Characterization and genomic analysis of an efficient dibutyl phthalate degrading bacterium *Microbacterium* sp. USTB-Y

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Abstract

A promising bacterial strain for biodegrading dibutyl phthalate (DBP) was successfully isolated from activated sludge and characterized as a potential novel *Microbacterium* sp. USTB-Y based on 16S rRNA sequence analysis and whole genome average nucleotide identity (ANI). Initial DBP of 50 mg/L could be completely biodegraded by USTB-Y both in mineral salt medium and in DBP artificially contaminated soil within 12 h at the optimal culture conditions of pH 7.5 and 30 °C, which indicates that USTB-Y has a strong ability in DBP biodegradation. Phthalic acid (PA) was identified as the end-product of DBP biodegraded by USTB-Y using GC/MS. The draft genome of USTB-Y was sequenced by Illumina NovaSeq and 29 and 188 genes encoding for putative esterase/carboxylesterase and hydrolase/alpha/beta hydrolase were annotated based on NR (non redundant protein sequence database) analysis, respectively. Gene3781 and gene3780 from strain USTB-Y showed 100% identity with *dpeH* and *mpeH* from *Microbacterium* sp. PAE-1. But no phthalate catabolic gene (*pht*) cluster was found in the genome of strain USTB-Y. The results in the present study are valuable for obtaining a more holistic understanding on diverse genetic mechanisms of PAEs biodegrading *Microbacterium* sp. strains.

Keywords *Microbacterium* sp. · Degrading characteristics · Degrading genes · Dibutyl phthalate · Soil remediation

Introduction

Phthalate esters (PAEs) composed of a benzene ring and two side chains at the ortho-position are the most widely used plasticizers in plastic products, medical supplies, home appliances and toys (Daiem et al. 2012). In 2014 the global consumption of plasticizers reached 8.4 million tons, in which PAEs account for 70% (Ren et al. 2018). Due to physical bond (such as molecular forces and polymerization forces) to plastic products, PAEs are easily released into environment (Benjamin et al. 2015), such as agricultural soil (Lü et al. 2018), water and sediments (Adeogun et al. 2015), atmospheric aerosols (Philip et al. 2018). PAEs are classified as endocrine disrupting chemicals (EDCs) due to their estrogenic and endocrine disrupting properties which may cause fertility problems (Latini et al. 2006; Mathieu-Denoncourt et al. 2015), respiratory diseases, childhood obesity, abortion, and neuropsychological disorders (Stojanoska et al. 2017). Six of PAEs including dimethyl phthalate (DMP), diethyl phthalate (DEP), dibutyl phthalate (DBP), dinonyl phthalate (DOP), diethylhexyl phthalate (DEHP), and butyl benzyl phthalate (BBP) have been listed as the priority pollutants by the United States Environmental Protection Agency (USEPA), the European Union (EU), and the China National Environmental Monitoring Center (US EPA 1992; European Union 1993; Xu et al. 2005).

Conventional available methods for removing PAEs include biodegradation, advanced oxidation (Tichonovas et al. 2017) and photocatalysis (You et al. 2018), and biodegradation is considered to be an efficient, low-cost, safety and stability method (Gao et al. 2016). In the past 40 years, lots of PAEs biodegrading bacteria mainly belonging to genera *Pseudomonas*, *Arthrobacter*, *Comamonas*, *Gordonia*, *Rhodococcus*, and *Sphingomonas* have been isolated from water, sediment, soil, etc., but microbial resources of high-efficiency and applicable strains were limited (Wang et al. 2003; Wu et al. 2011; Ren et al. 2018; Shen et al. 2019). Although *Microbacterium* sp. strains were reported to biodegrade or transform DEHP and DBP (Chen et al. 2007;...
Lu et al. (2020), few studies on the mechanism and genomic analysis of *Microbacterium* sp. were found up to now.

The present work described the isolation and identification of a DBP biodegrading *Microbacterium* sp. USTB-Y which could transform DBP to PA. It explored the mechanism of biodegradation and characterized the environmental factors influencing the degradation process. In addition, we analyzed the whole genome shotgun sequencing to putatively assign genes expected to be involved in PAEs metabolism and also proposed the reason why *Microbacterium* sp. USTB-Y was unable to degrade PA.

### Materials and methods

#### Chemicals and culture medium

DMP, DEP, DBP, BBP, DEHP, DOP, monoethyl phthalate (MEP), monomethyl phthalate (MMP), monobutyl phthalate (MBP), phthalic acid (PA), protocatechuic acid (PCA) and n-butyl alcohol (analytical grade, above 98% of purity) used for biodegradation experiments was purchased from Aladdin Chemistry Co. (Shanghai, China). Standard stock solution of PAEs in methanol was prepared. All organic solvents used were of HPLC grade while other reagents were of analytical grade.

Improved Luria–Bertani (LB) medium contained (g/L) NaCl (10), beef peptone (10), yeast extract (5), glucose (5) and its final pH was adjusted to 7.5.

Mineral salt medium (MSM) contained (g/L) NH₄Cl (0.5), Na₂HPO₄ (0.3), KH₂PO₄ (0.05), MgSO₄ (0.1), CaCl₂ (0.01), ammonium ferric citrate (0.01), 0.5 mL trace elements (Karn et al. 2010), and its final pH was adjusted to 7.5. The media and vessels (e.g., Erlenmeyer flask) were sterilized in an autoclave at 121 °C for 20 min.

#### Enrichment and isolation of DBP biodegrading bacteria

Activated sludge samples were collected from Weifang Kangda sewage treatment factory of Shandong in China. Standard stock solution of DBP was added to a 250-mL triangular flask and the solvent methanol was allowed to evaporate overnight before adding 50 mL MSM and 10 g of activated sludge. These flasks were incubated at 30 °C and 200 rpm. An aliquot of 5 mL was subcultured to fresh medium every week and the concentration of DBP increasing from 50 to 1000 mg/L (50, 100, 200, 400, 600, 800, and 1000 mg/L). After 7 weeks, some bacteria could grow in the medium with 1000 mg/L DBP; then serial dilutions of the final bacterial culture were conducted and inoculated with spread method onto the LB agar plates and cultured for 48 h at 30 °C. Different isolates were screened with the ability to biodegrade DBP, and a promising bacterial strain USTB-Y was isolated and used.

#### Identification and whole genome sequencing of strain USTB-Y

The morphology of strain USTB-Y was observed by a microscope (OLYMPUS DP72, Japan). Total DNA was extracted from the purified bacterium using the Bacterial Genome Extraction kit (Tiangen, Beijing) following the manufacturer’s instructions. Following centrifugation, the supernatant was used as template for polymerase chain reaction (PCR) with the primer pair of 27F (5′-AGAGTTTGTATCCTGGCTAG-3′) and 1492R (5′-GGTACCTTGTTACGACTT-3′) (Zhao et al. 2021). The PCR products were sequenced by Shenggong Biotechnology Co., Ltd. (Shanghai, China) and deposited in the GenBank database under the accession number MW828316. The resulting sequence of strain USTB-Y were aligned and compared with the known gene sequences in EzBioCloud database. The nearest neighbor sequences were aligned using Clustal W and a phylogenetic tree based on the 16S rRNA sequence data was constructed by the neighbor-joining method with MEGA 7 software. To define the phylogenetic relationship, the genome sequences of available *Microbacterium* sp. strains in EzBioCloud database were downloaded from NCBI and whole genome average nucleotide identity (ANI) values were obtained from pairwise comparison online (EzBioCloud database).

The draft genome was sequenced by Illumina NovaSeq platform of Beijing Fixgene Co., Ltd. Low reads were trimmed by fastp software. High quality reads were then assembled by Spades software. The genome sequence was annotated by the PGAP on NCBI (October, 2020), and submitted to NCBI Sequence Read Archive (https://www.ncbi.nlm.nih.gov/sra/) with SRA number SRR14179763.

#### Preparation of the bacterial suspension

Strain USTB-Y was pre-cultured in LB liquid medium at 30 °C and 200 rpm for 18 h (at logarithmic phase) and cell pellets were harvested by centrifugation (6000 rpm for 10 min), washed three times with sterile physiological saline water and resuspended in MSM to obtain the inoculum suspension (approximately 1.2 × 10⁸ CFU/mL determined by colony counting method). Inoculation proportion of 4% (volume ratio) was used in the following biodegradation experiments.

#### Effect of culture conditions on DBP biodegradation by strain USTB-Y

In this experiment three single factors including pH, temperature and substrate concentration were used as independent
variables to investigate their effects on the biodegradation ability of DBP by strain USTB-Y. The pH of culture medium was set at 5.5, 6.5, 7.5, 8.5 and 9.5 (30 °C, initial concentration of DBP at 50 mg/L); temperature at 15, 25, 30, 37 and 40 °C (pH at 7.5, initial concentration of DBP at 50 mg/L); and initial concentrations of DBP at 50, 100, 500 and 1000 mg/L (30 °C, pH at 7.5).

Bacterial suspension (0.8 mL) was inoculated into a 150 mL Erlenmeyer flask with 20 mL liquid MSM while the MSM without inoculation served as the control. They were then incubated with triplicates on an incubator at 200 rpm in dark for 24 h and samples of culture solution were treated to analyze the residual DBP concentration. Samples with different initial DBP concentrations were collected at regular time intervals to get the biodegradation curves.

Biodegradation of DBP, MBP and PA by strain USTB-Y in MSM

MBP and PA were reported as main intermediates of DBP biodegradation by Microbacterium sp. (Chen et al. 2007; Lu et al. 2020). To investigate biodegradation ability of DBP, MBP and PA by strain USTB-Y, the initial concentration in MSM was set at 500 mg/L. Culture solution was sampled every 24 h to obtain the growth curve of strain USTB-Y and the degradation curve. The experiment was performed at optimal conditions following the procedure above.

Analysis of residual substrates by HPLC

DBP and its product were quantified using HPLC followed an improved method described by Lu et al. (2020). Twice the volume of methanol was added to the culture solution, and the mixture was vortexed for 5 min before it was centrifuged at 10,000 rpm for 5 min, then the supernatant was filtered through a 0.22 μm organic nylon micropore membrane and analyzed by an Dionex Ultimate 3000 HPLC (Thermo Fisher Scientific Inc., USA) which was equipped with a C18 reverse phase column (250×4.6 mm², 5 μm particle size, Dikema Technology Co. Ltd., China) and a UV-detector at 228 nm. For DBP analysis, methanol and an aqueous solution of 0.5% acetic acid (80:20, v/v) were used as the mobile phase with a flow rate of 1.0 mL/min. The temperature of column oven was 30°C. For quantification, an external standard method was applied and the standard curve was built following a six-point calibration curve (ranged from 25 to 500 mg/L and the R² was above 0.99). All experiments were conducted in triplicates and were subjected to statistical analysis.

Identification of DBP biodegradation product by GC–MS

To investigate the degradation pathway of DBP, the culture samples containing 100 mg/L DBP were collected at 0, 4, 10 and 12 h, respectively and treated to analyze the residual DBP and its biodegradation product. Cultured solution was extracted twice with equal volume of dichloromethane, the combined organic phase was concentrated to near dryness by a rotary evaporator and then the residue was redissolved in acetone for GC-MS (QP2010 Plus, Shimadzu, Japan) analysis. A capillary column (HP-5MS, 0.25 μm × 0.25 mm × 30 m) was used for separation. Helium (> 99.99% of purity) was employed as carrier gas at a flow rate of 1.0 mL/min. The temperatures of injection and ion source were set at 250 °C and 220 °C, respectively. The GC oven temperature was programmed as follows: 100 °C held for 2 min, raised at 15 °C/min to 129 °C, then at 40 °C/min to 280 °C (held for 5 min). The extract (1 μL) was injected in the splitless mode. Mass spectra were acquired in the electron ionization (EI) mode using an electron impact ionization of 70 eV and scanning at 45–500 amu.

Substrate utilization tests

Liquid MSM of 20 mL was supplemented with each compound of PA, PCA, MMP, MEP, DMP, DEP, DOP, DEHP and BBP (100 mg/L) as the sole carbon source to test the substrate utilization of strain USTB-Y. After culturing at optimal condition for 72 h, both the substrate concentration and the cell quantity (CFU) in the culture solutions were determined. The growth of USTB-Y in MSM supplemented with n-butyl alcohol as the sole carbon source was also determined.

Bioremediation of DBP artificially contaminated soil by strain USTB-Y

Soil samples were taken from top 20 cm of soil profile on a botanical garden in Weifang and contained no detectable residual PAEs. The soil was mixed thoroughly, sieved through 2-mm mesh to remove stones and debris and stored at room temperature overnight. Soil properties were measured as follows: pH 6.83, organic carbon 15.4 g/kg, total nitrogen 1.63 g/kg, total phosphorus 0.79 g/kg, total potassium 13.75 g/kg. Soil samples were autoclaved and treated with DBP stock solution thoroughly to get a concentration of 50 mg/kg dry soil and followed by methanol evaporation for 12 h at room temperature. Prepared soil of 50 g was put into a 150 mL flask sealed with a gas-permeable membrane and inoculated with the prepared inoculum to give an initial bacterial population of 6×10⁹ CFU/g as the biodegradation treatment. All soil samples were cultured at 30 °C in the
dark for 12 h. DBP-contaminated soil without inoculation served as a blank control and all treatments were prepared in triplicate. Soil samples of 2 g were collected and soaked with 20 mL of a mixture of acetone and hexane (1:1, v/v) overnight in a glass centrifuge bottle and then extracted ultrasonically three times to collect organic phase. After centrifugation at 6000 rpm for 10 min, the supernate solution was concentrated to near dryness with a rotary evaporator. The residue was redissolved in 5 mL methanol which was diluted to a suitable concentration for HPLC analysis.

Statistics analyses

The data were calculated, analyzed and plotted by Microsoft Excel 2010 (Microsoft Co., USA). All the statistical analysis was performed by using statistical analysis tools SPSS 28.0 using one-way ANOVA (analysis of variance). The data was evaluated in 95% significance interval (p < 0.05 was considered as the threshold for statistical significance).

The degradation ratio of DBP was calculated by Eq. (1):

\[
\text{Degradation ratio (\%) } = \left( \frac{C_0 - C_t}{C_0} \right) \times 100\%
\]

where \( C_0 \) is the initial concentration of DBP in culture (or soil) and \( C_t \) is the residual concentration of DBP after biodegradation experiment.

The biodegradation process of DBP was confirmed by first-order kinetic model as Eq. (2), the half-life of first-order reaction could be calculated by Eq. (3) (Suzuki et al. 1998; Heo et al. 2020):

\[
\ln C = -kt + A
\]

\[
t_{1/2} = \frac{\ln 2}{k}
\]

where \( C \) is the DBP concentration (mg/L) at time \( t \) (h), \( k \) is the first-order kinetic constant (h\(^{-1}\)) and \( A \) is a constant. \( t_{1/2} \) is the half-life corresponding to the time interval of the DBP concentration to decrease to half of its initial value.

Results

Isolation and identification of the bacterial strain USTB-Y

Strain USTB-Y capable of utilizing DBP as the sole carbon and energy source was isolated from activated sludge. On a LB plate, its colonies were regular round, opaque, smooth and glossy surface, and turned to yellow after 72 h. The morphological examination under the light microscope revealed short rods cells (Online Resource 1 Fig. S1-1). The physiological and biochemical assays indicated that strain USTB-Y was a Gram-positive strain, capable of producing catalase and starch hydrolase (Online Resource 1 Table S1-1). Strain USTB-Y was deposited in culture collection of CGMCC (China General Microbiology Culture Collection Center, No. 1.18977). The 16S rRNA gene sequence (1393 bp) of strain USTB-Y was searched against the EzBioCloud database. It showed 99.14% identity to Microbacterium azadirachtae DSM 23848 (T). Phylogenetic analyses based on 16S rRNA sequences within genus Microbacterium suggested strain USTB-Y was closest to the species Microbacterium panacieterrae DCY56 (T) (Fig. 1a). ANI values between USTB-Y and the members of genus Microbacterium were in the range of 74.98 to 86.61% (Online Resource1 Table S1-2). Nine nearby neighbor species to strain USTB-Y were chosen to phylogenetic tree analysis on the basis of the whole genome sequences by using OAT. The result revealed that strain USTB-Y shared highest identities (86.61%) to Microbacterium azadirachtae DSM 23848 (Fig. 1b). According to current bacterial taxonomy, strain USTB-Y is a potential novel species within Microbacterium genus for their ANI < 95% (Kim et al. 2014).

Effect of culture conditions on DBP biodegradation by strain USTB-Y

Figure 2 shows effects of culture conditions (temperature, pH and substrate concentration) on the biodegradation ratios of DBP (50 mg/L) by strain USTB-Y within 24 h. DBP biodegradation ratios were not significant different among the two tested temperatures (i.e., 25 and 30 °C) and they were all higher than 96% (Fig. 2A), but declined to 60%, 58% and 48% when the temperature was at 37, 15 and 40 °C, respectively. These results imply that DBP could be easily biodegraded under tropical temperature. When pH values were within 5.5–9.5, DBP could be efficiently biodegraded by strain USTB-Y with the biodegradation ratios generally > 96% and at pH values 6.5–8.5 its biodegradation percentage reached about 98.5% (Fig. 2B). It implied that strain USTB-Y could adapt to a wide range of pH to biodegrade DBP. The optimal culture conditions (pH 7.5, 30 °C) were used in further biodegradation experiments. Additionally, within the initial concentrations of 50–100 mg/L, DBP could be completely biodegraded by strain USTB-Y within 12 h (Fig. 2C). Within the initial concentrations of 500–1000 mg/L, DBP could be completely biodegraded within 120 h (Fig. 2D). These results demonstrated that strain USTB-Y could adapt to the environment with high concentration of DBP with ideal degradation efficiency. Moreover, Kinetic analysis reveals that DBP biodegradation by strain USTB-Y was best fitted to the first-order kinetics (\( R^2 > 0.9 \)). When DBP initial concentrations were 50 and 100 mg/L, the first-order biodegradation constant (\( k \)) was 0.3331 and 0.393 h\(^{-1}\) and the \( t_{1/2} \) was 3.61 and 3.35 h, respectively. When the initial concentrations of DBP were increased to 500 and 1000 mg/L,
the first-order degradation constant ($k$) fell to 0.0628 and 0.0607 h$^{-1}$ with the half-life increasing to 21.00 and 24.71 h, respectively (Online Resource 1 table S1-3). The biodegradation ratio of DBP was dose-dependent and the initial concentration of DBP may play an important role in the biodegradability of DBP.

Biodegradation of DBP, DMP and PA by strain USTB-Y in MSM

Initial MBP and DBP up to 500 mg/mL could be completely biodegraded by strain USTB-Y within 72 h and 96 h, respectively and the cells grew vigorously with the biodegradation progressed (Online Resource 1 Fig. S1-2). However, PA was hardly biodegraded by strain USTB-Y and the cell quantity was not increased in the PA medium. It indicated that strain USTB-Y could utilize DBP and MBP individually as the sole carbon source, but not PA.

Identification and bioconversion analysis of DBP to PA

Results in Fig. 3 showed that DBP (retention time at 11.925 min) was completely biodegraded and a product (retention time at 4.17 min) was accumulated in the end. It indicated that strain USTB-Y transformed DBP to another compound whose retention time was same with the PA standard. The product was further identified by GC–MS (Fig. 4). By comparing with the authentic standard solution and the published mass spectra from National Institute of Standards and Technology database (NIST14s library), the compound I at 0 h and 10 h sample was DBP and the compound II at 10 h was corresponding to phthalic acid (PA). The peak around position 33 min is a polymer which is both appeared at 0 h and 10 h.

In the bioconversion of DBP to PA, with the concentration of PA increased the concentration of DBP decreased indicating a progressive bioconversion of DBP to PA by
strain USTB-Y (Online Resource 1 Fig. S1-3). DBP with 100 mg/L was biodegraded completely at 12 h and the concentration of PA reached 46.85 mg/L and almost no change occurred after approximately 7 days. In the control group without strain USTB-Y the concentration of DBP was constant. In addition, the pH value of the medium solution was gradually declined from 7.49 to 6.84 with the proceeding of DBP biodegradation which indicated that the accumulation of acidic compound (PA).

Substrate utilization of strain USTB-Y

The biodegradation of individual PAEs (DMP, DEP, DOP, BBP and DEHP) and their intermediates (MMP, MEP, PA and PCA) were measured (Online Resource 1 Fig. S1-4). Strain USTB-Y could completely biodegrade tested dialkyl PAEs with shorter alkyl-chains such as DMP, DEP and BBP; but hardly biodegraded tested dialkyl PAEs with longer alkyl-chains such as DEHP and DOP (biodegradation ratio < 5%). For tested intermediates, it showed relatively higher biodegradation ratio against MEP (79%) but showed lower biodegrading efficiency against MMP (26%) and hardly biodegraded PA and PCA (biodegradation ratio < 5%). Results of growth characteristics showed that strain USTB-Y had a vigorous growth on DMP, DEP and BBP but hardly grew on PCA, DOP, DEHP and PA, which agreed well with the biodegradation results above. Meanwhile, it showed that strain USTB-Y grew vigorously in MSM supplemented with n-butyl alcohol, which indicated that it could use n-butyl alcohol as the sole carbon source for growth.

Bioremediation of DBP artificially contaminated by strain USTB-Y

DBP at initial concentration of 50 mg/kg decreased sharply by 100% in the biodegradation treatment but did not change remarkably in the control (about 11%) within 12 h (Online Resource1 Fig. S1-5). The biodegradation ratio of DBP in the treatment inoculated with strain USTB-Y was significantly higher than the control (without inoculation) after 12 h of incubation. This result indicated that USTB-Y could biodegrade DBP efficiently in artificially contaminated soil.

Overview of genome analysis and molecular mechanism for DBP biodegradation

The draft genome was sequenced using the Illumina Hiseq platform with a paired-ends sequencing. After quality control, the theoretical coverage ratio of clean data was > 100 x and both quantity and quality met the analysis requirements and sequencing statistics was shown in table S1-4 (Online Resource1). It revealed a total length
Fig. 3  HPLC chromatograms for DBP biodegradation by USTB-Y in MSM after the following times: a 0 h; b 4 h; c 10 h; d 12 h
of 4,109,641 bp with an average GC content of 70.67%. The reads were assembled into 20 scaffolds with a N50 of 745,149 bp. Genome properties and statistics of strain USTB-Y were listed in Online Resource1 table S1-5. The results of genome annotation showed that 81.59% of genes (3200) were assigned to 20 different categories of COG (Online Resource1 Fig. S1-6). The genes number of carbohydrates transport and metabolism (G), secondary metabolites biosynthesis, transport and catabolism (Q) and intracellular trafficking, secretion, and vesicular transport function (U) were 268, 58 and 30, respectively. According to the KEGG analysis, 75 genes were involved in the Xenobiotics biodegradation and metabolism among which 25, 13, 26, and 9 genes were predicted to participate in benzene metabolic pathway, biodegradation of aromatic compounds, phenylalanine metabolism and aminobenzoate degradation, respectively (Online Resource1 Fig. S1-7, Online Resource 2 and 3).

In the present study, Microbacterium strain USTB-Y was found to be able to transform DBP to PA. According to NR analysis, there were 29 and 188 genes encoding for putative esterase/carboxylesterase and hydrolase/alpha/beta hydrolase, respectively (Online Resource 4). Among these genes encoding for esterases or hydrolases, gene3780 and gene3781 showed 100% identity with mpeH (GenBank accession no. MK165157) and dpeH (GenBank accession no. MK165156) from Microbacterium sp. PAE-1, respectively (Lu et al. 2020). It was reported that DpeH could only hydrolyze DBP to MBP while MpeH could only hydrolyze MBP to PA (Fig. 5). Furthermore, gene3777 and gene3778 in the upstream of gene3780 were annotated to transposase (Online Resource 6). These findings provide important information of the molecular mechanism for DBP transformation to PA. Additionally, no phthalate catabolic gene cluster (pht) was identified in the genome of strain USTB-Y. Moreover, there were no enzymes annotated to participate in the phthalate biodegradation in the pathway map of aromatic compounds in KEGG (Online Resource 5). These results elucidated the molecular mechanism for inability to biodegrade PA by strain USTB-Y.

### Discussion

The genus Microbacterium was characterized as Gram-stain positive, asporogenous and rod-shaped with optimum growth observed at 20–30 °C (Suzuki et al. 1998), and distributes extensively in various environments including soil, seawater,
Fig. 5 Genome map of reference genes (dpeH and mpeH) in Microbacterium sp. PAE-1 and genes of scaffold 11 in Microbacterium sp. USTB-Y (sequences of gene3780 and gene3781 showed 100% identity with the sequences of mpeH and dpeH, respectively.) (Lu et al. 2020). Note The length and direction of the arrow on the map represent the length and coding direction of the gene, respectively. If a gene is staggered, it means it overlaps with upstream or downstream genes.

Members of genus Microbacterium were reported to biodegrade various organic contaminants such as crude-oil (Schippers 2005), sulfonamide antibiotics (Ricken et al. 2015), carbendazim (Lei et al. 2017), benzo[a]pyrene and pyrene (Qin et al. 2017), PAHs (Cazals et al. 2019), decabromodiphenyl ether (Yu et al. 2019), etc. In 2007, Chen et al. first reported that Microbacterium sp. CQ0110Y could biodegrade DEHP (Chen et al. 2007). Yang et al. reported that a stable DBP-biodegrading bacterial consortium B1 was mainly composed of Pandoraea sp. and Microbacterium sp. (Yang et al. 2018). In this study, a newly isolated Microbacterium sp. USTB-Y from activated sludge could efficiently transformed DBP into PA and biodegraded DMP, DEP and BBP in MSM, which is consistent with the results of Microbacterium sp. PAE-1 (Lu et al. 2020).

Strain USTB-Y could biodegrade DBP at a broad range of temperatures (15–40 °C) and pH (5.5–9.5) and better adapted to low temperature and acidity. Both the degradation ratios at 15 °C and pH 5.5 reached more than 58% (Fig. 2), higher than that of consortium B1 (Yang et al. 2018) and consortium LV-1 (Wang et al. 2017). In this study, Microbacterium sp. USTB-Y could biodegrade almost all DBP of 100 mg/L within 12 h, far higher than that by bacterial consortium LV-1 (Wang et al. 2017), Gordonia sp. QH-12 (Jin et al. 2016) and Bacillus subtilis N-1 (Huang et al. 2018) (100% within 3 days, 100% within 20 h, and 100% within 5 days, respectively). DBP degradation constants (0.0607–0.3331 h⁻¹ with half-life of 3.61–24.71 h for 50–1000 mg/L of DBP) by strain USTB-Y was far higher than those obtained by endphytic Bacillus subtilis N-1 (0.0122–0.0959 day⁻¹ with half-life of 7.23–56.8 h for 100–200 mg/L of DBP) (Huang et al. 2018), Spingobium yanoikuyae SHJ (0.1867 day⁻¹ with half-life of 101.4 h for 50 mg/L of DBP) (Feng et al. 2018a, b), Providencia sp. 2D (0.0265–0.0800 day⁻¹ with half-life of 8.66–26.16 h for 50–1000 mg/L of DBP) (Zhao et al. 2016), Strain USTB-Y could biodegrade 50 mg/L DBP from artificially contaminated soil within 12 h which was much better than the biodegradation of DBP by Bacillus subtilis N-1 (5 day) and Rhodococcus ruber CQ0301 (10 day) (Huang et al. 2018; Li et al. 2006). It suggested that this strain has great potential in bioremediation of DBP artificially contaminated soil.

Strain USTB-Y could easily biodegrade tested dialkyl PAEs with shorter alkyl-chains (DMP, DEP, DBP and BBP) but hardly biodegraded tested dialkyl PAEs with longer alkyl-chains (DEHP and DOP). It may be due to biodegradability differences in short-versus long-chain PAEs and the steric effects of long phthalate ester side chains avoid the binding of hydrolytic enzymes to phthalate, thereby inhibiting their hydrolysis (He et al. 2013; Liang et al. 2008). For tested intermediates, strain USTB-Y could easily utilize n-butyl alcohol, MBP and MEP but hardly utilized PA and PAC. It indicated that n-butyl alcohol and MBP were the possible intermediates in the PAEs biodegradation by strain USTB-Y and it lacked related genes for PA and PAC biodegradation.

PAEs biodegradation could be divided into two steps: transformation of PAEs into PA and utilization of PA. The bacterial biodegradation of DBP entailed sequential hydrolysis of the ester bonds between the alkyl chains and the aromatic ring, involving the formation of MBP (hydroxylation) or DEP (β-oxidation) followed by PA or direct formation of PA (Cazals et al. 2019; Huang et al. 2018), then the aromatic ring cleavage occurred before entering the TCA cycle (Lei et al. 2017; Feng et al. 2018a, b; Zhao et al. 2016). In this study, PA was identified as the end-product of DBP and the other metabolites (such as DMP, DEP etc.) were
not detected, probably because they had disappeared owing to immediate biodegradation by strain USTB-Y once they were formed.

Several encoding genes involved in PAEs biodegradation have been reported, especially PAEs hydrolase (Wu et al. 2013; Chen et al. 2015; Iwata et al. 2016; Nahurira et al. 2017). Two esterase/hydrolase genes named dpeH and mpeH located in the same transcription unit were cloned from Microbacterium sp. PAE-1 by Lu et al. in 2020. DpeH could only hydrolyze dialkyl PAEs to the corresponding monoaalkyl PAEs, which were then hydrolyzed to PA by MpeH. In this study, it was confirmed that gene3780 and gene3781 from strain USTB-Y showed 100% identity with mpeH and dpeH, respectively. This provided biological information base for bioconversion of DBP into PA by strain USTB-Y. And it was firstly reported that there are genes (gene3777 and gene3778) for transposase in the upstream of dpeH and mpeH (Online Resource 6). These finds are helpful in understanding the molecular mechanism of PAEs biodegradation in Microbacterium sp.

It is well known that PA could be converted to PCA by phthalate catabolic gene cluster (pht) (Li et al. 2016). In general, the whole pht cluster consisted of seven functional genes (phtAaAbAcAd) and an unknown ORF (phtU). Of the seven functional genes in Gram-positive bacteria, phtAaAbAcAd genes encode dioxygenase which can oxygenate phthalate to 3, 4-dihydro-3, 4-dihydroxyphthalate, and then 3, 4-dihydro-3, 4-dihydroxyphthalate was dehydrogenated to 3, 4-dihydroxyphthalate by dehydrogenase (phtB) and finally 3,4-dihydroxyphthalate was decarboxylated to form protocatechuic acid, dehydrogenase (phtC). In this study, the reported pht genes were not found in the draft genome of strain USTB-Y (Online Resource 4) and there were no enzymes annotated to participate in the pathway map of aromatic compounds degradation in KEGG (Online Resource 5). Besides Microbacterium sp. USTB-Y, Microbacterium sp. PAE-1 was also reported to be unable to utilize PA. The reason for this phenomenon may be attributed to gene lacking. Construction of genetically engineered bacteria can overcome the incomplete degradation of phthalate esters during bioremediation process. Further experiments have to be carried out to elucidate this possibility.

**Conclusions**

Microbacterium sp. USTB-Y is a high efficient bacterium for biodegrade DBP. It could biodegrade 100% of DBP of 50 mg/L both in MSM and in DBP artificially contaminated soil within 12 h. The optimal conditions for the strain were 30 °C and pH 7.5. The end-product of DBP was further identified as PA using GC/MS. Importantly, the draft genome analysis revealed that many genes in strain USTB-Y encoded hydrolases involved in transforming DBP into PA, especially gene3871 and gene3870. This study is helpful to the development of microbe resources for DBP biodegradation and the understanding on genetic mechanisms of DBP biodegradation in Microbacterium sp. strains.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1077/s11274-021-03181-5.

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**Author contributions** ZZ and HY conceived and designed the experiments. ZZ performed the experiments. CL and SA were involved in sample preparation. QX, AA, HZ, YL and YP were involved in identification of biodegradation products and genome analysis. Data was statistically analyzed by ZZ. The paper was written by ZZ, reviewed and edited by HY.

**Declarations**

**Conflict of interest** The authors declare that there is no conflict of interest.

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