Complete genome sequence of *Bacillus* sp. HBCD-sjtu, an efficient HBCD-degrading bacterium

Syed Bilal Shah¹ · Fawad Ali¹ · Ling Huang¹ · Weiwei Wang¹ · Ping Xu¹ · Hongzhi Tang¹

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Abstract

Environmental pollution caused by the release of industrial chemicals is currently one of the most important environmental harms. Manufacturing chemicals can be biodegraded, and valuable intermediates can be used as pharmacophores in drug targeting and have several other useful purposes. Hexabromocyclododecane (HBCD), a non-aromatic brominated flame retardant, is a toxic compound that consists of a cycloaliphatic ring of 12 carbon atoms to which six bromine atoms are attached. It is formed by bromination of *cis-trans-trans*-1,5,9-cyclododecatriene, but its use is now restricted in several countries, because it is an environmental pollutant. Little is known about whether bacteria can degrade HBCD. A bacterial strain that degrades HBCD was recently isolated using enrichment culture techniques. Based on morphological, biochemical and phylogenetic analysis this isolate was categorized as *Bacillus cereus* and named strain HBCD-sjtu. Maximum growth and HBCD-degrading activity were observed when this strain was grown at 30 °C, pH 7.0 and 200 RPM in mineral salt medium containing 0.5 mM HBCD. The genome of strain HBCD-sjtu, which consists of only one circular chromosome, was sequenced. This whole genome sequence will be crucial for illuminating the molecular mechanisms of HBCD degradation.

Keywords HBCD · Brominated flame retardant · BLAST · Degradation · Whole genome sequence · *Bacillus cereus*

Introduction

Brominated flame retardants (BFR), which represent approximately 80% of all fire retardants, are extensively applied in the production of several materials such as plastics, textiles and electronic circuitry, to avoid fires (Fonsecal et al. 2005). HBCD (1, 2, 5, 6, 9, 10-hexabromocyclododecane), an important type of BFR, has become a widely distributed environmental pollutant (Stubbings et al. 2014). A wide range of terrestrial and marine environments contain HBCD (Law et al. 2014). For example, HBCD is commonly found in sea water (Morris et al. 2004), lake water (Stuart et al. 2009), sediment, fish, (Eljarrr et al. 2004), soils (Eguchi et al. 2013), and sewage sludge (Morris et al. 2004), at concentrations ranging from 0.1 to 36,000 ng/g dry weight. Currently HBCD is listed as a high production volume chemical,

and about 16,700 tons were produced in 2001 (Birnbaum et al. 2004).

Due to their toxic and negative effects on human health and the environment, BFRs have attained global attention. Presently, the risk of HBCD toxicity has reached an alarming level due to the increase in HBCD pollution, and also because during the last decade levels of residual HBCD have increased in the food chain of vertebrates (Covaci et al. 2006). Though its exact toxicity is low, toxicological studies have shown that HBCD may induce cancer (Yvonne and Inga 2009; Koch et al. 2015), affect thyroid hormone levels (Koch et al. 2015), distraction of thyroid hormone and liver (Palace et al. 2008; Van der et al. 2006) and cause reproduction disorders (Ema et al. 2008). Likewise, some studies have revealed the toxic effects of HBCD on mammals (Covaci et al. 2006). The harm from HBCD bioaccumulation in food cannot be discounted; for example, the concentration of HBCD in marine organisms ranges from 4.1 to 519 ng/g fat weight and increases by 5% every year (James et al. 2009).

Thus, it is crucial to remove HBCD from the environment. Several techniques have been used to remediate HBCD, such as electro-chemical reduction (Elizabeth et al.

✉ Hongzhi Tang
tanghongzhi@sjtu.edu.cn

¹ State Key Laboratory of Microbial Metabolism, and School of Life Sciences and Biotechnology, Shanghai Jiao Tong University, Shanghai 200240, People's Republic of China

2014), photodegradation (Zhou et al. 2014), and anaerobic microbial degradation (Davis et al. 2005). Because photocatalysis is easily inactivated and the HBCD are difficult to separate, the application of photodegradation is difficult. The drawback of using electro-chemical reduction to degrade HBCD is that it is inefficient. In contrast, microbial degradation, in which living microorganisms are used to degrade harmful substances, is highly efficient and inexpensive (Yamada et al. 2009).

Here, a *Bacillus* sp. Strain, HBCD-sjtu, which efficiently degrades HBCD was isolated from a landfill. This strain could use HBCD as a carbon source and degrade approximately 0.5 mM HBCD in 4 days (Fig. 1). The HBCD present in the culture medium were quantified by HPLC (Agilent 1200 series) equipped with an Eclipse XDB-C18 column (column size, 250 × 64.6 mm; particle size, 5 µm; Agilent). A mixture of methanol and H₂O (95:05 v v⁻¹) was used as the mobile phase, at a flow rate of 0.3 ml min⁻¹. To determine the identity of this strain, we performed Comparative BLAST analysis (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) of the 16S rRNA gene of *Bacillus* sp. HBCD-sjtu. We found that this gene shares 99% similarity with the 16S rRNA genes of *Bacillus cereus* strains S2-8 and L12.

To date, no genome of a *Bacillus* species with the capability to degrade HBCD has been completely sequenced. In this study, we sequenced the whole genome of strain HBCD-sjtu, to obtain useful information about the genes that may be involved in HBCD degradation. The HBCD-sjtu genome was sequenced on the Pacific Biosciences (PacBio)

RS II sequencer (Pacific Biosciences, USA). The generated sequencing reads were de novo assembled using the PacBio RS hierarchical genome assembly process (HGAP) version 2 in SMRT analysis version 2.3 (<https://github.com/PacificBiosciences/SMRT-Analysis>). The 5,230,501-bp genome is composed of one circular chromosome with a G + C content of 35.42%. The coding region is about 4,414,071 bp, and there are 5360 ORFs (Open Reading Frames). The genome also encodes 13 5s rRNAs, 13 16s rRNAs, 13 23s rRNAs, 107 tRNAs, and 127 ncRNAs, which together make up 1.7% of the genome. The genome also contains 10 genes responsible for the metabolism of aromatic compounds. Moreover, 80 genes associated with motility and chemotaxis were found; these genes may help the bacterium to target and degrade chemical compounds (Sampedro et al. 2013). The features of the *Bacillus* sp. HBCD-sjtu whole genome sequence are summarized in Table 1.

The CAZy (Carbohydrate active enzymes) family includes enzymes related to the degradation, modification

Table 1 Genomic features of *Bacillus* sp. HBCD-sjtu

Features	Chromosome
Size (bp)	5,230,501
GC content (%)	3542.00%
rRNAs (5S, 16S, 23S)	(13, 13, 13)
tRNA genes	107
ORF number	5360

Fig. 1 Cell growth and HBCD degradation by strain HBCD-sjtu. Utilization of HBCD as a carbon and energy source by strain HBCD-sjtu. Cell growth and the degradation curve of strain HBCD-sjtu at 30 °C, pH 7 and 200 r/min in time 4 days. The values are the means of 3 replicates, and the error bars indicate the standard deviations

Table 2 Number of different carbohydrate active enzymes in whole genome sequence

Property	Number of GENES	Percentage (%)
GHs	25	0.466
GTs	30	0.56
PLs	0	0
CEs	36	0.672
AAs	4	0.075
CBMs	18	0.336
Others	20	0.373
Total	120	2.481

and production of glycosidic bonds (Lombard et al. 2014). There are five major categories of enzymes in this family: Glycoside Hydrolases (GHs), Glycosyl Transferases (GTs), Polysaccharide Lyases (PLs), Carbohydrate Esterases (CEs), Auxiliary Activities (AAs). We used HMMER (version 3.0) software to predict CAZy genes in the genome of strain HBCD-sjtu, and a total 120 genes were identified (Table 2).

Haloalkane dehalogenases (EC 3.8.1.5), which belong to the α/β -hydrolase fold superfamily (Prokop et al. 2003), are another important class of enzymes capable of cleaving carbon–halogen bonds in halogenated aliphatic compounds. There is increasing interest in these enzymes because of their potential use in bioremediation and as industrial biocatalysts and biosensors. The structures of HBCD and hexachlorocyclohexanes (HCHs) are quite similar, and both are biodegraded by haloalkane dehalogenases linA2 and linB (Heeb et al. 2012, 2014). Genes encoding linA2 and linB and eleven other dehalogenases were annotated in the HBCD-sjtu genome sequence.

Oxygenases are members of the oxidoreductive group of enzymes (E.C. Class I). Using NADH/ NADPH/FAD as the cosubstrate, these enzymes oxidize substrates by shifting one atom of oxygen from molecular oxygen (O_2) to the substrate, while the other oxygen atom is reduced by electrons from cofactors, producing water. Oxygenases are classified as monooxygenases and dioxygenases based on the number of oxygen atoms consumed during oxidation. Oxygenases are very important, because they play a crucial role in the degradation/detoxification of compounds. They contribute to the breakdown of organic compounds by breaking down the aromatic ring and thus increasing the water solubility or reactivity of organic compounds. Without oxygenase activity, the mineralization of xenobiotic compounds would not be possible. Even though oxygenases have such a vital role, there is only limited information about these enzymes (Arora et al. 2009). We identified 20 oxygenase genes in the whole genome sequence of strain *Bacillus* sp. HBCD-sjtu.

The CRISPR recognition tool (CRT 1.2) was used to predict DRs (forward repeats) and spacers (Bland et al.

2007). One CRISPR with a length of 3234 bp, representing 0.0618% of the genome sequence, was identified in strain HBCD-sjtu. The Comprehensive Antibiotic Resistance Database (CARD) can be used to identify antibiotic resistance genes in genomic sequences. At present, the database contains 3008 genes involved in antibiotic resistance and biosynthesis and 4120 genes associated with resistance inhibition. BLAST searches against CARD revealed that the HBCD-sjtu genome contains 51 antibiotic resistance genes (0.951%), 25 antibiotic target genes (0.466%) and 3 antibiotic biosynthesis genes (0.056%) (McArthur et al. 2013).

In conclusion, the WGS of *Bacillus* sp. HBCD-sjtu will enrich the dehalogenase genome database and facilitate studies aimed at elucidating the mechanism of biodegradation of HBCD.

Accession and CCTCC Patent number(s)

The genome sequence of *Bacillus* sp. HBCD-sjtu has been deposited in GenBank under the accession number CP025122. The strain is also has been submitted to China Center for Type Culture Collection (CCTCC) for patent and reservation under the patent number CCTCC M 2017771.

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Compliance with ethical standards

Conflict of interest The authors declared no conflict of interest.

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