C. The injector temperature was 250 °C and the detector was kept at 325 °C. Automatic splitless injection was used with 2 μL injection volume; flow of the nitrogen carrier gas was maintained at a constant pressure of 25 psi. Three point calibration curves, ranging from 0.5 to 50 ng/mL were run daily for all individual congeners to adjust for variation in ECD response. Extraction efficiencies, measured as the amount of PBDE recovered compared to the amount of PBDEs added to the control samples, for the di- through hexa-BDEs ranged from 73 to 118%. The extraction efficiency for mono-BDE was much less, around 54%. Extraction efficiency for the internal standard averaged 93%. Identification of hydroxylated intermediates was performed using a Waters (Milford, MA) GC-MS in electron impact mode with a 30 m DB-5 column with 0.25 mm i.d. and 0.25 μm film thickness. A splitless injection of 2 μL was made and the helium carrier gas flow was 2 mL/min. The temperature program was the same as described above. To ensure good recoveries of acidic metabolites, the samples were acidified to pH4 with H₂SO₄ before extraction. Some of the sample aliquots were derivatized with heptafluorobutyric acid anhydride (HFBA) (Supelco, Bellefonte, PA) for improved detection. Briefly, the 3 mL samples were extracted with 1 mL of ethyl acetate by shaking overnight. The ethyl acetate was then transferred to a new vial and evaporated to dryness with a stream of nitrogen. A 200 μL amount of acetonitrile and 50 μL of HFBA were added and the vial was placed in a 55 °C oven for 90 min. After incubation, the acetonitrile was evaporated to dryness and the contents of the vial were resuspended in 100 μL of isooctane.

Determination of Bromide. The relatively low concentrations of bromide released during PBDE transformation and complex matrix precluded the use of conventional bromide measurement techniques. To measure bromide at low concentrations in the presence of organic matter a new sensitive analytical technique was developed in which bromide was converted to hypobromous acid (HOBr), which subsequently was detected by quantification of brominated phenols formed after reaction with phenol. This method is similar to methods by Mishra et al. (21) and Reddy-Noone et al. (22) which could not be used in our experiments due to interference from the presence of cells. A bromide calibration curve was created using standards of NaBr in deionized organic-free water. The samples were buffered with 50 μL of a 1 M KH₂PO₄ solution adjusted to pH 7. We then added 250 ng of phenol (10 μL from a 25 ng/μL phenol

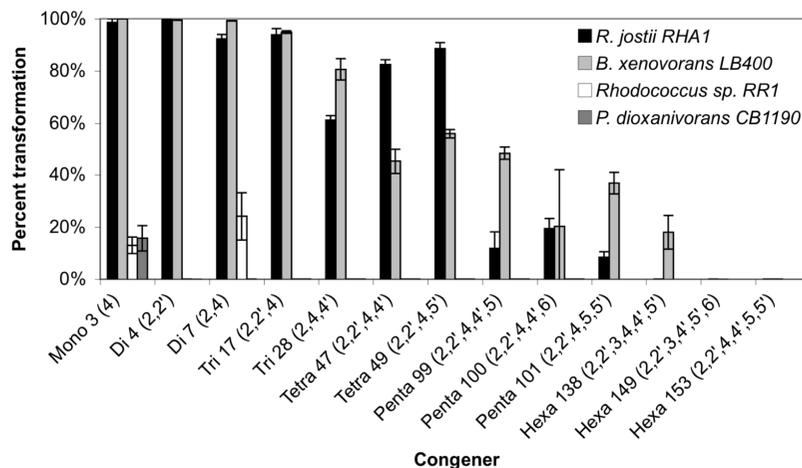


FIGURE 1. Percent transformation of thirteen PBDE congeners by *R. jostii* RHA1, *B. xenovorans* LB400, *Rhodococcus* sp. RR1, and *P. dioxanivorans* CB1190. Values are averages of triplicates. The percent degradation was calculated as the difference between the average PBDE concentration in the controls versus the live samples. Error bars represent the total error. Strains RR1 and CB1190 were not tested for the ability to degrade hexa-BDEs.

solution) and 5 μg of NaOCl (10 μL from a 0.5 $\mu\text{g}/\mu\text{L}$ NaOCl solution). The samples were briefly vortexed and allowed to react in the dark for 10 min. The reaction was stopped by adding 50 μL of a 0.3 M $\text{Na}_2\text{S}_2\text{O}_3$ solution. The samples were extracted with 100 μL of isooctane by vortexing for 5 min prior to analysis by a Varian 3800 GC-ECD with a 30 m DB-1 column with 0.25 mm I.D. and 0.25 μm film thickness with automatic splitless injection. The following temperature program was used: 80 $^\circ\text{C}$ for 2 min, then ramp at 20 $^\circ\text{C}/\text{min}$ until 300 $^\circ\text{C}$ and hold for 2 min. As shown in Supporting Information (SI) Figure SI-S1, six products are formed in this method: 2,4,6-trichlorophenol, 2-bromo-4,6-dichlorophenol, 4-bromo-2,6-dichlorophenol, 2,6-dibromo-4-chlorophenol, 2,4-dibromo-6-chlorophenol, and 2,4,6-tribromophenol. The formation of 2,4,6-trichlorophenol and 2,4,6-tribromophenol was verified with standards from Sigma-Aldrich. Standards for the intermediate partially brominated and partially chlorinated phenols were not commercially available, so their formation was verified by GC-MS (SI Figure SI-S1). In the absence of bromide, 2,4,6-trichlorophenol was the predominant product with small amount of a second peak (A) likely attributable to trace amounts of bromide in the NaOCl stock (SI Figure SI-S2). As the concentration of bromide increased, the areas of three peaks (2,4,6-tribromophenol and peaks A and B) increased as the area of 2,4,6-trichlorophenol decreased. A calibration curve in deionized water, in which the sum of the peak areas of peak A,B and 2,4,6-tribromophenol were used was linear with a modest background signal attributable to bromide in the NaOCl stock solution (SI Figures SI-S3 and SI-S4). To create a calibration curve with cells grown in minimal medium, the samples were filtered with a 0.2 μm filter. The filtrate was diluted 1:100 in deionized organic-free water and then spiked with bromide. A linear calibration curve was obtained for the diluted filtrate with a larger intercept related to interference from the bacterial medium and cellular material (SI Figure SI-S3). The yield of this method could not be ascertained directly, as standards for three of the brominated and chlorinated phenols are not commercially available. However, using 2,4,6-trichlorophenol (TCP) as a surrogate, the yield could be determined comparing the amount of TCP produced to the theoretical amount that should have been formed given the NaOCl and phenol concentrations, using rate constants from Acero et al. (23). The method was determined to be 98% efficient in nanopure water. When the reaction was repeated in the presence of 1/100 diluted filtered cells spiked with bromide, the efficiency of 2,4,6-trichlorophenol formation dropped to 60%. The limit of detection was 2.5 nM bromide

in nanopure water. Over the course of an experiment, samples were collected and filtered with a 0.2 μm filter. The filtrate was then diluted 1:100 into a final volume of 2.5 mL of deionized organic-free water to minimize interference from bacterial protein in the medium. After measuring the concentrations of bromide in the diluted samples, the values were multiplied by 100 to determine the true concentration of bromide in the experimental samples. For each experiment, a new bromide calibration curve was created using filtered cells collected at the start of the experiment and covering the range of bromide expected based on PBDE transformation.

Results

Four bacterial isolates were exposed to 13 PBDE congeners ranging from mono- to hexa-BDEs for three days under aerobic conditions. The four strains were two PCB degrading bacteria, *Rhodococcus jostii* RHA1 and *Burkholderia xenovorans* LB400; a related strain known to degrade aromatics, *Rhodococcus* sp. RR1 (19); and an additional ether-degrading bacterium, *Pseudonocardia dioxanivorans* CB1190 (24). RHA1 and LB400 were initially grown on biphenyl, whereas RR1 and CB1190 were grown on pyruvate and 1,4-dioxane, respectively.

Figure 1 shows the percent transformation for the thirteen tested congeners for each of the four tested isolates. The percent of PBDE transformed was determined by comparing the PBDE concentrations remaining in the live samples with those present in the autoclaved controls in order to account for extraction efficiencies and any potential mass losses. The two PCB-degrading strains, RHA1 and LB400, transformed greater than 90% of the mono- and di-BDE congeners within three days, while they transformed only 10–45% of the penta-BDE congeners. Hexa-BDEs were found to be the most resistant to transformation by these strains. Of the three tested congeners, LB400 was only able to transform 18% of hexa-BDE 138 but not hexa-congeners 153 and 149, whereas RHA1 was not able to degrade any of the three tested congeners even at increased cell densities of 0.7 (OD600). *Rhodococcus* sp. strain RR1 transformed the mono and di-BDE 7 (2,4-dibromodiphenyl ether), whereas it was unable to transform other congeners including di-BDE 4 (2,2'-dibromodiphenyl ether). CB1190 transformed only about 16% of the mono-BDE and none of the more brominated congeners.

RHA1 was tested for the ability to transform a combination of PBDE congeners by exposure to the DE-71 industrial penta-BDE mixture. RHA1 transformed 95% of the tetra-BDE 47, 78% of the penta-BDE 99, and 45% of the penta-BDE 100.

Although the exact concentrations cannot be compared with those from the individual PBDE experiments due to differing PBDE and cell concentrations, the same general transformation pattern is evident, indicating that the transformation ability of RHA1 is not significantly affected by the presence of multiple PBDEs.

Strain RHA1 was observed to release stoichiometric quantities of chloride during the transformation of 2,2'-dichlorobiphenyl (17). LB400 has also been shown to dechlorinate certain PCBs by hydroxylation of the chlorinated carbon in the ortho position (25, 26). Therefore, we analyzed for bromide generation concurrent with PBDE disappearance in order to confirm PBDE transformation by RHA1 and LB400. Since bromide detection is analytically challenging at low concentrations, mono-BDE 3 was added to RHA1 and LB400 bacterial cells at 68 μM , one thousand-fold higher than previously tested concentrations, and bromide release was monitored over a 24 h period. The same experiment was repeated using tetra-BDE 47 at a concentration of 7.6 μM . Because of the elevated PBDE concentrations, the PBDEs were not transformed to the same extent as in the lower concentration experiments. RHA1 and LB400 transformed 99% and 66%, respectively, of the 68 μM mono-BDE 3 they were exposed to, whereas RHA1 only degraded 20% of the 7.6 μM tetra-BDE 47 it was exposed to and LB400 did not degrade the elevated concentrations of the tetra sufficiently to detect production of bromide. Figure 2 shows the formation of bromide by RHA1 and LB400. SI Figure SI-5 shows chromatograms from the bromide analysis for the RHA1 samples and controls exposed to mono-BDE at zero and 18 h. No significant formation of bromide was detected in the autoclaved controls. Concurrent with the degradation of mono-BDE and tetra-BDE by RHA1, 80 and 100% respectively, of the stoichiometric bromide concentrations were recovered, indicating that RHA1 is capable of cleaving all the carbon-bromine bonds under aerobic conditions. Conversely, when LB400 transformed mono-BDE 3, only 10% of the stoichiometric quantities of bromide were recovered, indicating that LB400 transforms mono-BDE 3 to brominated intermediates rather than completely debrominating it. Since the bromide analytical method relies upon converting bromide to brominated phenols, any bromophenols generated as byproducts of PBDE transformation could skew the bromide quantification. However, no production of 4-bromophenol or 2,4-dibromophenol was detected in RHA1 samples transforming 4-bromodiphenyl ether and 2,2',4,4'-tetrabromodiphenyl ether respectively. Nor were any bromophenols detected as byproduct in the LB400 samples.

During aerobic PCB degradation by these strains, one of the rings is initially hydroxylated at two adjacent carbon positions to form a dihydrodiol which is then dehydrogenated to a dihydroxylated PCB (27). The chromatograms generated by the GC-ECD during LB400 exposure to mono-BDE contained a peak with a longer retention time than the original mono-BDE in all three of the live samples. This peak was not present in the autoclaved controls. The peak area of this unidentified compound was approximately as large as the substrate peak in the autoclaved controls. Analysis by GC-MS of acidified aliquots from these samples and comparison of the spectrum with that of a known standard identified this compound to be a hydroxylated mono-BDE. The M+ ions were 264 m/z and 266 m/z and were present at the same ratio as expected for brominated compounds containing equal proportions of the 79 and 81 bromine isotopes (Figure 3). The exact position of the hydroxyl group on the ring is unknown, although the MS fragments suggest that the hydroxyl group is on the nonbrominated ring. No other brominated compounds, including dihydroxylated mono-BDE, were detected by GC-MS. Aliquots from the LB400 samples exposed to mono-BDE 3 were also derivatized with

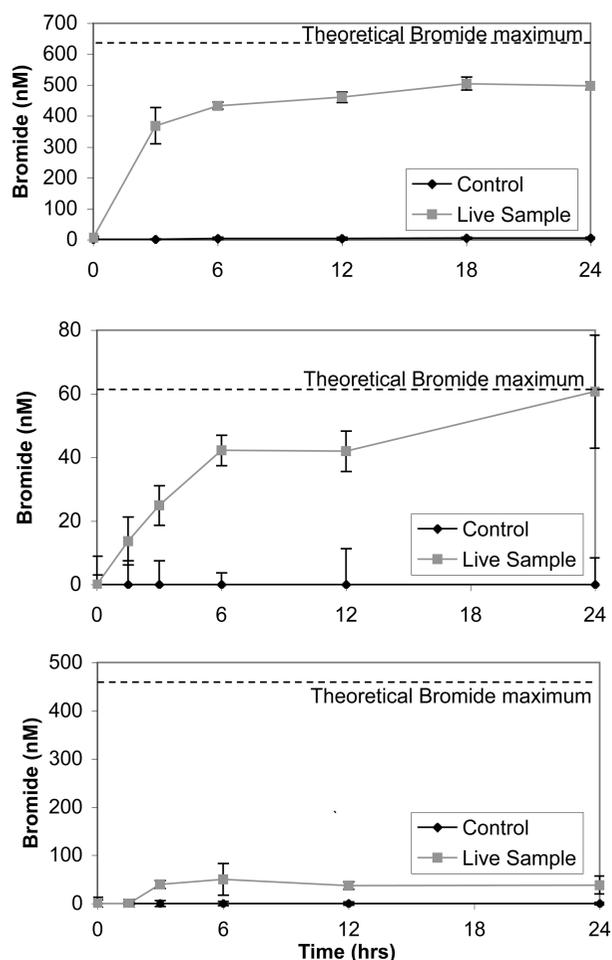


FIGURE 2. Bromide production by (A) RHA1 exposed to mono-BDE 3, (B) RHA1 exposed to tetra-BDE 47, and (C) LB400 exposed to mono-BDE 3 over the course of 24 h. The values are the averages for triplicates and the error bars are standard deviations. The dotted line indicates the maximum amount of bromide that could theoretically be released from the amount of PBDEs transformed.

HFBA for further verification (SI Figure SI-6). The spectrum yielded M+ ions of 460 m/z and 462 m/z which correspond to the molecular weight of a hydroxylated mono-BDE with the addition of HFBA. No other fluorinated peaks containing bromine were detected. These results indicate that a hydroxylated mono-BDE was the dominant product of the 90% of mono-BDE transformed by LB400.

Samples of RHA1 transforming 68 μM mono-BDE 3 were analyzed for the presence of metabolites. Aliquots were acidified to pH 4 and analyzed by GC-ECD and GC-MS, but no peaks were detected that were not present in the acidified autoclaved control.

Discussion

Experiments exposing four bacterial strains to 13 mono-through hexa-BDE congeners over the course of three days found that PBDEs can be aerobically transformed. The aerobic PCB-degrading species *Rhodococcus jostii* RHA1 and *Burkholderia xenovorans* LB400 were found to be capable of PBDE transformation, active toward some fraction of all of the mono- through penta-BDE congeners. In addition, LB400 was able to transform 18% of hexa-BDE 138, although none of the other tested hexa-BDEs were transformed by either strain. Aerobic biotransformation of PBDEs by bacteria had been previously described only for mono- through tri-BDE congeners by *Sphingomonas* strains (10, 11, 13, 27). This is

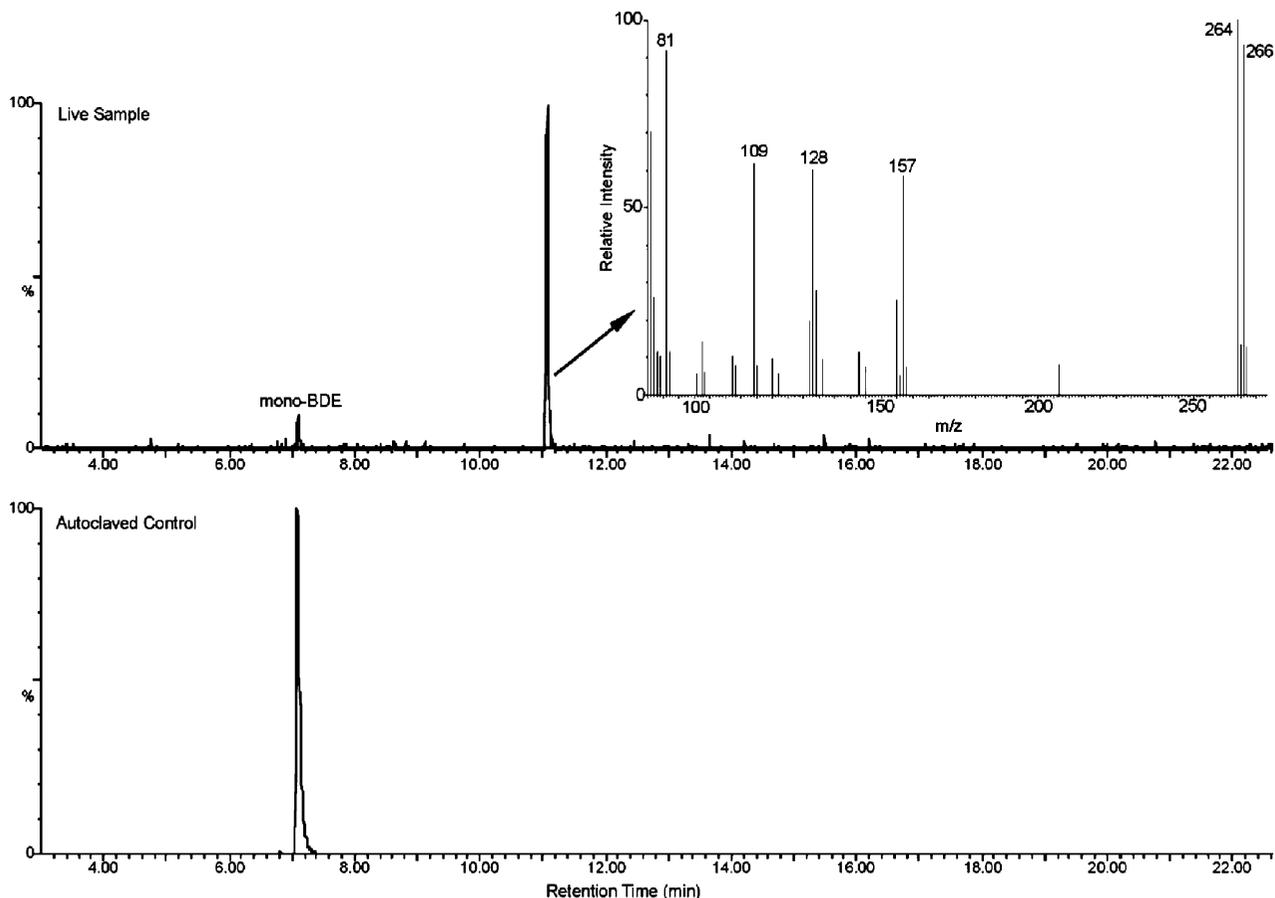


FIGURE 3. GC-MS chromatograms of underivatized extracts of an autoclaved control and a live sample of LB400 degrading 68 μM mono-BDE 3 after 24 h. The insert is the MS spectrum of the peak at 11 min identified as a hydroxylated mono-BDE.

the first report of aerobic transformation of the environmentally relevant tetra- and penta-BDE congeners as well as one hexa-BDE. RHA1 and LB400 catalyzed much greater removal of PBDEs than the previously described *Sphingomonas* sp. strain PH-07. Kim et al. observed at most 20% degradation of mono-BDE 3 and di-BDEs 7 and 15 over the course of eight days and only trace removal of tri-BDE 28. By comparison, RHA1 and LB400 transformed all of the mono-BDE 3 and di-BDE 7 and transformed between 60 and 80% of tri-BDE 28 within three days.

The other two species tested in this study, *Rhodococcus* sp. RR1 and *Pseudonocardia dioxanivorans* CB1190, were unable to transform most of the tested PBDEs. Although strain RR1 degrades aromatics, it only partially transformed mono-BDE 3 and one of the di-BDEs. It appears that RR1 can only transform PBDEs with one nonbrominated ring, such as mono-BDE 3 and di-BDE 7 (2,4-dibromodiphenyl ether), whereas all of the congeners that were not transformed have both rings brominated. Although CB1190 is an ether-degrading bacterium, capable of breaking the ether bond in 1,4-dioxane and tetrahydrofuran, it was able to transform only the mono-BDE 3 and only to a small extent (17%). Clearly, although they are ethers, PBDEs have very different chemical structures from the known ether substrates of CB1190. Furthermore, CB1190 has not been observed to degrade other halogenated compounds (24).

In general, transformation of the mono to hexa-BDE congeners by these bacteria was inversely proportional to the degree of bromination. Plotting the logarithm of the octanol-water partition coefficients obtained from Braekvelt et al. (28) for various PBDEs against the transformation ability of RHA1 and LB400 gives a correlation coefficient of 0.85. The inverse correlation between biotransformation and degree of PBDE bromination was expected due to the

increased size and hydrophobicity of more brominated congeners, reducing availability to the cell. Additionally, increased bromination also decreases the susceptibility of the carbons to hydroxylation and can sterically hinder enzymatic attack. This trend has also been observed with PCB transformation by RHA1 and LB400 (15–17). RHA1, however, is able to transform PCBs more effectively than the brominated diphenyl ether counterparts. For example, whereas RHA1 transformed about 82% of tetra-BDE 47 and 12% of the penta 99 within a three day incubation, it transformed, on average, 95% of tetra-CB 47 and 90% of penta-CB 99 in three days when tested at comparable cell densities and concentrations. Furthermore, RHA1 is able to transform hexa- and hepta-CBs, whereas no hexa-BDE transformation was observed for this strain even at higher cell densities. Although comparisons with LB400 studies are difficult because the cell densities and durations of the experiments are significantly different, Bopp found that LB400 readily transformed 59% of hexa-CB 153, whereas BDE 153 was not transformed in this study (15). This is perhaps in part due to the order of magnitude greater hydrophobicity ($\log K_{ow}$ of 7.9 for hexa-BDE vs 6.8 for hexa-CB) and the larger molecular volume of the PBDEs compared to the PCBs, greater steric hindrance of bromide compared to chloride, as well as other factors (28).

It is difficult to determine the effect of bromine position on the transformation extent in this study due to the small variability observed among the different congeners in the same homologue group. Tri-BDE 28 (2,4,4'-tri-BDE) was transformed to a lesser extent than tri-BDE 17 (2,2',4-tri-BDE) and tetra-BDE 49 (2,2',4,5'-tetra-BDE) was transformed slightly more than tetra-BDE 47 (2,2',4,4'-tetra-BDE). In the case of hexa-BDEs the effect of bromine position is more evident since only one congener was transformed. LB400

transformed 18% of 138 (2,2',3,4,4',5'-hexa-BDE) but not the other two congeners 153 (2,2',4,4',5,5'-hexa-BDE) and 149 (2,2',3,4',5',6-hexa-BDE). Hexa-BDE 138 is the only tested congener that has two adjacent nonbrominated carbons at the ortho and meta position where ring hydroxylation of PCBs by LB400 typically begins (29, 30).

Experiments in which RHA1 and LB400 were exposed to elevated concentrations of mono-BDE 3 and tetra-BDE 47 found that bromide is released during PBDE transformation. Between 80 and 100% of the stoichiometric quantities of bromide were released when RHA1 transformed PBDEs. Because this occurred with both a mono-BDE and a tetra-BDE, which has bromines on both rings, this indicates that RHA1 completely debrominated the starting molecule. PCB dechlorination has been observed with RHA1 where stoichiometric concentrations of chloride were released from 2,2'-di-CB (17). Strain LB400 has also been shown to dechlorinate ortho-substituted PCBs (25, 26). This is the first report of complete aerobic debromination of PBDEs with stoichiometric bromide. Previously tested PBDE-transforming bacteria formed a variety of products. Schmidt et al. found that *Sphingomonas* strain SS3 released 35% of the transformed 4-bromodiphenyl ether as bromide (11) while generating 4-bromophenol and 4-bromocatachol as byproducts. Kim et al. showed that *Sphingomonas* strain PH-07 converted 4-bromodiphenyl ether to 4-bromophenol and 2-hydroxymuconic acid (13).

Results with LB400 were significantly different as only 10% of the transformed mono-BDE was released as bromide with the remaining substrate transformed to a hydroxylated mono-BDE as identified by GC-MS. Previous studies with PCBs showed that LB400 could dechlorinate certain PCBs by initial hydroxylation at the chlorinated carbon (31). In this work, however, LB400 hydroxylated the mono-BDE on the nonbrominated ring, suggesting that bromide is being released during subsequent transformation of the hydroxylated metabolite. Why the majority of the substrate is only transformed to the hydroxylated form is unclear, however, the release of approximately 10% bromide suggests that there may be some product toxicity or that the steps beyond the hydroxylation reaction are kinetically rate-limiting. Given that the metabolite 2,3-hydroxybiphenyl from biphenyl degradation by LB400 is known to be toxic to this strain (32), it is possible that the hydroxylated mono-BDE is toxic to the cell as well. The chlorobenzoates formed during breakdown of PCBs by LB400 are also toxic (33). The generation of hydroxylated PBDEs by this strain is potentially problematic to higher organisms as hydroxylated PBDEs are more endocrine-disrupting than their nonhydroxylated counterparts (34–36). Interestingly, LB400 might use a different enzyme to transform PBDEs than PCBs. A biphenyl dioxygenase enzyme (*Bph*) catalyzes the first step of PCB transformation in LB400 by adding two hydroxyl groups to the ring (37–39). With the accumulation of monohydroxylated mono-BDE by LB400, it appears instead that monooxygenase activity may be involved in PBDE transformation by this strain.

Aerobic PCB transformation is typically cometabolic, as has been shown with RHA1 and LB400, although certain species have been found that can metabolize 4-chlorobiphenyl (40). *Sphingomonas* sp. SS3 has been shown to grow on 4-bromodiphenyl ether (10, 11). The ability of RHA1 and PB400 to grow on PBDEs should be investigated, as metabolic growth on PBDEs would eliminate the necessity for a primary substrate such as biphenyl.

The results of this study demonstrate that PBDEs with six bromines or less can be aerobically transformed by PCB-degrading bacteria. Depending on the species, we found that PBDEs can be transformed and release stoichiometric quantities of bromide or lead to an accumulation of hy-

droxylated derivatives with increased endocrine disruption characteristics. This has potential implications for the environment, as PBDE transformation could occur naturally, although further studies are required to assess the potential for these reactions to occur under environmental conditions.

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Supporting Information Available

A detailed description of the bromide analytical method and associated figures as well as the chromatograms of the derivatized hydroxylated mono-BDE. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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