PROPERTIES OF A PHTHALATE ESTERS HYDROLASE FROM *ARTHROBACTER* SP. ZJUTW AND COMPARISON OF ITS TRANSESTERIFICATION AND ESTER HYDROLYSIS ABILITY

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Abstract. Microbial degradation is suggested to be the principal mechanism for removing Phthalate esters (PAEs) from the environment. PAEs esterase is one of key enzymes in the degradation pathway. In this study, a phthalate esters hydrolase gene, *pehA*, that displays good alkali stability, is cloned from *Arthrobacter* sp. ZJUTW and characterized. The enzyme showed a monomeric structure with a molecular mass of approximately 26.17 kDa and pI of 4.42. Its optimal pH and temperature were pH 10.0 and 50 °C, respectively. The enzyme was stable in a pH ranging from 8.0 to 10.0. In addition, the ester hydrolysis and transesterification catalytic ability of the esterase were compared through high performance liquid chromatography (HPLC) and Gas Chromatography-Mass Spectrometer (GC-MS). When performing the ester hydrolysis function, PehA has the catalytic ability to hydrolyze two ester bonds at the same time. The kinetic parameters of transesterification and ester hydrolysis conditions showed that the catalytic efficiency of transesterification is significantly higher than that of ester hydrolysis, and PehA preferentially catalyzed transesterification with PAEs and methanol as a substrate. This is the first report on the comparison of transesterification and ester hydrolysis ability of the PAEs esterase.
Keywords: *PehA, gene cloning, endocrine disrupting chemicals, degradation, purification*

Introduction

Phthalate esters (PAEs) are important synthetic organic compounds used as plasticizers in the industrial production of plastics. However, PAEs have become dangerous pollutants in environmental samples in recent years as endocrine disrupting chemicals which influence the genitals. Among rats and adult men, researches have shown that phthalate exposure increased DNA damage in sperm, induced hepatocellular tumors and in some cases reproductive toxicity (Fatoki and Ogunfowokan, 1993; Wang et al., 2004). Some of the PAEs including dimethyl phthalate (DMP), di-n-butyl phthalate (DBP), and di-n-octyl phthalate (DOP) have been listed as priority pollutants by the China National Environmental Monitoring Center (Fenner et al., 2013) and the US Environmental Protection Agency (Xu et al., 2005).

Since the photolysis and chemical hydrolysis rates of phthalate esters are very slow, microbial degradation is suggested to be the principal mechanism for removing PAEs from the environment. In recent years, some microorganisms have been reported to be capable of PAE degradation, these include *Gordonia* sp. (Jin et al., 2012), *Pseudomonas* sp. (Wang et al., 2003), *Arthrobacter* sp. (Wang et al., 2012), *Rhodococcus* sp. (Jin et al., 2010), *Camelimonas* sp. (Chen et al., 2014), *Pelotomaculum* and *Desulfotomaculum* species (Qiu et al., 2004). Numerous studies have demonstrated the microbial biodegradation pathways of phthalate esters. The metabolism of phthalate esters is initiated in bacteria by their hydrolysis converting them into mono-phthalate esters,

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which are further degraded by ester-hydrolysis to phthalate (PA). PA is further metabolized in aerobic bacteria by two different dioxygenase-initiated pathways through the common intermediate, protocatechuate (3,4-dihydroxybenzoate). Protocatechuate is further degraded into organic acids through either ortho- or meta-cleavage pathway by ring cleavage enzymes, which eventually converted them into CO_2 and H_2O through Krebs cycle (Eaton, 2001; Stingley et al., 2004; Ren et al., 2018).

Hydrolysis of the ester bond is a common key initial step in the microbial degradation of PAEs. PAEs esterase is one of key enzymes in the degradation pathway. However, only a few enzymes involved in this reaction have been reported. These enzymes include dimethyl terephthalate (DMT) esterase from *Fusarium* sp. DMT-5-3 (Luo et al., 2012), two distinct PAE hydrolases in *Micrococcus* sp. YGJ1 (Akita et al., 2001; Maruyama et al., 2005), mono-2-ethylhexyl phthalate hydrolase from *Gordonia* sp. P8219 (Nishioka et al., 2006; Iwata et al., 2016), ester hydrolase PatE from *Rhodococcus jostii* RHA1 (Hara et al.,2010), DBP hydrolase CarEW from *Bacillus* sp. K91 (Ding et al.,2015), DBP hydrolase from *Acinetobacter* sp. M673 (Wu et al., 2013), and Esterase EstB and EstG from *Sphingobium* sp. SM42 (Whangsuk et al., 2015). The esterase can either cleave the ester bond by ester hydrolysis or transesterify with alcohols. Previous studies have only reported the ester hydrolysis catalytic ability of PAEs esterase, but not explored its transesterification ability. Due to the complex composition of pollutants in the environment, it is possible that both alcohols and PAEs exist simultaneously. It is necessary to explore the transesterification of microorganisms and their esterases.

The PAEs esterase play important roles in the decontamination of PAEs. Studying the catalytic mechanism and recombinant expression of PAEs esterase can help improve the efficiency of PAEs pollutants treatment. In this paper, we described a basophilic enzyme from *Arthrobacter* sp. ZJUTW isolated from sludge of the river of Hangzhou city. This enzyme displayed specific hydrolase activity toward PAEs. The experimental data from HPLC and GC-MS led us to compare the ester hydrolysis and transesterification catalytic ability of the esterase.

Materials and methods

Strains and reagents

Arthrobacter sp. ZJUTW was isolated from sludge in Shangtang River of Hangzhou City, using dibutyl phthalate as sole carbon and energy source. The strain was deposited at the China Center for Type Culture Collection (CCTCC) under the accession number CCTCC M2012246.

DMP, diethyl phthalate (DEP), DBP, di (2-ethylhexyl) phthalate (DEHP) were purchased from Sinopharm Chemical Reagent Co., Ltd, all were > 98% pure. HPLC-grade methanol was purchased from Tianjin Siyou Fine Chemicals Co., Ltd. (Tianjin, China). Nickel-NTA agarose and pET28a expression kit were purchased from Shanghai Sangon Biological Engineering Technology Co., Ltd. (Shanghai, China). Other chemicals were analytical-reagent grade, and were purchased locally.

Genome sequencing and sequence analysis

Genomic DNA of *Arthrobacter* sp. ZJUTW was extracted using a genomic DNA isolation kit by Dalian Takara Biomedical Technology Co., Ltd (Dalian, China).

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Genome sequencing was performed by Zhejiang Tianke Biological Technology Co., Ltd. (Zhejiang, China) using Illumina Hiseq 2000 Sequencing platform, and a partial genomic sequence was obtained. Oligonucleotide primers were synthesized by Shanghai Sangon Biological Engineering Technology and Services Co., Ltd. (Shanghai, China). The full-length salicylate esterase gene *pehA* was revealed based on the prediction of ORFs from the partial genomic sequence by the Glimmer 3.02 (http://www.cbcb.umd.edu/software/glimmer/). Putative functions were inferred using the Basic Local Alignment Search Tool (BLAST) (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Protein similarity search and alignment were performed using the data from CLUSTAL W (Thompson et al., 1994). The signal sequence for peptide cleavage in the amino acid sequences of *pehA* was predicted using SignalP 4.0 (www.cbs.dtu.dk/services/Signal P) (Petersen et al., 2011). ESPript output was used to render the analysis of multiple sequence alignment (Gouet et al., 1999). The neighbor-joining method in the molecular evolutionary genetic analysis software package MEGA (version 6.0) was used to construct a phylogenetic tree. The theoretical molecular mass and isoelectric point of the deduced *pehA* protein sequence was calculated using the Compute pI/Mw tool on the ExPASy proteomics server (available at http://ex pasy.org/tools/p itool.html).

Expression and purification of recombinant PehA

The *pehA* gene was amplified by primers P1 (forward): 5'-CGCGGATCCATGGAGATCGTACTGGTGCA-3' and P1 (reverse): 5'-CCCAAGCTTCCA GTCCTGTTAGGCAATGAC-3'. Initial activation of the *Taq* DNA polymerase was performed for 5 min at 94 °C, followed by 32 cycles as follows: 94 °C for 45 s, then 55 °C for 45 s, 72 °C for 90 s, followed by a final extension at 72 °C for 10 min. Then following the manufacturer's instructions, the expression of *pehA* gene was performed using the *pET*28a expression kit. The PCR product was ligated to the *pET*28a vector and introduced into *E. coli* BL21 (DE3) cells. Positive colonies were picked and grown in Luria-Bertani (LB) at 37 °C in the presence of 50 μg/ml of kanamycin to an OD600 of 0.6. The culture was then induced with 1.0 mM IPTG and grown at 22 °C with shaking at 180 rpm for 18 h. The cells were harvested, re- suspended in 20 mM potassium phosphate buffer (pH 8.0), and disrupted by sonication. After centrifugation at 12,000 × g at 4 °C for 15 min, the supernatant was collected and further purified by a 2 ml volume of NTA-Ni^{2+} agarose following the manufacturer's instructions. The following purification was then performed by size exclusion chromatography on a Q Sepharose XL 10/30 column (Amersham Bioscience) equilibrated with 20 mM sodium phosphate buffer (pH 8.0) at a flow rate of 1.0 ml min. The purified recombinant PehA was examined using sodium salt-polyacrylamide gel electrophoresis (SDS-PAGE). The protein concentration was quantified using the Bradford procedure with bovine serum albumin as a standard.

Assay of enzyme activity

Enzyme activity was quantified at 40°C, with 120 uL of 10 mM ρ-nitrophenyl butyrate (ρ-NPC4) substrates, 1.68ml of 50 mM phosphate buffer (pH 8.0), and 200 μl of the purified PehA. Blank reactions were performed with every measurement to subtract appropriate values for non-enzymatic hydrolysis of the substrate. The production of ρ-nitrophenol (ρ-NP) was monitored in triplicate every minute for 5 min

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at 405 nm. One unit of esterase activity was defined as the amount of enzyme that produced 1 μmol ρ-NP in 1 min under the assay conditions

Properties of the PehA

The effect of pH and temperature on enzyme activity and stability was measured on ρ-NPC4 ester substrates for relative activity, which was expressed as a percentage of the initial activity. The optimum pH was determined by measuring the activity at 40 °C over the pH region 3.0-11.0. The stability at different pH (5.0-10.0) was determined after incubating the enzyme for 30 min and by measuring the relative activity at 40 °C and pH 8.0. The optimum temperature was determined by assaying the enzyme activity at various temperatures (30-70 °C) for 20 min in 0.02 mol/l potassium phosphate buffer (pH 8.0). The thermostability was determined by measuring the remaining activity at 30 °C and pH8.0, after incubation of the purified esterase between 30 and 70 °C and pH 8.0 for 30 min.

The effect of different potential inhibitors or activators (metal ions and organic solvents) on the enzyme activity was also determined by measuring the relative activity using a standard assay with ρ-NPC4 as the substrate. The reaction system was 120 μL of 10 mM ρ-NPC4 ester substrates, 1.68 ml of 50 mM phosphate buffer (pH 8.0), 200 μl of the purified PehA, and a final concentration of 1.0 mM of different metal or a final concentration of 1.0% (volume to volume ratio) of organic solvent. The enzyme was preincubated at 40 °C for 5 min. The remaining activity was assayed was described. The activity assayed in the absence of inhibitors or activators was defined as the control.

Comparison of transesterification and ester hydrolysis ability

For comparing the transesterification and ester hydrolysis ability of PehA, the enzyme kinetic constants were determined by measuring the initial rate of enzymic reaction for DBP, DEP and DMP, which were firstly dissolved in methanol and dimethyl sulfoxide (DMSO), respectively. Parametric identification of maximum velocity (V_{max}) and Michaelis-Menten constants (K_m) was used from the equation for initial reaction velocity. The recombinant PehA assays were performed in 50 mM Tris-HCl buffer (pH 8.0) with 10 mM of DBP, DEP or DMP (firstly dissolved in methanol and DMSO, respectively) at 40 °C for 10 min. The substrate amount was then determined by HPLC/MS analysis. The substrate-free assay system was also used as blank simultaneously. Kinetic values were calculated from nonlinear regression data analysis against various substrate concentrations.

HPLC and GC-MS analytical methods

The mixture of enzymatic degradation was extracted with equal volume dichloromethane, then the extract was evaporated, and the residue was dissolved in 1 ml methanol. The amount of remaining substrate was determined by HPLC (Agilent 1260 series, USA) equipped with a Diamonsil-C18 column (4.6 mm × 250 mm × 5 μm; Dikma Technologies Inc, China). A mixture of methanol and H_2O (90:10 by volume) was used as the mobile phase at a flow rate of 1.0 ml min^{-1}. The UV detector wavelength was 235 nm and the sampling quantity was 20 μl.

After collecting the HPLC peaks separately, enzymatic degradation products were identified using a Hewlett Packard 6890N gas chromatograph (Hewlett Packard, USA) equipped with an Agilent 5975C mass selective detector (Agilent, USA). The column

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used was a HP-5MS (30 m × 250 m × 0.25 m) capillary column. The temperature program consisted of 1 min hold at 60 °C, an increase to 220 °C at 30 °C min^{-1}, and 2 min hold, an increase to 250 °C at 5 °C min^{-1}, and 2 min hold, an increase to 280 °C at 5 °C min^{-1}, and 3 min hold. The injection volume was 1 μl and the carrier gas was helium (1.0 ml min^{-1}). The mass spectrometer was operated at an electron ionization energy of 70 eV. Instrumental library searches, comparison with available authentic compounds, and mass fragmentation pattern were used to identify the degradation products.

Statistical analysis methodology

Each treatment in the experiment was performed in triplicates. Software Origin 8.0 was used to draw the figures with error bars. Data are expressed as the mean ± standard deviation (SD).

Results and discussion

Sequence analysis of PehA

A gene annotated as "salicylate esterase" with a 723-long ORF that encoded 240 amino acid proteins was found and we named it as *pehA*. No signal sequence was found. Sequence alignment revealed that amino acid sequence of *pehA* was 100% sequence identity to alpha/beta hydrolase from *Gordonia* QH-11 (*Fig. 1*), which was not reported about catalytic ability of PAEs. In addition, these sequences all contain the typical catalytic triad composed of Ser75-Glu194-His221 and the consensus motif (Gly-X-Ser-X-Gly) around the active-site serine (*Fig. 2*). Some PAEs esterases sequences were retrieved and compared with known representative esterases' sequences of eight families (Ren et al., 2018). The catalytic triad containing Ser, Glu (or Asp), and His was widely reported in families IV, V, and VII. Most enzymes in family V possess the typical α/β-hydrolase fold with a catalytic triad (Arpigny and Jaeger, 1999), and existed basic amino acid (H and R) in GX1SX2GG motif, suggesting PehA belongs to the family V. The sequence similarities and conservation of typical catalytic triads suggest that these enzymes may have a number of important common functions that were conserved during the course of evolution.

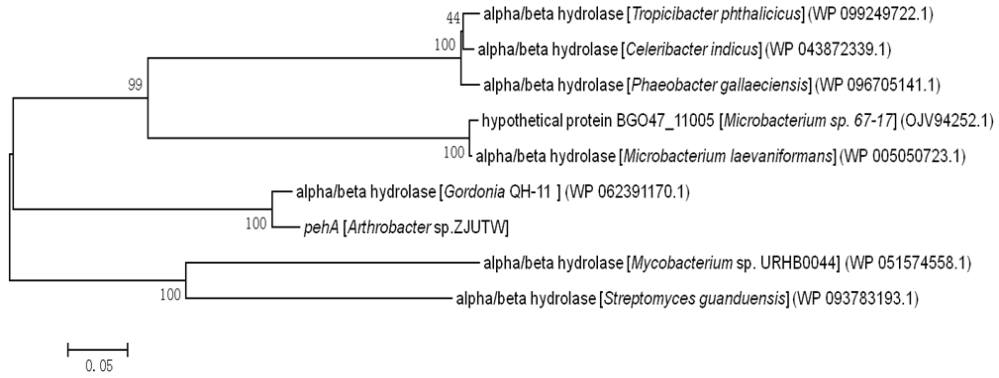

Figure 1. *Neighbor-joining tree of esterases. Protein sequences were aligned using the built-in CLUSTAL W (default parameters), the tree was built using the neighbor-joining method with default parameters and 1000 bootstrap replications*

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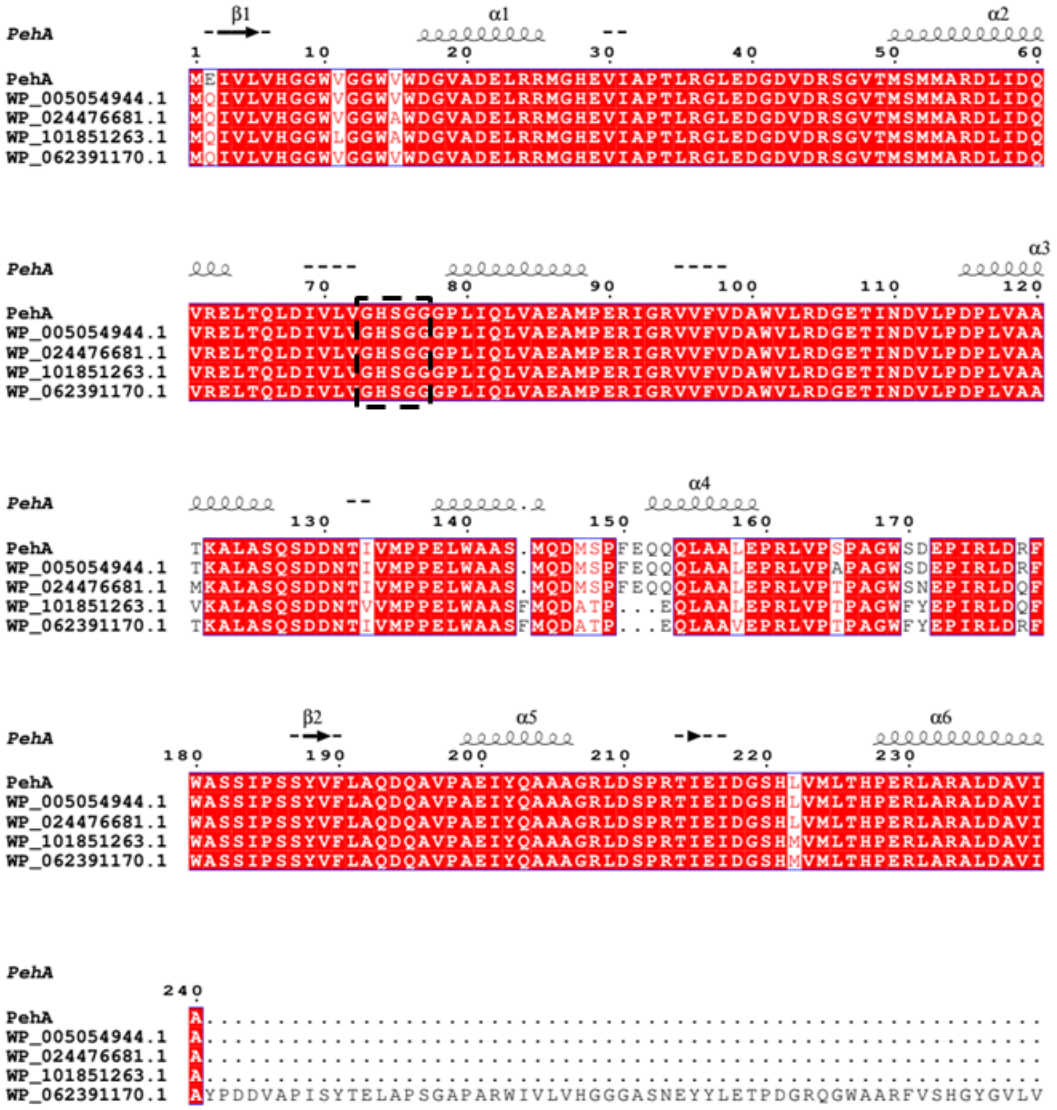

Figure 2. *Protein sequences alignment between PehA and homologs from the carboxylesterase family. ESPript outputs obtained with the sequences from the SWISSPROPT databank and alignment with CLUSTAL W. Sequences are grouped according to similarity. WP_005054944.1 from alpha/beta hydrolase of Microbacterium; WP_024476681.1 from alpha/beta hydrolase of Actinobacteria; WP_101851263.1 from alpha/beta hydrolase of Kocuria flava; WP_062391170.1 from alpha/beta hydrolase of Gordonia phthalatica. A conserved pentapeptide (GXSXG), containing the serine residue of the catalytic triad, was framed by a dotted box. Symbols above blocks of sequences represent the secondary structure, springs represent helices, and arrows represent β-strands*

Enzyme cloning, overexpression and purification

To investigate the biochemical properties of the enzyme, the *pehA* gene was expressed in the *pET*-28a vector as a 6 × His tagged fusion protein and induced with 1 mM IPTG or without IPTG at 20 °C for 18 h. The crude enzyme extracted from recombinant *E. coli* BL21 cells was purified using Ni^{2+}-NTA metal-chelating affinity chromatography and analyzed by SDS–PAGE. As shown in *Figure 3* (lane 3), one band corresponded in size to the calculated molecular mass of PehA was detected

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(26.17 kDa). The band was absent in the control lane from the *E. coli* BL21 cells induced without IPTG (*Fig. 3*, lane 1). The isoelectric point (pI) was 4.42.

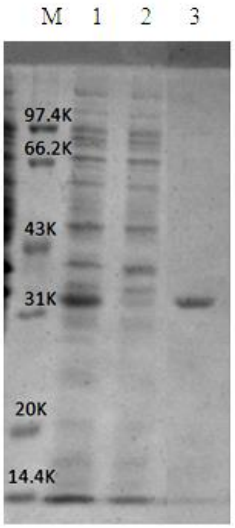

Figure 3. *Analysis of the protein expressed in E. coli BL21 cells following purification on a 12% SDS- PAGE. Lane M, protein molecular marker; Lane 1, after induction with 1 mM IPTG and grown at 20 °C for 18 h; Lane 2, before induction with IPTG; and Lane 3, purified recombinant PehA (molecular weight without histidine tag is 26.17 kDa)*

Effect of pH and temperature on enzyme activity and stability

The effect of pH on PehA activity was determined using ρ-NPC4 as the substrate at 40 °C with pH values ranging from 4.0 to 11.0. Optimum activity was observed at pH 10.0, with approximately no activity at pH 4.0 (*Fig. 4A*). The pH stability analysis revealed that the enzyme was very stable at pH 7.0- 10.0, retaining more than 70% of the original activity after pre-incubation at the given pH range for 60 min. However, PehA only maintained 41% and 32% of its activity at pH 6.0 and 11.0, respectively, after incubation for 60 min.

The effect of temperature on PehA activity was investigated using ρ-NPC4 as the substrate at pH 7.0 with the temperature ranging from 30 to 70 °C. Optimum activity was observed at 50 °C. When the temperature rises above 50 °C, the enzyme activity drops sharply. The temperature stability of PehA was examined by measuring its residual activity after incubating the purified enzyme for 0.5 h from 30 to 70 °C. PehA retained approximately 95%, 99%, 86% of its activity after incubation for 0.5 h at 30, 40 and 50 °C respectively. However, the enzyme was unstable above 70 °C, and retained approximately 33% of activity (*Fig. 4B*).

Effect of metal ions and organic solvents on enzyme activity

The effect of different metal ions on PehA activity was examined by addition of each metal ion into the reaction mixture at a final concentration of 1.0 mM. The results are presented in *Table 1*. Ni^{2+}, had a moderately inhibitory effect (86.2% residual activity); Mg^{2+}, K^{+}, Zn^{2+}, Ba^{2+}, Al^{3+} and Li^{+} had no apparent effect on enzyme activity; whereas Co^{2+}, Cu^{2+} and Mn^{2+} activated PehA, and Fe^{3+} strongly activated PehA with residual activities of 163%.

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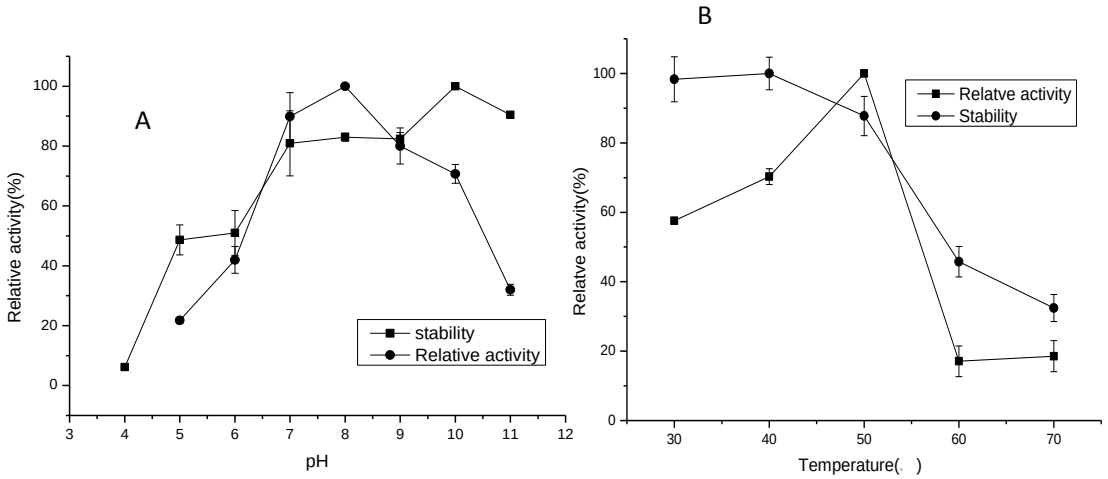

Figure 4. *Effect of temperature and pH on PehA activity and stability. Relative activity of purified PehA was determined at different pH (A) or temperatures (B) using ρ-NP butyrate (ρ-NPC4) as the substrate at 405 nm. Remaining enzyme activity was measured at 40 °C and pH 8.0 after incubating purified PehA at different temperatures (B) or pH for 60 min*

Table 1. *Effect of various metal ions and organic solvents on enzyme activity*

Metal ions/organic solvents	Concentration	Relative activity (%)
control	0 mmol/L	100
Ni^{2+}	1 mmol/L	86.2 ± 3.78
Mg^{2+}	1 mmol/L	98.0 ± 6.24
Co^{2+}	1 mmol/L	109.3 ± 3.21
Ba^{2+}	1 mmol/L	105.4 ± 2.61
Zn^{2+}	1 mmol/L	105.0 ± 7.43
Ca^{2+}	1 mmol/L	112.3 ± 6.37
Cu^{2+}	1 mmol/L	117.6 ± 4.45
Mn^{2+}	1 mmol/L	123.0 ± 3.54
Fe^{3+}	1 mmol/L	163.1 ± 4.59
K^{+}	1 mmol/L	107.3 ± 2.15
Al^{3+}	1 mmol/L	101.2 ± 2.07
Methanol	1%V/V	107.4 ± 7.74
Ethanol	1%V/V	109.9 ± 8.21
Acetone	1%V/V	105.5 ± 1.88
DMSO	1%V/V	97.4 ± 0.81
Formaldehyde	1%V/V	22.3 ± 1.33
Acetonitrile	1%V/V	93.8 ± 21.97
Trichloromethane	1%V/V	60.3 ± 2.87
Tween 80	1%V/V	80.5 ± 8.91

Organic solvents such as Formaldehyde (1%) strongly inhibited PehA (22.3% residual activity), and Trichloromethane (1%) and Tween 80 (1%) exhibited moderate

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inhibitory effects (approximately 60.3% and 80.5%, respectively). Acetonitrile (1%) and DMSO (1%) had little inhibitory effect on the enzyme activity (97.4% and 93.8% residual activities, respectively), whereas methanol (1%), ethanol (1%), and acetone (1%) little activated PehA with residual activities of 107.4%, 109.9% and 105.5%, respectively.

Comparison of transesterification and ester hydrolysis ability of PehA

Break of the ester bond is a common key initial step in the microbial degradation of phthalate esters. Esterase has two kinds of catalytic reactions involved transesterification and Ester hydrolysis to execute the Initial degradation step. To explore the catalytic ability for transesterification and Ester hydrolysis of PehA, The products of the reaction catalyzed were identified by HPLC and GC-MS. In the reaction system containing methanol, butyl methyl phthalate (BMP) was detected (*Figs. 5A* and *6A, B*), indicating that in this system, PehA exchanged a methyl group on methanol with butyl group of DBP through a transesterification reaction. In the reaction system containing no methanol (DBP dissolved in DMSO), PA was detected (*Figs. 5B* and *6C*), and mono-butyl phthalate (MBP) was not detected, indicating that the esterase hydrolyzes two ester bonds simultaneously by ester hydrolysis to produce PA. However, in most of the previous reports, the initial step in the degradation of PAEs is that esterase hydrolyzes one ester bond to produce a monoester, then monoesterase hydrolyze another ester bond to form PA, suggesting that PehA has the catalytic ability to hydrolyze two ester bonds at the same time. Kcat/Km is one of the specificity constants of the enzyme, and can represent the specificity of the same enzyme for several substrates that compete with each other. The Kcat/Km ratio is a measure of the priority of different substrates, and the substrate with the larger Kcat/Km ratio is the preferred target for the enzyme. The kinetic parameters of different substrates were determined at pH 8.0 and 40 °C using the purified recombinant PehA (*Table 2*). After comparing the ester hydrolysis and transesterification catalytic kinetic constants of several PAEs, the results showed that the ester hydrolysis catalytic activity of esterase was significantly stronger than that of transesterification. In *Table 2*, the Kcat/Km values of DEP (containing methanol) and DEP (no methanol) as substrates are 41.02 and 0.47, respectively, and that of DBP (containing methanol) and DBP (no methanol) are 8.95 and 0.213, respectively, indicating that the catalytic efficiency of transesterification is significantly higher than that of ester hydrolysis in the presence of methanol, and PehA preferentially catalyzed transesterification with PAEs and methanol as a substrate. In addition, PehA exhibited strong catalytic ability for DBP and DEP, weak catalytic ability for DMP, and no catalytic ability for DEHP. This result suggested that PehA might have an ester chain length-dependence of PAEs. The kinetic parameters of some PAEs hydrolase are reported, the Kcat/Km values of DBP as substrates are 0.109 s^{-1} μM^{-1} (Ding et al., 2015), 0.07 s^{-1} μM^{-1} (Jiao et al., 2013) and 10.11 s^{-1} μM^{-1} (Wu et al., 2013), respectively, but all these data could only be measured at the condition of hydrolyzation. There is no report on the kinetic parameters of transesterification of these PAEs esterase. Some studies have reported that microorganisms can degrade PAEs through the ester conversion reaction process (Cartwright et al., 2000; Amir et al., 2005; Okamoto et al., 2011), but did not separate the esterases from these strains. To our knowledge, this is the first report on the comparison of transesterification and ester hydrolysis ability of PAEs esterase, and can help to further understand the degradation mechanism of PAEs by microbial.

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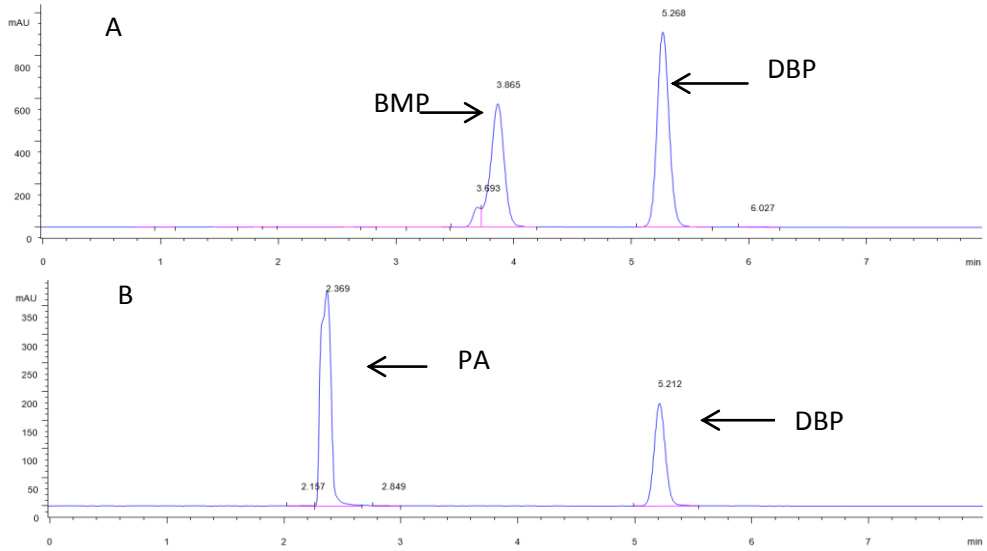

Figure 5. *The results of HPLC analyses of enzymatic products. (A) The results of DBP dissolved in methanol as a substrate after incubation with PehA. (B) The results of DBP dissolved in DMSO as a substrate after incubation with PehA*

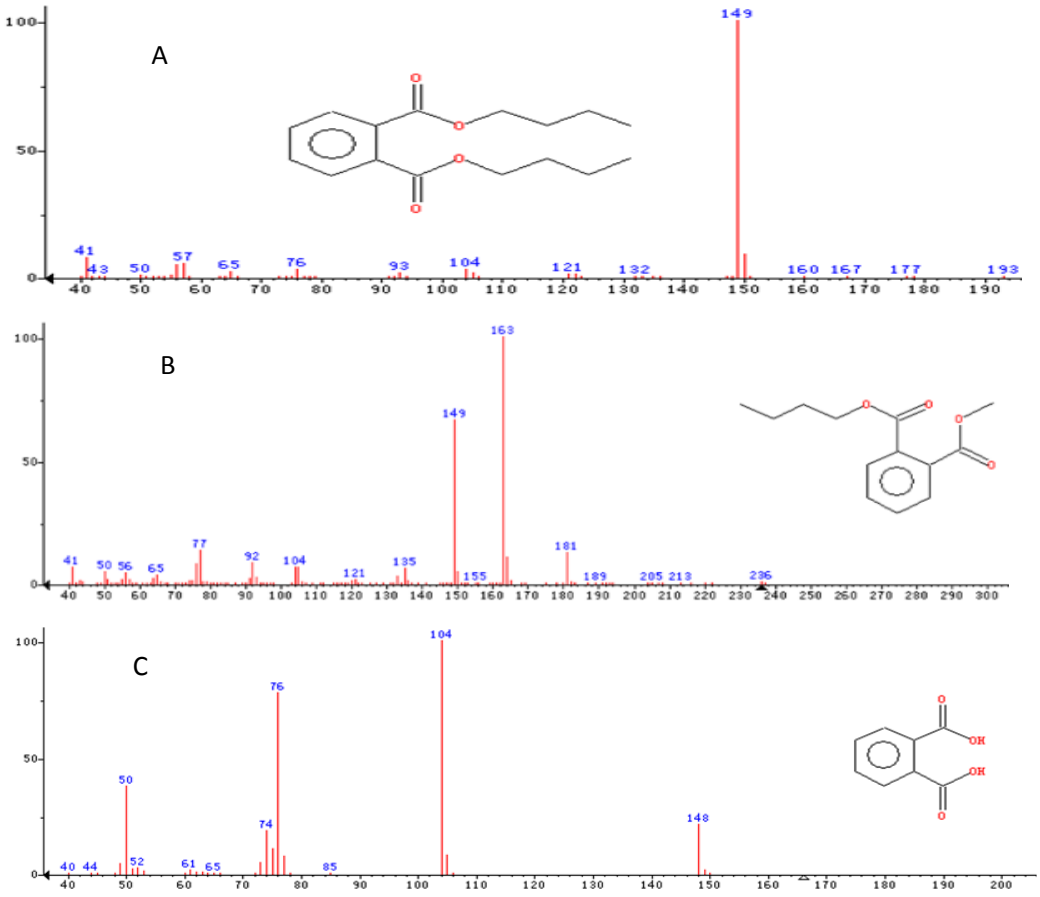

Figure 6. *GC-MS analysis of products identified in the PehA catalyzed reaction. (A) Mass spectrometry of peak time of 5.212 min and 5.268 min by HPLC in Figure 5. (B) Mass spectrometry of peak time of 3.865 min by HPLC in Figure 5. (C) Mass spectrometry of peak time of 2.369 min by HPLC in Figure 5*

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Table 2. *Kinetic parameters of the recombinant PehA on different substrates at pH 8.0 and 40 °C*

Substrate	Vmax (mmol/min·mg)	Km (mmol/l)	Kcat(/s)	Kcat/Km (l/s·mmol)
DEP (dissolved in methanol)	41.17 ± 1.32	0.44 ± 0.03	17.9 ± 1.44	41.02
DBP (dissolved in methanol)	54.90 ± 1.87	2.67 ± 0.02	23.8 ± 0.94	8.95
DMP (dissolved in methanol)	0.239 ± 0.01	10.79 ± 0.72	0.104 ± 0.01	0.0096
DEP (dissolved in DMSO)	373.02 ± 11.42	347.6 ± 9.35	162.2 ± 6.58	0.470
DBP (dissolved in DMSO)	319.11 ± 22.06	472.5 ± 13.18	100.64 ± 8.36	0.213
DMP (dissolved in DMSO)	1.032 ± 0.02	1288 ± 20.07	3.56 ± 0.4	0.00276

Conclusions

In this study, the *pehA* gene efficiently degraded several PAEs such as DBP, DEP and DMP, was cloned from *Arthrobacter* sp. ZJUTW and heterologously expressed in *Escherichia coli* BL21 using the *pET28a* expression system. The enzyme showed a monomeric structure with a molecular mass of approximately 26.17 kDa and pI of 4.42. The enzyme exhibited maximal activity at pH 10.0 and 50 °C, The enzyme was better stable within the pH range from 8.0 to 10.0. HPLC and GC-MS were employed to detect the catalytic ability of PehA. The results showed that this enzyme has two catalytic functions: transesterification and Ester hydrolysis. When performing the ester hydrolysis function, it has the catalytic ability to hydrolyze two ester bonds at the same time. Through comparing the kinetic parameters of transesterification and ester hydrolysis conditions, we found that the catalytic efficiency of transesterification is significantly higher than that of ester hydrolysis, and PehA preferentially catalyzed transesterification with PAEs and methanol as a substrate. The PAEs esterase play important roles in the decontamination of PAEs, the production of such recombinant enzyme can probably provide efficient biocatalysts at low costs for environmental protection purposes.

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