Biotransformations of HBCDs by Rhodococcus Strain Stu-38 and Identification of Transformation Products

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Abstract

1, 2, 5, 6, 9, 10-Hexabromocyclododecanes (HBCDs) are new brominated flame retardants causing serious environmental pollution. Dozens of degradative bacteria have been found with capacity to transform HBCDs. In the present study, an aerobic functional bacterium *Rhodococcus* strain stu-38 was isolated from enriched culture of mangrove sediment using HBCDs as carbon source. This strain could stereoselectively transform HBCDs, the removal rate was α-γ-β-HBCD in the mineral salt medium, but was β-α- and γ-HBCD in the growth medium, and it selectively transformed γ- HBCD in the seawater. Transformation rate of strain stu-38 was lower than other functional strains, however, seven potential debrominated products of HBCDs were identified by using GC-MS. These debrominated products, included dibromocyclododecadiene, bromocyclododecadienol and bromocyclododecatriene were formed through reductive debromination, hydrolytic debromination and dehydrobromination. Overall, *Rhodococcus* sp. stu-38 diastereoisomer-specifically transformed HBCDs to various debrominated products in the different cultural media, which highlighted the complicated stereoselective biotransformation of HBCDs.

Introduction

Hexabromocyclododecanes (HBCDs) are a new type of additive brominated flame retardants (BFRs) applied in extruded (XPS) and expanded (EPS) polystyrene foams. HBCDs are used to improve the flammability resistance or chemically bound to synthetic matrices such as plastics, textiles, electronic circuitry and other materials (Koch et al. 2015). HBCDs released from industry and waste products can infiltrate various ecosystems, leads to serious contamination (Cao et al. 2018). The first detection of environmental HBCDs was in fish and sediment samples from the river Viskan in Sweden, 1995 (Sellström et al. 1998). Since then, they have been found in various environmental media (Cao et al. 2018), even in the human body (Lu et al. 2018; Roosens et al. 2009). Since HBCDs were recalcitrant to degradation and could lead to neurotoxicity (Mariussen and Fonnum et al. 2003), disruption of the inflammatory response in the immune cells (Yasmin and Whalen et al. 2018) and the bronchial epithelial cells (Koike et al. 2016), they had been listed in Annex A of the Stockholm Convention on Persistent Organic Pollutants (POPs) in May 2013.

Over three tons of HBCDs were released into the environment in Europe each year (Koch et al. 2015). The environmental distribution of HBCDs is mainly in the soil and sediment (Cao et al. 2018; Zhang et al. 2018b), which benefits for biodegradation. Biotransformation of HBCDs has been observed in the sludge, soil and sediment. Half-lives of HBCDs in the environment varying from days to months (Davis et al. 2005, 2006; Gerecke et al. 2006; Stiborova et al. 2015). Both biotic and abiotic transformation contribute to removal of HBCDs while the biological activity can greatly enhance HBCDs transformation in the environment (Davis et al. 2006; Gerecke et al. 2006; Morris et al. 2004; Stiborova et al. 2015).

Numbers of isolated bacterial strains and enzymes have been found to transform HBCDs effectively. *Bacillus* sp. HBCD-sjtu could consume 90% of 321.0 μg/mL HBCDs in 4 days (Shah et al. 2018; Shah et

al. 2019). *Pseudomonas aeruginosa* HS9 could degrade 69% HBCDs in four days and had been used for remediation test in plant soil (Huang et al. 2019). Anaerobic dehalorespiring strain *Dehalobium chlorocoercia* DF-1 was added to the sediment to remove γ-HBCD (Demirtepe and Imamoglu et al. 2019). LinB, an hexachlorocyclohexanes (HCHs) haloalkane dehalogenase, converts HBCDs via hydrolytic debromination; LinA2, a HCHs dehydrochlorinase, converts HBCDs through HBr-elimination (Yu et al. 2021).

Three main diastereisomers of HBCDs in commercial products and environmental materials are α-, β- and γ-HBCD. Bacterial debromination of HBCDs are generally diastereoisomer-specific (Yu et al. 2021). *Sphingobium chinhatense* IP26 could transform 27, 20, 78, 63, 39 and 41% of (-)α-, (+)α-, (-)β-, (+)β-, (-)γ- and (+)γ-HBCDs at 1.0 μg/mL in 6 days (Heeb et al. 2017). The degradation rates of α-, β-, γ-HBCD by *Pseudomonas* sp. GJY were with small differences (85.38, 82.64 and 75.50% in 8 days, respectively), but this strain could transform three diastereoisomers to different products (Geng et al. 2019). Strain HB01 selectively debrominated 81% γ-HBCD at a high concentration of 642.0 μg/mL in 5 days (Yamada et al. 2009). Anaerobic strain *Dehalococcoides mccartyi* 195 stereoselectively transformed HBCDs, and the transformation rate of three diastereoisomers followed the order of α-HBCD > β-HBCD > γ-HBCD (Zhong et al. 2018). Diastereoisomer selection had been observed in dehalogenases too. (-)α-, (+)β-, and (+)γ-HBCDs were transformed faster by LinB than their enantiomers, and LinA2 selectively catalyzed the transformation of β-HBCDs (Heeb et al. 2012; 2013; 2014; 2015). It may conclude that the diastereoisomer-specific transformation of HBCDs are result from structurally selection of dehalogenase on the substrates (Heeb et al. 2021; Heeb et al. 2012; Heeb et al. 2013; Suar et al. 2005). However, the debromination behavior of bacterial strain can be different from that of their dehalogenases. For example, though *S. chinhatense* IP26 has genes encoding LinA and LinB, this strain transforms HBCDs through hydrolytic pathway (Heeb et al. 2012, 2017, 2021), which may suggest that the transformation pathway is selectable for functional bacteria.

Moreover, the long half-lives of HBCDs (Davis et al. 2005, 2006) along with the absence of known degradative bacteria in the contaminated material (Stiborova et al. 2015) indicated that the efficient degradative bacteria might not contribute to degradation of HBCDs *in situ*. Dozens of lower effective strains were reported but uncharacterized in the literature (Chang et al. 2020; Yamada et al. 2009), leaving the role of strains with lower efficiency were unclear in the remediation.

Present research investigated the HBCDs transformation by *Rhodococcus* sp. stu-38 in the different materials including mineral salt medium, seawater and the nutrient seawater. In three media, different diastereoisomer-specific transformation trends of HBCDs by this strain were found yielding different debrominated products identified by using GC-MS. Therefore, the results obtained here provided the primary knowledge about the diastereoisomer-specific transformation patterns of a functional strain in various environmental materials, and might contribute to the bioremediation of HBCDs contamination.

Materials And Methods

Chemicals and media

The HBCDs used in this study was a composite of a commercial sample supplied by Adamas-beta (Adamas Reagent Co., Ltd. Switzerland). The composition is about 17.7%: 10.6%: 71.7% for α-, β- and γ-HBCD. Mineral salt medium (MSM) contained 1.0 g/L $(NH_4)_2SO_4$, 0.8 g/L Na_2HPO_4, 0.2 g/L KH_2PO_4, 0.2 g/L $MgSO_4$, 10.0 g/L NaCl, 0.005 g/L $FeCl_3$, 0.001 g/L $(NH_4)_2MoO_4$, and 10-15 g agar for solid media, pH 7.0. For the liquid media containing HBCDs, HBCDs were dissolved in dichloromethane, injected into flasks. Dichloromethane was volatilized before injection of liquid media. Solid MSM was prepared with 1.5 mg HBCDs on the surface.

Seawater was obtained from the offshore area of Shantou, China. Seawater media was filtered through 0.45μm and sterilized. Seawater-LB medium was composed of seawater, 10 g/L peptone, 5 g/L yeast extract powder, pH 7.2-7.5. 100 μg HBCDs were dissolved in dichloromethane, injected into each 50 mL centrifugal tube. Dichloromethane was volatilized before addition of media and bacteria.

Strain isolation and identification

Mangrove sediment was collected from Zhanjiang, China. Sediment was mixed with 100 mL of MSM media using 30.0 mg/L HBCDs as the sole carbon source to enrich functional bacteria. The mixture was cultured at room temperature around 25 ℃, 150 r/min. After 30 days, 2 mL of mixture was transferred to a new flask with 100 mL MSM and 30.0 mg/L HBCDs. In the fifth transfer, the culture was used for isolation on solid media contain HBCDs. Six strains were obtained and named 38-43 in order. The 16S rRNA gene of strains was amplified using the universal primers 27F and 1492R, and sequenced by BGI (Guangzhou, China). The 16S rRNA sequence was aligned using Nucleotide Blast on the NCBI web (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The phylogenetic tree was generated using the neighbor-joining method with MEGA 5.0. Strain 38, renamed stu-38 was used for further research.

Batch experiments

Five groups were set up. In the freshwater group (FW), bacteria were cultured in the seawater-LB media for two days. Cells were harvested by centrifuge at 6000 rpm/min, resuspended with 1mL MSM. Then the cells were inoculated to a 50 mL tube containing 100 μg HBCDs and 5mL MSM. The final concentration of HBCDs was 16.7 μg/mL. Controls (FW-C) were without addition of cells. In the other two groups, seawater and seawater-LB were used to replace MSM, designed as SW and SWLB which were containing cells of strain stu-38. SWLB-C and SW-C were controls without cells. All groups were cultured at room temperature (25-30℃), 150 rpm/min. Each group was triplicate. The residual HBCDs in the FW and FW-C were determined after two months. The residual HBCDs in the SWLB, SWLB-C, SW and SW-C were determined at one month.

Determination of HBCDs residues and identification of debrominated products

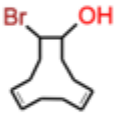
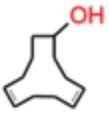
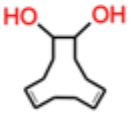
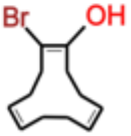
To analyze the residues and debrominated products of HBCDs, the cultures were frozen and dried, and extracted by 15 mL of n-hexane, dichloromethane and acetone in the proportion of 1:1:1. The extracts were dried and dissolved in the methanol, determined by Liquid Chromatograph Mass Spectrometer (LC-MS, Thermo TSQ-Endura, USA) equipped with a dual-mode discrete-dynode detector and a Hypersil GOLD 1.9 μ column (50×2.1 mm). Solvent A (methanol: acetonitrile = 80% :20%) and solvent B (10 mmol/L NH_4Ac) were used as mobile phase. The mobile phase composition started with solvent A: B at 50%: 50%, then to 90%: 10% in 9 minutes, ended up with 100% solvent A in 13 minutes (Morris et al. 2004). The transformation of HBCD was followed by monitoring the HBCD molecular precursor ion (m/z = 640.6 amu) and its fragment ions (m/z = 79.1, 81.1 and 560.5 amu). Concentrations of HBCD were quantified based on the HBCD molecular ion (m/z = 81.1 amu) (Davis et al. 2005). The recoveries of HBCDs in the MSM, SW and SWLB were in the range of 111.1-122.5%, 64.0-70.7% and 140-146%, respectively. The transformation efficiency η was defined as

$$\eta = (C_0 - C_t)/C_0 \times 100\%,$$

where C_0 was the concentration of control at time t in the FW or initial concentration of substrate in other groups, μg/L; C_t was the concentration of HBCD at time t, μg/L.

After determination, the extracts were dried and dissolved in the mixture of n-hexane, dichloromethane and acetone, analyzed by Gas Chromatography/ Mass Spectrometer (GC-MS, QP-2010ULTRA model, Shimadzu, Japan) equipped with a HP-5ms Ultra Inert Capillary Column (30 m×0.25 mm×0.25 μm film thickness). The procedures were 80 ℃ for 1 min, then increase to 250 ℃ in 13 min by 10 ℃/min, and end up with 250 ℃for 10 min. Full scan of molecules in the range of 30-700 m/z was performed in select ion monitoring (SIM) mode to detect possible brominated degradation products (Davis et al. 2005; Zhong et al. 2018). HBCDs dissolved in the acetone and placed in the room temperature for 48 hours to harvest debrominated products (Zhong et al. 2010) were used as the positive control for identification.

Table 1 transformation products identified by GC-MS

	proposed products	structure	m/z	retention time	culture
1	$C_{12}H_{17}Br$ bromocyclododecatriene		241	7.945 10.230	MSM SW-LB
2	$C_{12}H_{18}BrOH$ bromocyclododecadienol		259	8.355	MSM
3	$C_{12}H_{18}Br_2$ dibromocyclododecadiene		322	11.280 14.065 13.085	MSM SW SW-LB
4	$C_{12}H_{19}OH$ cyclododecadienol		180	6.115 7.280	SW SW-LB
5	$C_{12}H_{18}O$ 1,2-epoxy- 5,9-cyclododecadiene		178	6.845	SW
6	$C_{12}H_{18}(OH)_2$ cyclododecadiendiol		196	7.395	SW
7	$C_{12}H_{16}BrOH$ bromocyclododecatrienol		257	10.910	SW-LB

Results

Isolation and identification of bacteria

Six of bacterial strains grew on MSM containing HBCDs were obtained. The colony of strain 38 turned slight red on solid SWLB medium after several days of culture (Fig. 1a). Its 16S rRNA gene sequence displayed 99% identity with *Rhodococcus* strains through Nucleotide Blast analysis on the NCBI website. The GenBank accession number of its 16S rRNA was MT815909. The isolated strain 38 was renamed *Rhodococcus* sp. stu-38. Strain 39 to 43 were identified as *Marinobacter*, *Nitratireductor*, *Brucella*, *Sinomonas* and *Ochrobactrum* in order, as displayed in the Fig. 1.

Transformation of HBCDs by *Rhodococcus* sp. stu-38

The transformation rates of HBCDs by isolates in the FW were determined. Strain *Rhodococcus* sp. stu-38 showed better removal ability than others. As was shown in Fig. 2. 37% α-HBCD and 24% γ-HBCD were removed in comparison with control in two months. And the lowest removal rate was on β-HBCD. Strain *Marinobacter* slightly transformed HBCDs. *Nitratireductor*, *Brucella*, *Sinomonas*, *Ochrobactrum* did not showed transformation ability. Therefore, strain *Rhodococcus* sp. stu-38 were used for further research.

The residual HBCDs in the SW were determined in one month (Fig. 3). At the end of incubation, 42% and 36% of γ-HBCD were removed in the SW and SW-C, respectively. Only about 6% loss of γ-HBCD was related to strain stu-38. However, the decrease of α- and β-HBCD were hardly observed. 33%, 51% and 34% of α-, β- and γ-HBCD were removed in SWLB in one month (Fig. 4). In the SWLB-C, about 15%, 19%, and 16% of α-, β- and γ-HBCD were removed abiotically in one month. Removal of 18%, 32%, 18% of α-, β- and γ-HBCD were related to strain stu-38. In total, 39% of HBCDs in the SWLB was removed in four weeks, higher than 17% in the SWLB-C.

Identification of the debrominated products

GC-MS was used to identify the debrominated products of HBCDs since the m/z of fragment ions were clear (Fig. S1). In the acetone, HBCDs can be chemically debrominated to tribromocyclododecadiene ($C_{12}H_{15}Br_3$) and dibromocyclododecadiene ($C_{12}H_{14}Br_2$) which were used as positive controls (Fig. S2 and S6). The character ions were $C_{12}H_{15}Br_3$, m/z=400.0; $C_{12}H_{14}Br_2$, m/z=319.1; $C_{12}H_{13}Br$, m/z=238.2; $C_{12}H_{12}$, m/z=157.3; C_6H_7, m/z=79. Together, seven debrominated products with carbon frame of HBCDs were identified (table 1). There were two lower brominated alkenes, including dibromocyclododecadiene, bromocyclododecatriene; two lower brominated alkenols, including bromocyclododecadienol and bromocyclododecatrienol; the carbon skeleton of HBCDs, cyclododecatriene; two hydroxylated cyclododecatrienes, including cyclododecadienol and cyclododecadiendiol; one oxidized cyclododecatriene, 1,2-epoxy-5,9-cyclododecadiene. These debrominated products were present at low level (Fig. S6-S9), and were absent in the FW-C, SW-C, SWLB-C and the HBCDs standard solution.

Discussion

Transformation of HBCDs by *Rhodococcus* strain stu-38

In the present research, six bacterial strains grew on MSM containing HBCDs were isolated. Strain *Rhodococcus* sp. stu-38 showed better transformation ability than the others. Stu-38 could not use HBCDs as the sole carbon source and might survive the oligotrophic HBCDs-containing media by living on CO_2 (Feisthauer et al. 2008; Ohhata et al. 2007; Yano et al. 2015). Some *Rhodococcus* strains had been found with dehalogenation capacity, for example, strains 1CP, JT-3 and EK2 could transform various organohalides (Khosrowabadi and Huyop et al. 2014; Roth et al. 2013; Zhang et al. 2018a). This is the first study demonstrated the capacity of *Rhodococcus* strain to convert HBCDs.

The HBCDs removal ability of strain stu-38 was lower than functional strains from other research. For example, *Bacillus* sp. HBCD-sjtu was reported to consume 90% HBCDs at 321.0 μg/mL in four days (Shah

et al. 2018; Shah et al. 2019). *Sphingobium chinhatense* IP26 could transform 78% and 63% of (-) β- and (+) β-HBCDs from initial concentration of 1.0 μg/mL in six days (Heeb et al. 2017). The low efficient transformation ability of stu-38 could be a heritage of converting natural organohalides *in situ* (Verma et al. 2014; Kaster et al. 2014; Yu et al. 2021). The sediment applied for enrichment culture was rarely contaminated HBCDs (unpublished data), which might not be a selective pressure for indigenous bacteria.

Rhodococcus sp. stu-38 selectively removed α-HBCD and γ-HBCD in the FW. The selective transformation by strain stu-38 was also found in SWLB where 33%, 51% and 34% of α-, β- and γ-HBCD were removed. β-HBCD was rarely transformed by strain stu-38 in the FW and SW (Fig. 2 and 3). But in SWLB, strain stu-38 could removed 32% β-HBCD, and showed better transforming ability on α- and γ-HBCD (Fig. 4). Biotransformation rates of a functional strain in different media were not well studied. *P. aeruginosa* HS9 could degrade 69% HBCDs in the MSM in 14 days, but removed 88% HBCDs in the plant soil (Huang et al. 2019). It indicated that the different factor in growing environment of bacteria could affect their transformation ability, in corresponding to biodehalogenation of other organic halogenated compounds (Wang et al. 2015). Diastereoisomers-specific biotransformation of HBCD was also observed in other functional strains. *S. chinhatense* IP26 could transform 27, 20, 78, 63, 39 and 41% of (-)α-, (+)α-, (-)β-, (+)β-, (-)γ- and (+)γ-HBCDs at 1.0 μg/mL in 6 days (Heeb et al. 2017). *Achromobacter* sp. HBCD-1 (Peng et al. 2015), *D. mccartyi* 195 (Zhong et al. 2018), *Pseudomonas* sp. GJY (Geng et al. 2019) were more effective on debromination of α-HBCD. *Pseudomonas* sp. strain HB01 (Yamada et al. 2009) selectively transformed 81% γ-HBCD in five days. The stereoselection on transforming HBCDs was result from the fitness of substrates to the active site of enzyme as revealed by the investigation of dehalogenases LinB, LinA1, LinATM and LinA2 (Heeb et al. 2021; Heeb et al. 2012; Heeb et al. 2013; Suar et al. 2005). As indicated in this study, bacterial diastereoisomer-specificity could also affect by the cultural media.

R. sp. stu-38 facilitated the abiotic transformation of HBCDs

Biotransformation half-lives of HBCDs are varying from a few days to over 100 days in the sludge, soil and sediment (Yu et al. 2021). Present study shew that abiotic transformation of HBCDs had a major contribution in the seawater media, which was corresponding to previous reports that abiotic loss was a large contribution in transformation of HBCDs in the aquatic sediment and active sludge (Davis et al. 2005, 2006). The removal rates of SW and SWLB were higher than SW-C and SWLB-C, indicated that the presence of strain stu-38 could facilitate the removal of HBCDs, which was corresponding to other research (Huang et al. 2019). Chemical and physical factors can lead to abiotic transformation of HBCDs, for example, FeS, nanoscale zero-valent aluminum, sulfidated nanoscale zerovalent iron, and ultraviolet light (Franke et al. 2017; Palau et al. 2017; Yu et al. 2015). Abiotic transformation of HBCDs could have been mediated by chemicals since the cultures were placed in the dark environment.

The augmentation of glucose increased bacterial diversity and improved the removal of HBCDs in the suspension of planted soil (Le et al. 2017). Biostimulation of carbon source could improve the removal of γ-HBCD in the sediment (Demirtepe and Imamoglu et al. 2019). Present research shew that the addition

of nutrition enhanced the removal of HBCDs by stu-38 because bacteria might maintain high activity in the nutrient media. It suggested the augmentation of carbon source as a strategy to improve bioremediation in the contaminated sites. This was different from previous research using pure bacterial strain to transform 3-chlorobenzoate (Chobchuenchom et al. 1996).

The formation of the debrominated products

HBCDs could be chemically debrominated to tribromocyclododecadiene and dibromocyclododecadiene in the acetone (Fig. S2 and S6) (Zhong et al. 2010). At the same retention time, the intensity of tribromocyclododecadiene (m/z=401, 321, 241 and 159) and dibromocyclododecadiene (m/z=321, 241 and 159) were much smaller in the SW than in the acetone (Fig. S2). It was because of the high levels of chemicals from seawater and cells, which could result in the difficulties for the identification of penta- and tetra- brominated products and the detection of α- and β-HBCD (Fig. S1, S7-S9). Moreover, the low transformation efficiency of strain stu-38 leaded to the debrominated products presented at low concentration and weak intensity (Fig. S3-S5, S7-S9).

By using GC-MS, seven biodebrominated products were identified relying on mass spectra of GC-MS (Table 1; Fig. S3-S5, S6-S9). But the accurate identification and further determination were difficult because the limit of standards. Based on the debrominated products observed, the possible transformation pathways of HBCDs by strain stu-38 in the FW, SW and SWLB were proposed (Fig. 5).

The debromination pathways of HBCDs include HBr-elimination (dehydrobromination), HBr-dihaloelimination and hydrolytic debromination (Ang et al. 2018; Yu et al. 2015). HBr-elimination of HBCDs yields lower brominated compounds with an HBr removed to form a carbon-carbon double bond (Kunze et al. 2017). Dihaloelimination involves electron transfer in which HBCDs serve as electron acceptor. Hydrolytic debromination yields lower brominated alkanol or alkenol. These debromination pathways were all observed in the transformation of HBCDs by *R.* sp. stu-38 (Fig. 5). As the toxicity of debrominated products were unknown, it was unclear if the toxicity of debrominated products resulted in the low efficiency of *R.* sp. stu-38 (Heeb et al. 2017; Lal et al. 2010; van Hylckama Vlieg et al. 2000).

Dibromocyclododecadiene, formed through HBr-dihaloelimination, were found in FW, SW and SWLB (Fig. 5). Full debromination via dihaloelimination and the cleavage of cyclododecatriene was not found in this research. The ring opening intermediate was observed in the study of strain GJY (Geng et al. 2019). Strain HS9 (Huang et al. 2019) and GJY (Geng et al. 2019) could convert HBCDs through both reductive and hydrolytic debromination yielding various intermediates. Stu-38 shew similar debromination patterns in the FW and SW, but the biotransformation of HBCDs in the SWLB was more complicated (Fig. 5, S5).

Anaerobic strains *D. mccartyi* 195 (Zhong et al. 2018) and *A.* sp. HBCD-1 (Peng et al. 2015), aerobic strains *P. aeruginosa* HS9 (Huang et al. 2019) and *P.* sp. GJY (Geng et al. 2019) could convert HBCDs through dihaloelimination. The oxygen-tolerant nonrespiratory reductive dehalogenase was also reported (Payne et al. 2015). However, genes encoding such enzymes were not annotated in the genome of stu-38 except several genes encoding haloalkane dehalogenase and haloacid dehalogenase (data not

shown). Haloalkane dehalogenase LinB is known for converting HBCDs via hydrolytic debromination (Heeb et al. 2012). Therefore, further research needs to identify the functional dehalogenases in the *R.* sp. stu-38.

Conclusion

In summary, a new HBCDs-transforming bacteria *Rhodococcus* sp. stu-38 was identified in the present research. This strain selectively transformed HBCDs diastereoisomers in the mineral salt medium, seawater and nutrient seawater. Seven debrominated products were identified by using GC-MS. The formation of debrominated products were partially depended on the culture media. Together, this study demonstrated a functional *Rhodococcus* originated from mangrove sediment which could diastereoisomer-specifically transform HBCDs depending on its living environment, which highlight the monitoring of various lower brominated products of contaminants during bioremediation.

Declarations

Authors' contributions

Wenqi Luo, Xianbin Lin, Shanshan Meng, Lele Li performed the serial diluting culture with HBCDs supplied as the sole carbon source. Fei Yu isolated the bacteria, performed the rest experiment and completed the manuscript. Yuyang Li analyzed the genomic sequence of bacteria. Xueying Ye, Tao Peng, Hui Wang, Tongwang Huang, Zhong Hu provided the directions. All authors read and approved the final manuscript.

Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

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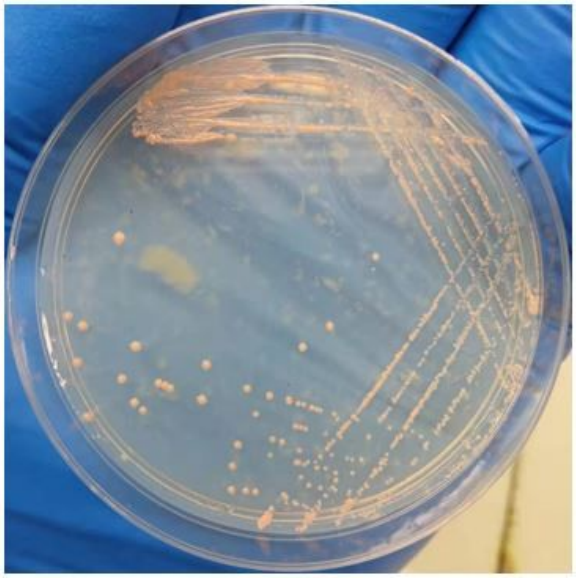
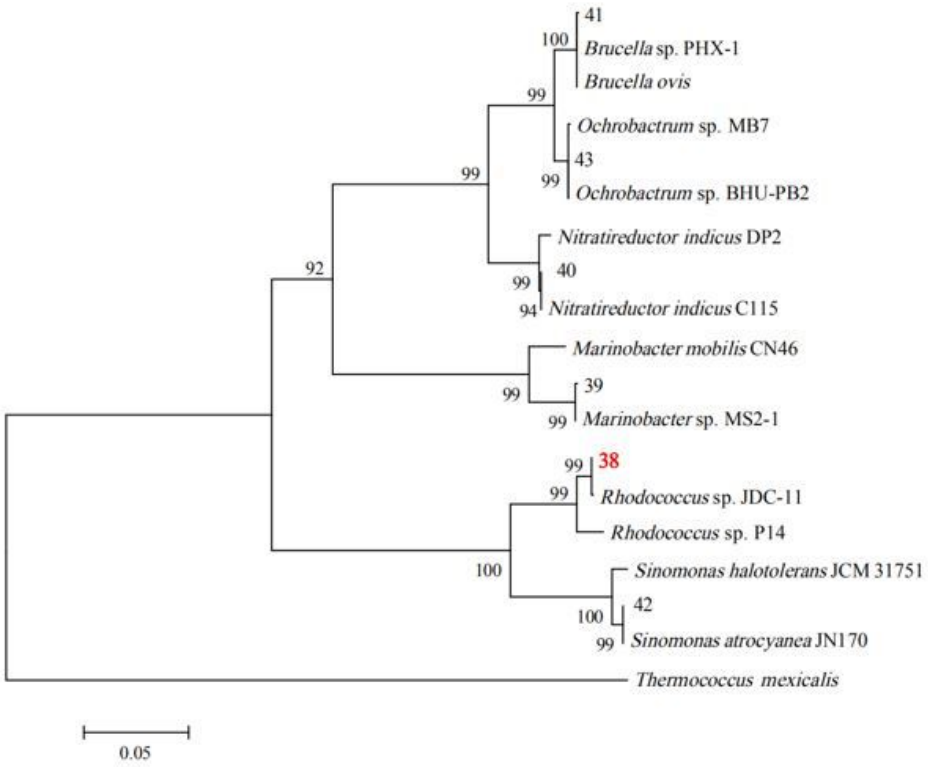
Figures



(a)



(b)

Figure 1

(a) The morphology of colony of strain stu-38. (b) The neighbor-joining tree of six isolated bacteria

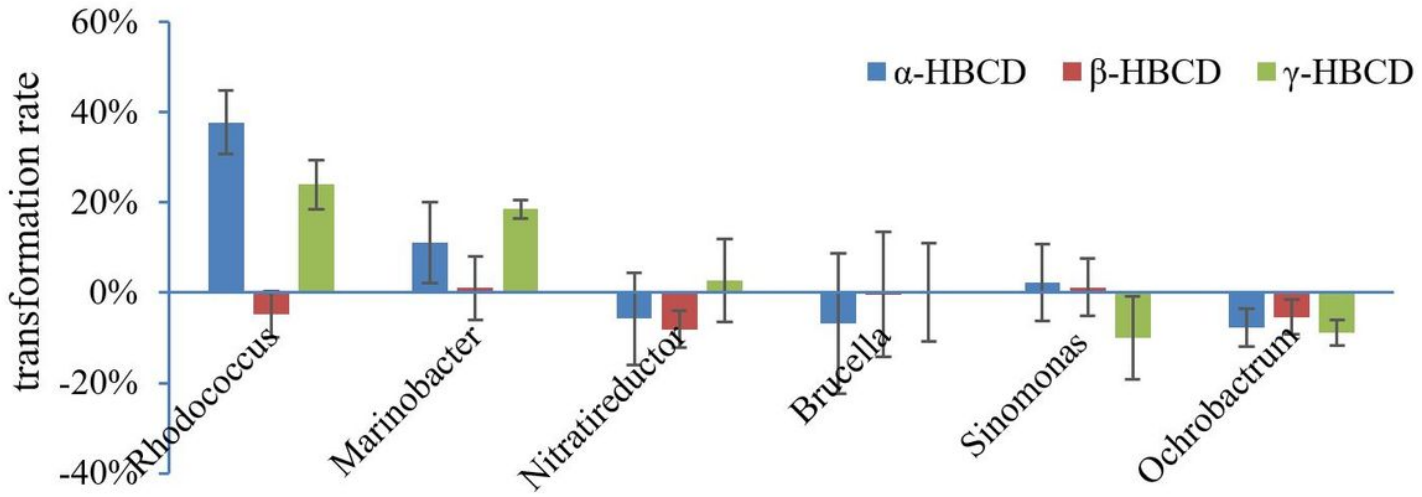


Figure 2

The HBCDs transforming rates of six isolated strains

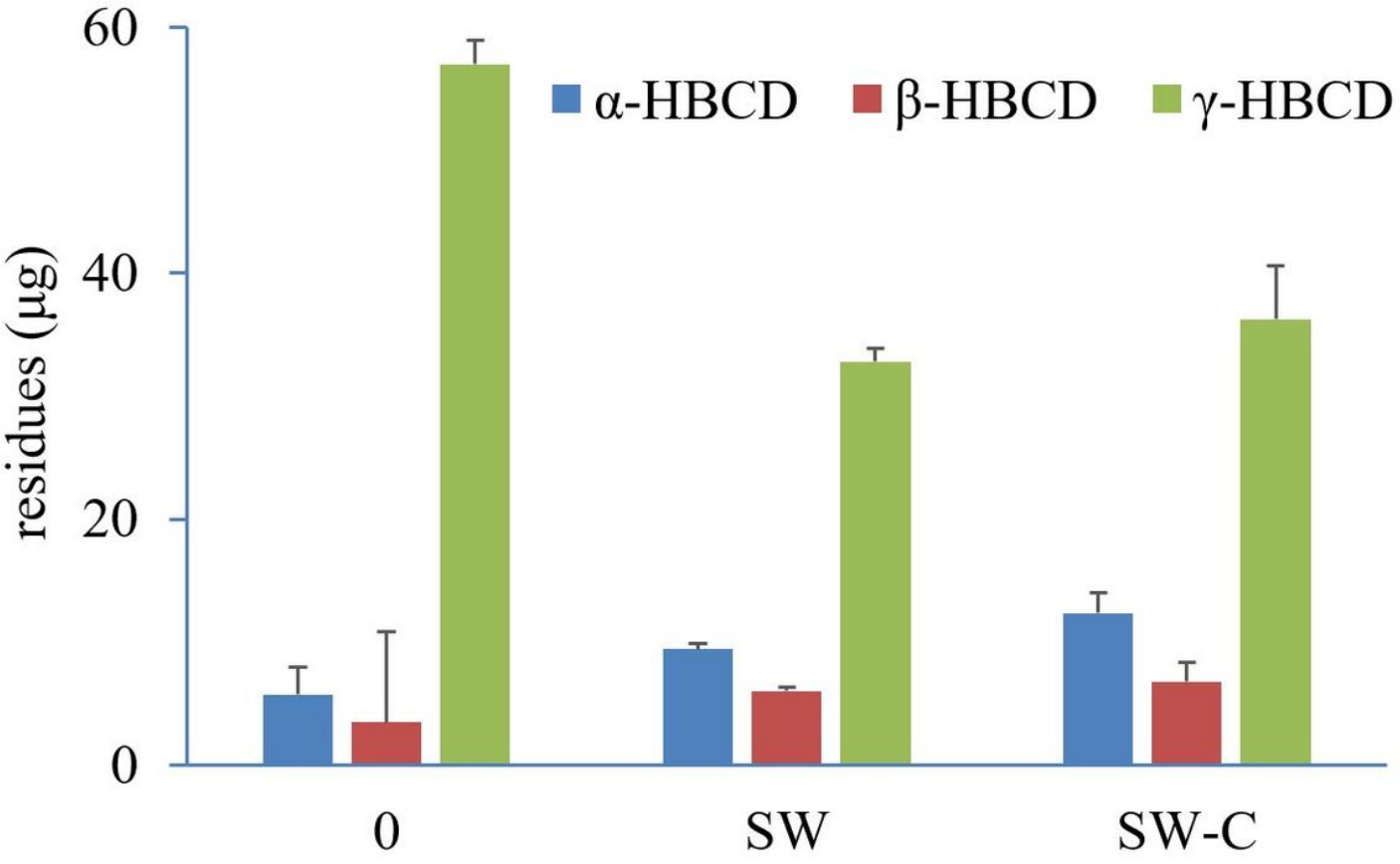


Figure 3

Residues of HBCDs in the SW and SW-C

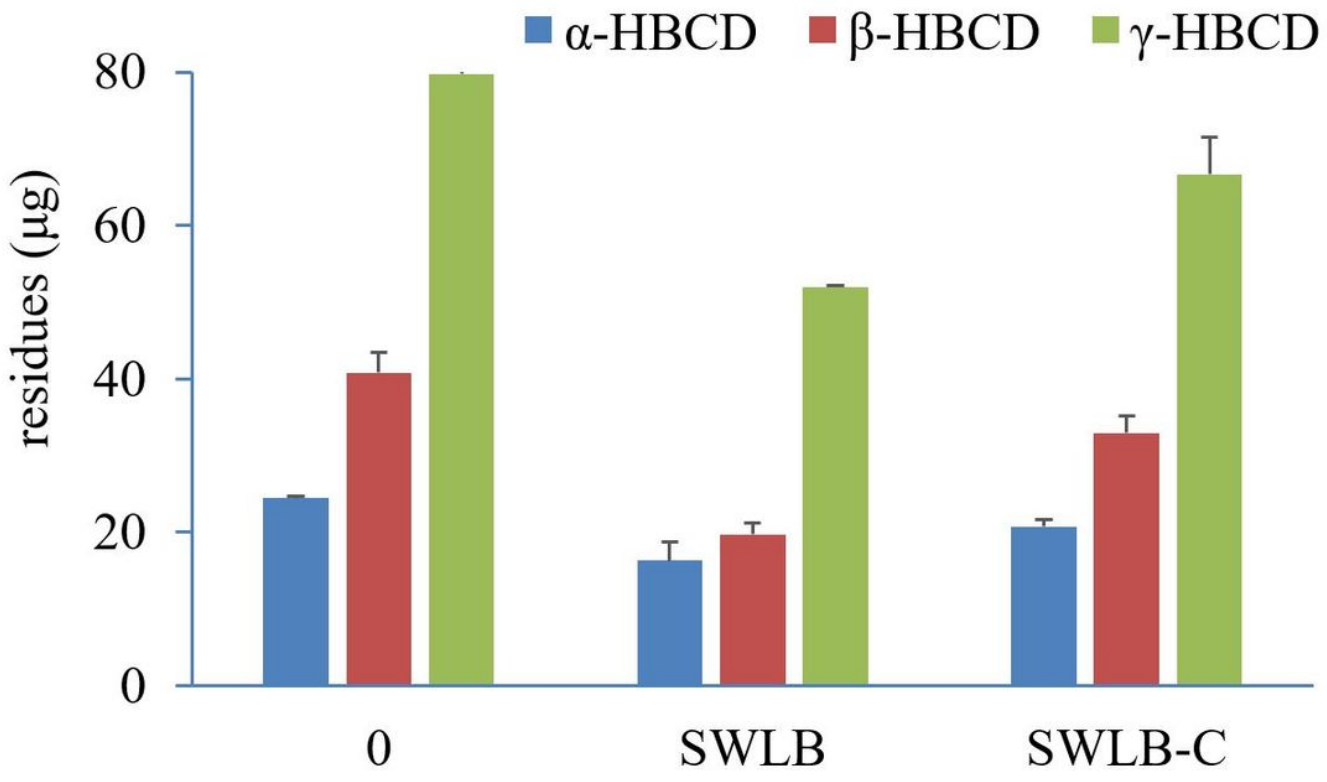


Figure 4

Residues of HBCDs in the SWLB and SWLB-C

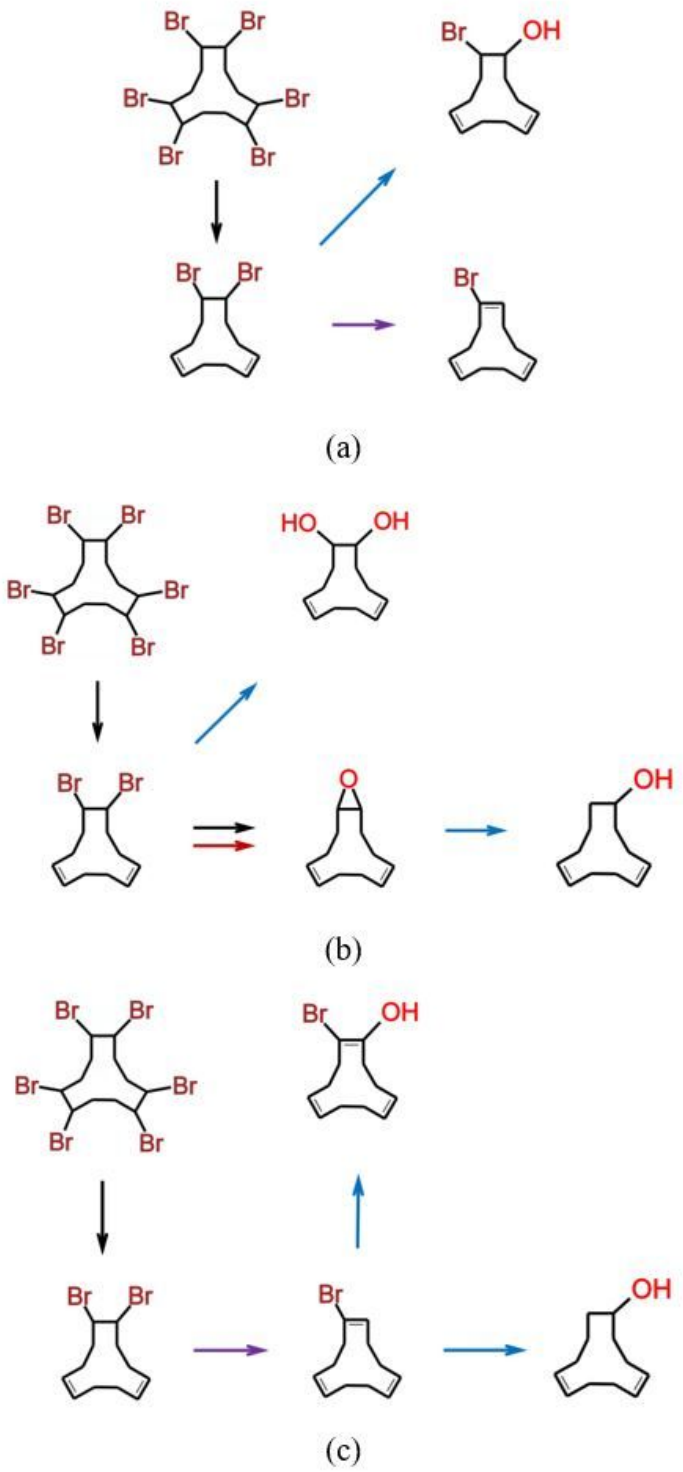


Figure 5

The putative transformation pathway of HBCDs by stu-38 in the (a) FW, (b) SW and (c) SWLB. Black arrow indicates the reductive debromination, blue arrow indicates hydrolytic debromination, the purple arrow shows the HBr-elimination, red arrows represents oxidative reaction

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