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Molecular cloning, purification and biochemical characterization of a novel pyrethroid-hydrolyzing carboxylesterase gene from *Ochrobactrum anthropi* YZ-1

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ABSTRACT

Strain YZ-1 was isolated from activated sludge and identified as *Ochrobactrum anthropi*. This strain was capable of degrading pyrethroids pesticides, suggesting the presence of degrading enzymes. In the present study, a novel esterase gene *pytZ* was cloned from the genomic library of YZ-1 successfully. The *pytZ* contained an open reading frame of 606 bp encoding a pyrethroid-hydrolyzing carboxylesterase. Deduced amino acid sequence showed moderate identities (39–59%) with most homologous carboxylesterase, except a putative carboxylesterase from *O. anthropi* ATCC 49188 with the highest identity of 85%. Phylogenetic analysis revealed that PytZ belonged to esterase VI family. The gene *pytZ* showed no any sequence similarity with reported pyrethroid-hydrolyzing genes and was a new pyrethroid-degrading gene. PytZ was expressed in *Escherichia coli* BL21 (DE3) and purified using Ni-NTA Fast Start. PytZ was able to degrade various pyrethroids. The optimal temperature and pH were 35 °C and 7.5. This enzyme was very stable over a wide range of temperature and pH. No cofactors were required for enzyme activity. Broad substrate specificity, high enzyme activity, and the favorable stability make the PytZ a potential candidate for the detoxification of pyrethroid residues in biotechnological application.

1. Introduction

Pyrethroids are mostly used insecticides throughout the world. They are synthetic analogs of the natural pyrethrin, which is one kind of toxin with insecticidal activity deriving from the flowers of *Chrysanthemum cinerariaefolium*. Pyrethroids were classified as type I or type II based on the toxic symptoms and the absence or the presence of a cyano group at the carboxyl alpha position [1]. Pyrethroid pesticides have been used for more than 30 years, and accounted for more than 30% of insecticide market [2]. With the withdrawal of organophosphorus insecticide from the market, their usage is continuing to grow.

Pyrethroid pesticides are widely used for their high insecticidal activity and were generally considered low toxic for mammalian [3]. However, extensive application of this kind of pesticides has caused many problems, such as pest resistance, soil and water contamination, high residue in agricultural product, and human exposure. Previous studies reported that the high exposure to pyrethroids might cause reproductive toxicity [4], cytotoxicity [5,6], and adverse effects on many tissues [7,8]. Some of them have been classified as a possible human carcinogen by the Environmental Protection Agency (EPA) of US [9,10]. In addition, most pyrethroid insecticides possess acute toxicity to some no-target organisms, such as been, silkworm, especially aquatic invertebrates, often at the concentration less than 1 $\mu g\,L^{-1}$ [11,12]. These findings revealed that pyrethroids are potentially harmful to human health and ecosystem. Great concerns have been raised about the pyrethroid residues and persistence in the environment. Therefore, it is urgent to develop some efficient strategies to solve these problems caused by pyrethroid residues.

In natural environment, pyrethroid pesticides are degraded mainly by photolysis, hydrolysis and microbial decomposition [13]. Microbial degradation plays an important role in the elimination of pyrethroid residues. Biodegradation is an important environment biotechnology for the treatment of organic pollutants. One strategy of which is using some key enzymes to break down pesticide residues. For pyrethroid pesticides, they are a large class of compounds that contain an ester bond structure in molecular, which was formed by an alcohol and acid moieties. The major metabolic pathway of pyrethroids in resistant insects and degrading microorganisms involves in oxidation by cytochrome P450s and hydrolysis by esterases [14]. Carboxylesterases are subtypes of esterases and included within subgroup 3.1.1 of the International Union of Biochemistry. They are able to hydrolyze a large number of ester-containing compounds, such as carbamates and pyrethroids [15]. It has been confirmed that carboxylesterase is responsible for the detoxification of pyrethroids by hydrolyzing the ester bond [16]. Many pyrethroid-degrading strains have been isolated, and degradation studies were also carried out [9,10,17–19].

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Some pyrethroid-degrading carboxylesterases were purified and characterized from pyrethroid-degrading microorganisms [14–16], animal liver microsomes [20,21] and pyrethroid-resistant insects [22]. However, the reports about pyrethroid-degrading genes are still rare. So far, only several degrading genes were cloned, i.e. pyrethroid-hydrolyzing genes from *Klebsiella* sp. ZD112, *Sphingobium* sp. JZ-1 and metagenome [23–25].

In this study, we mentioned the isolation and identification of a pyrethroid-degrading strain *Ochrobactrum anthropi* YZ-1, the cloning and expression of the gene *pytZ* which encoded a novel pyrethroid-hydrolyzing carboxylesterase, and the characterization of the purified enzyme, such as substrate specificity, stability, optimal temperature and pH.

2. Materials and methods

2.1. Strains, plasmids and media

Escherichia coli DH5α and *E. coli* BL21 (DE3) were purchased from Tiangen, and were used as cloning and expression host cell respectively. The plasmids pUC18 (Tiangen) and pET30a (+) (Novagen) were used for cloning and expression vectors accordingly. Mineral salt medium (MSM) that containing (g L^{-1}) NH_4NO_3, 1.0; NaCl, 0.5; $(NH_4)_2SO_4$, 0.5; KH_2PO_4, 0.5; and K_2HPO_4, 1.5 was used for the isolation and determination of degrading ability. Luria-Bertani (LB) medium containing (g L^{-1}) peptone, 10; yeast extract, 5.0 and NaCl, 10.0 was used for the genomic library construction and recombinant protein expression. Appropriate antibiotics were added when required.

2.2. Chemicals and enzymes

Lambda-cyhalothrin (98%), beta-cypermethin (95%), beta-cyfluthrin (96%), deltamethrin (98%) and permethrin (95%) were provided by Jiangsu Yangnong Chemical Group Co. Ltd. (Yangzhou, China). All the enzymes used were purchased form Takara. *P*-nitrophenyl esters were purchased from Sigma. All other chemicals were of analytical grade and purchased from commercial sources. Protein molecular mass marker was product of Tiangen.

2.3. Analysis condition of gas chromatography (GC)

Pyrethroid residue was extracted with equal volume *n*-hexane twice. The organic layer was filtrated, dried and re-dissolved in *n*-hexane and determined by GC. The analysis conditions were as follows: ECD, RTX-1301, carrier gas of N_2 (99.999%) at 1.0 mL min^{-1}; inlet temperature of 260 °C, temperature programming of 230 °C 8 min, increasing to 280 °C at 25 °C min^{-1} and remaining 6 min, detector temperature of 300 °C; 1 μL sample with split ratio of 49:1.

2.4. Isolation and identification of pyrethroid-degrading strains

Activated sludge was collected from an aerobic pyrethroid manufacturing wastewater treatment system. To isolate pyrethroid-degrading bacteria, 5 g of activated sludge as initial inoculum was inoculated into MSM which was only added 100 mg L^{-1} lambda-cyhalothrin as sole carbon source for microorganism growth. After six days of incubation at 30 °C and 180 rpm on a rotary shaker, 5 mL of culture was transferred into fresh MSM medium containing 200 mg L^{-1} lambda-cyhalothrin and incubated for another six days. The transfer was conducted for six times successively until the concentration of lambda-cyhalothrin in MSM gradually increased to 800 mg L^{-1}. Then the culture was diluted and spread on MSM agar plate containing 600 mg L^{-1} lambda-cyhalothrin. The target strain was selected and purified. Their degrading ability to pyrethroids was determined by GC. The strain which possessed the highest degrading ability to lambda-cyhalothrin was identified by morphological, physiological and biochemical characteristic [26], and 16S rDNA gene analysis [27].

2.5. Genomic library construction and screening

Genomic DNA extraction was conducted using TIANamp Bacteria DNA Kit (Tiangen) according to the manufacturer's instructions. To construct a size-fractionated genomic library, genomic DNA of *O. anthropi* YZ-1 was partially digested with *Sau*3AI. DNA fragments from 1 to 3 kb were pooled and ligated into the pUC18 plasmid vector which had been previously digested with *Bam*HI. The recombinant plasmids were used to transform competent cells of *E. coli* DH5α. Constructed genomic library was plated onto LB agar plates containing 100 μg mL^{-1} ampicillin. The plates were incubated at 37 °C for about 48 h. The initial screening of transformants was performed as described previously [28]. Then the obtained clones by initial screening were transferred to MSM agar plate containing 600 mg L^{-1} lambda-cyhalothrin as sole carbon scours for the second screening based on the growth status. Finally, the screened clones were tested for their degrading ability to lambda-cyhalothrin by GC.

2.6. Sequence analysis

The recombinant plasmid was extracted and sequenced. Open reading frame (ORF) search was performed by ORF Finder online tool http://www.ncbi.nlm.nih.gov/gorf/gorf.html and DNASTAR software. The searches for nucleotide and protein sequence homology were conducted with BLAST program at NCBI http://www.blast.ncbi.nlm.nih.gov/. Multiple sequence alignment was carried out using CLUSTAL W program [29]. The result was visually highlighted with BoxShade Server program http://www.ch.embnet.org/software/BOX_form.html. Phylogenetic tree was constructed using MEGA 4.0 software by neighbor-joining method. Bootstrapping (1000 replicates) was used to estimate the confidence levels of phylogenetic reconstructions [27]. All the sequences used were retrieved from GenBank and SWISS-PROT database.

2.7. Expression and purification of pyrethroid-hydrolyzing carboxylesterase

For expression of pyrethroid-hydrolyzing carboxylesterase, the complete open reading frame of *pytZ* was amplified by PCR (Peltier Thermal Cycler, Bio-Rad) using primers LF (5′-CG**GGATCC**ATGAGCGTAGTGACGATGATCGTAAAA-3′, *Bam*HI restriction site was highlighted as bold) and LR (5′-CG**GAATTC**TCAGTTGTTTTTGACGATGGGAGC-3′, *Eco*RI restriction site was highlighted as bold). PCR product was digested with *Bam*HI and *Eco*RI simultaneously. Purified target fragment was inserted into pET30a (+) expression vector which had been previously digested with the same restriction enzymes. The recombinant was used to transform *E. coli* BL21 (DE3) cells. The expression procedure was carried out according to the standard method [30]. *E. coli* BL21 (DE3) containing the pET30a-*pytZ* plasmid was inoculated in 30 mL LB medium supplemented with 50 μg mL^{-1} kanamycin and incubated at 37 °C and 180 rpm on a rotary shaker for 12 h. Culture was transferred into fresh LB medium in the proportion of 1:100 and incubated for 2 h. Then isopropyl-β-D-thiogalactopyranoside (IPTG) was added with a final concentration of 1 mM. After incubation at 30 °C for another 3 h, cells were harvested by centrifugation at 10,000 × *g* for 10 min at 4 °C. Then the protein was purified using Ni-NTA Fast Start Kit (Qiagen) according to the manufacturer's instructions. The purified protein was analyzed by SDS-PAGE.

Protein concentration was quantified by the method of Bradford using bovine serum albumin as standard [31].

2.8. Substrate specificity assay

For the substrate specificity experiment, lambda-cyhalothrin, beta-cypermethrin, beta-cyfluthrin, deltamethrin, and permethrin were used. Hydrolytic activity of the enzyme was determined according to the method of Stock et al. [21], with slight modification. Briefly, 1 μL of substrate was added to 0.5 mL of the preincubated enzyme solution (10–60 μg mL^{-1}). The mixture was incubated from 2 to 10 min depending on the substrate. Then 0.5 mL of *n*-hexane was added to each sample and the mixture was vortexed. One activity unit was defined as the amount of enzyme to hydrolyze 1 nmol of substrate per minute. All the treatments were carried out in 50 mM sodium phosphate buffer (pH 7.5) at 35 °C. No more than 10% of the substrate was hydrolyzed during the assay, and solvent content never exceeded 1% of the total assay volume.

For enzyme kinetics study, stock solution of lambda-cyhalothrin was diluted in six different concentrations. Kinetic value constants were obtained by Lineweaver–Burk plots.

2.9. Effects of temperature and pH on the enzyme activity

The optimal temperature of the enzyme was investigated by incubating the enzyme and lambda-cyhalothrin at different temperatures (15–60 °C) in 50 mM PBS buffer (pH 7.5) for 10 min. The thermostability of the enzyme was determined by incubating the sample at different temperatures for 2 h, and then the residual activity was measured and expressed as a percentage of the activity obtained without pre-incubation. For the effect of pH on enzymatic activity, the enzyme was tested at different pH values (pH 4.0–10.0) in the following buffers: disodium phosphate–citric acid buffer (pH 4.0–7.0) and Tri–HCl (pH 7.5–10.0).

2.10. Effect of metal ions and chemical agents on the enzyme activity

The effect of potential inhibitors or activators on the enzymatic activity was also investigated by the addition of various metal ions and chemical agents, such as Na^+, K^+, Mg^{2+}, Zn^{2+}, Fe^{2+}, Ag^+, and Hg^{2+} (1 mM); Tween-20 and Tween-80 (1.0%), sodium dodecyl sulfate (SDS), phenylmethylsulfonyl fluoride (PMSF) and diethyl pyrocarbonate (DEPC) (1 mM); chelating agents ethylenediamine tetraacetic acid (EDTA), 1,10-phenanthroline (1 mM). The mixtures were incubated in PBS (pH 7.5) at 35 °C for 10 min. Residual activity was measured and expressed as a percentage of the enzymatic activity which was obtained from the treatments without the addition of compounds listed above.

3. Results and discussion

3.1. Isolation and identification of the pyrethroid-degrading strain

It was generally considered that environmental conditions are crucial for the isolation and screening of desired degrading stains [32]. In this study, activated sludge samples were collected as inoculum from a pyrethroid manufacturer, which had produced pyrethroid pesticides for more than 10 years. Lambda-cyhalothrin was used as sole carbon source in MSM for the enrichment and isolation of pyrethroid-degrading strains. As the result of isolation procedure, eight morphologically different strains were able to grow well on MSM agar plate containing 600 mg L^{-1} of lambda-cyhalothrin. The degrading abilities of these eight strains were determined, respectively. One of them showed the highest degrading ability to lambda-cyhalothrin. This strain was designated as YZ-1 and selected for the further identification and other studies. The colonial morphology of strain YZ-1 on MSM agar plate was circular, smooth, convex, white, opaque, moist and with entire margin. Results of the physiological and biochemical test are shown in Table 1. The 16S rDNA gene sequence of strain YZ-1 showed 99% similarity to that of *O. anthropi* LMG 5446 and more than 90% similarity to other strains from the *Ochrobactrum* genus (Fig. 1). Based on the morphology, physio-biochemical characteristics, and 16S rDNA sequence analysis, strain YZ-1 was identified as *O. anthropi* YZ-1. Usually, bacteria isolated from the *Bacillus* and *Pseudomonas* genera are active microorganisms which are able to degrade many organic compounds, such as pyrethroids and organophosphorus pesticides [33–35]. The reports about the degrading microorganisms isolated from *Ochrobactrum* genus are rare. It appears to be a new bacterium genus that participates in the degradation of pyrethroids.

Table 1
Physio-biochemical test results of strain YZ-1.

Physio-biochemical tests	Results	Physio-biochemical tests	Results
Gram-staining	–	Fructose utilization	+
Mobility	+	D-Sorbitol utilization	–
Lipid utilization	+	Arabinose utilization	+
Starch hydrolysis	+	Rhamnose utilization	+
Gelatin liquefaction	–	Fucose utilization	+
Fibrinolysis	–	Glycerol utilization	–
Voges–Proskauer (v.p.)	–	Cellobiose utilization	+
Indole reaction	–	Tween-40 utilization	–
Glucose utilization	+	Nitrate reduction	+
Sucrose utilization	+		

3.2. The construction and screening of the genomic library

The genomic library of *O. anthropi* YZ-1 was constructed successfully. Approximately 12,000 clones were generated in library. It is an efficient method to discover the new gene by construction and screening of genomic library. The direct cloning of pyrethroid-degrading gene is difficult. Therefore, we constructed the genomic library of strain YZ-1 in order to find the new pyrethroid-degrading gene. Pyrethroids are ester-containing compounds. The cleavage of ester bond by esterase is the major route of degradation [36]. In initial screening, six transformants displayed esterase activity. But only two of them showed the degrading ability to pyrethroids in subsequently further determination. This result indicated that not all the esterases were capable of degrading pyrethroids. And it also revealed that a good method is very important in screening the target gene. To some extent, it determines whether the desired gene can be obtained or not. In the present study, the used three-step screening strategy was proved an efficient method in screening the pyrethroid-degrading gene. One of the two target clones was further studied due to its relatively higher degradation ability.

3.3. Sequence and phylogenetic analysis

The inserted fragment in target clone was 1150 bp. ORF analysis revealed that this fragment contained two complete ORFs. The two ORFs were subsequently subcloned into pET30a (+) plasmid and used to transform *E. coli* BL21 (DE3) for their functional identification according to the method mentioned in Section 2.5. Result indicated that only one of the two ORFs possessed the degrading ability to pyrethroids. It was proved to be a target gene and designated as *pytZ*. For the other ORF, it was not able to degrade pyrethroids and was not a target gene. In addition, sequence analysis indicated that this ORF had no any type of conserved domain and

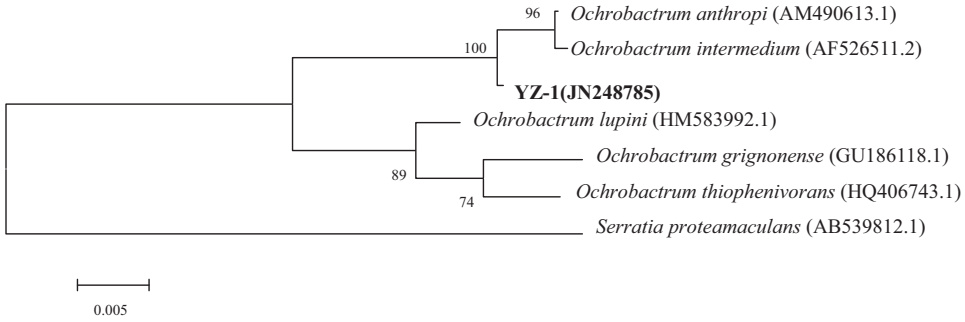

Fig. 1. The phylogenetic tree based on the 16S rDNA sequences of strain YZ-1 and related strains. Bootstrap values are given at branching points.

it only showed approximate 20–30% similarity with several hypothetic proteins. Which protein this ORF encoded was unknown. Sequence analysis revealed that the *pytZ* gene was composed of 606 bp which encoded a pyrethroid-hydrolyzing carboxylesterase of 201 amino acids. PytZ showed moderate identities (39–59%) with the most homologous carboxylesterase, e.g. carboxylesterase from *Mesorhizobium australicum* WSM2073 (ZP_08990834.1, 59% identity), carboxylesterase from *Paenibacillus curdlanolyticus* YK9 (ZP_07388827.1, 43% identity), carboxylesterase from *Enterococcus faecalis* T2 (ZP_05426330.1, 39% identity). However, it shared 95% identity with the putative carboxylesterase from *O. anthropi* ATCC 49188 (YP_001371865.1). The reason might be that the isolated strains YZ-1 and ATCC 49188 belong to the same genus. The catalytic triad formed by Ser-Asp-His and conserved pentapeptide motif of Gly-X-Ser-X-Gly is a typical feature of esterase family [37]. Multiple alignment result of PytZ with other carboxylesterases revealed that the enzyme contained the same catalytic triad, consisting of Ser^{105}, Asp^{154}, and His^{185} and conserved pentapeptide motif Gly-X-Ser-X-Gly (residues from 103 to 107). The Ser^{105} residue was in the center of the motif as catalytic nucleophile (Fig. 2). Furthermore, to clarify which esterase family the PytZ belongs to, a neighbor-joining phylogenetic tree was constructed

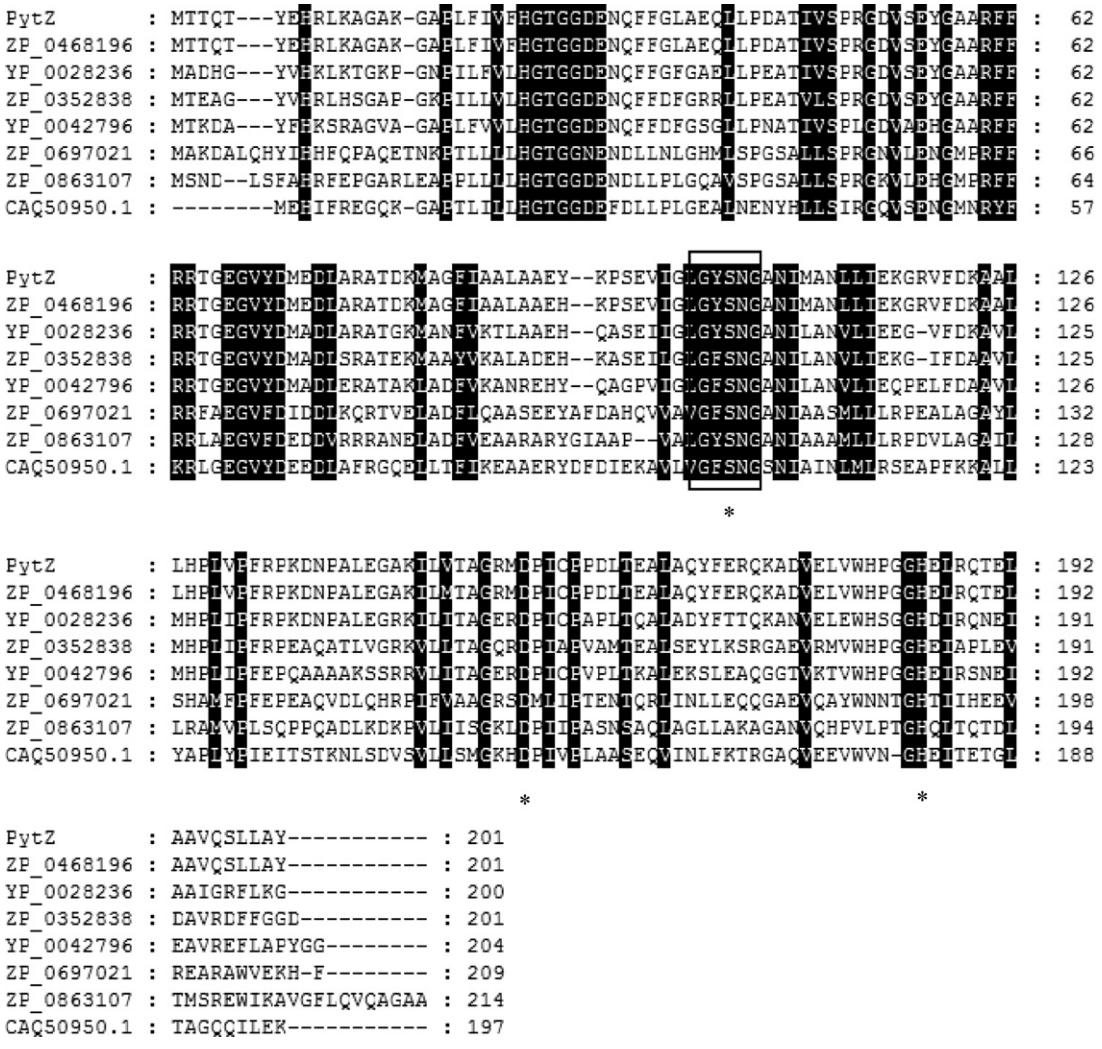

Fig. 2. Multiple alignment of amino acid sequences of the PytZ and other related proteins. The accession numbers of the protein sequences were given in brackets.

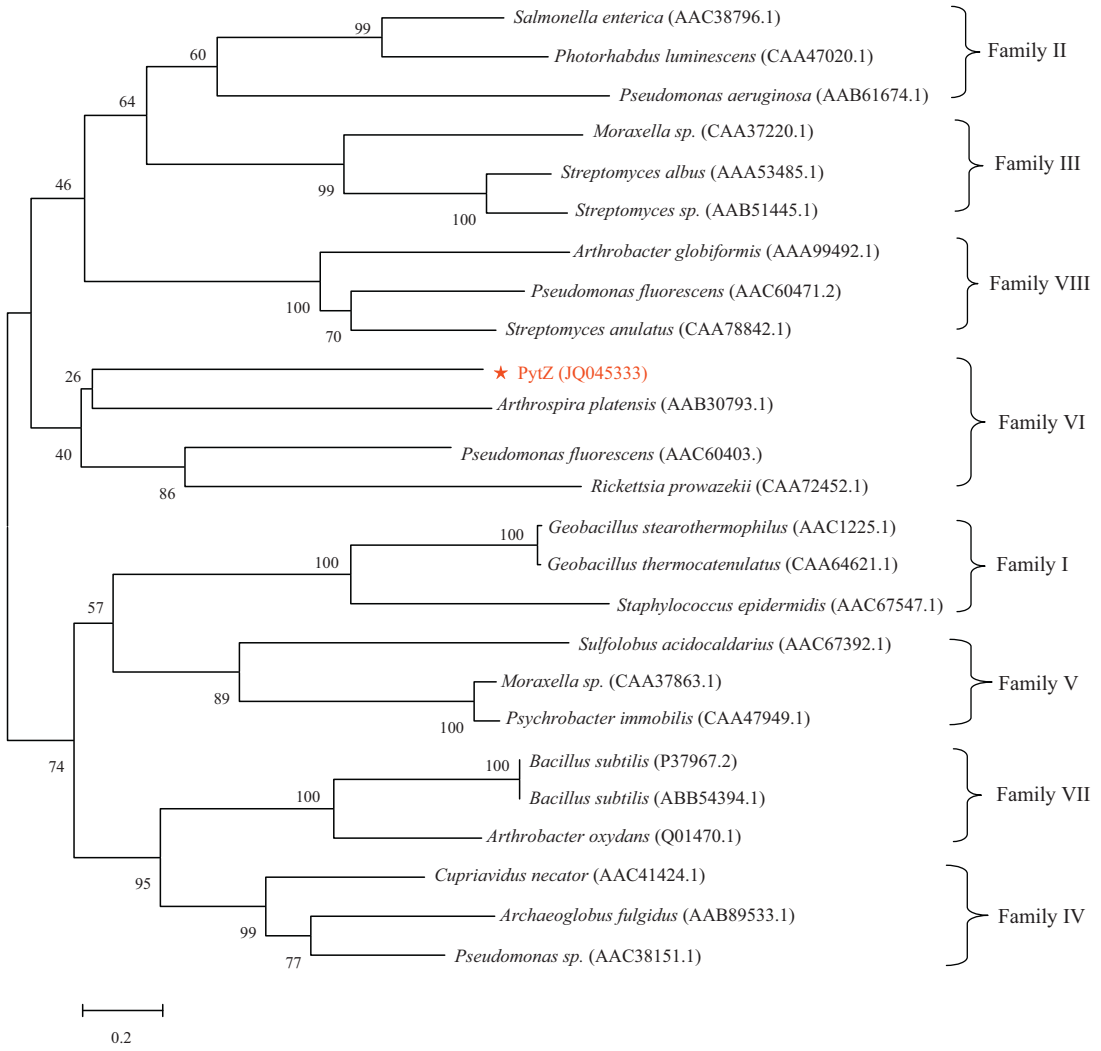

Fig. 3. Phylogenetic relationship of PytZ and other proteins of 8 different families. The accession numbers of the protein sequences were given in brackets.

using 24 bacterial esterase/lipase amino acid sequences which belonged to 8 different families, respectively [38]. As shown in Fig. 3, PytZ was clustered with *Arthrospira platensis* (AAB30793.1), *Pseudomonas fluorescens* (AAC60403.1) and *Richettsia prowazekii* (CAA72452.1), which belong to the family VI esterase, suggesting a new number of this family. Besides, *pytZ* showed no any similarity with reported degrading genes in deduced amino acid sequence. This revealed that the *pytZ* is a new pyrethroid hydrolyzing gene.

3.4. Expression and purification of recombinant PytZ

To study the enzymatic properties of PytZ, the *pytZ* gene was expressed in *E. coli* BL21 (DE3) using pET30a (+) expression system. Generally, the heterologous expression of recombinant protein is often influenced by many factors. In addition, the induced protein is prone to form the inclusion body in host cell due to excessive expression. In the present study, the protein of PytZ was expressed easily at a high level with the induction of 1 mM IPTG at 30 °C. More important, solubility analysis revealed that the recombinant protein was soluble. This facilitates its purification for the next function study. These characteristics make it possible for the mass production of PytZ and practical application in future. The purified recombinant protein showed a single band on SDS-PAGE (Fig. 4). The molecular mass of PytZ was approximately 25 kDa, in quite agreement with the molecular mass deduced from the amino acid sequence (24.2 kDa). It is smaller than the reported pyrethroid-hydrolyzing enzymes, such as permethrinase (61 kDa) from *Bacillus cereus* SM3 [14], pyrethroid hydrolase (56 kDa) from *Aspergillus niger* ZD11 [15] carboxylesterase (60 kDa) from mouse liver microsomes [21], carboxylesterase E3 (58.6 kDa) from *Nephotettix cincticeps* Uhler [22], EstP (73 kDa) from *Klebsiella* sp. ZD112 [23], carboxylesterase (31 kDa) from *Sphingobium* sp. JZ-1 [24], and esterase (31.15kDa) from the metagenome [25].

3.5. Substrate specificity of PytZ

The substrate specificity of the enzyme to various pyrethroids was determined. As shown in Fig. 5, the purified protein of PytZ was able to hydrolyze all the pyrethroids tested, which indicated that the enzyme was a broad-spectrum pyrethroid-hydrolyzing enzyme. The reason might be that the most pyrethroid pesticides possess the similar ester bond in their molecular structure [36]. In general, pesticide residues are mixtures in environment. Therefore, a broad-spectrum pyrethroid-degrading enzyme will have better practical application in biodegradation. Each enzyme has its optimal substrate. In the present study, the purified PytZ showed different degrading efficiency to various pyrethroids tested. Lambda-cyhalothrin was the optimal substrate and deltamethrin was the most persistent. This suggested that subtle differences in chemical structure of different substrates can

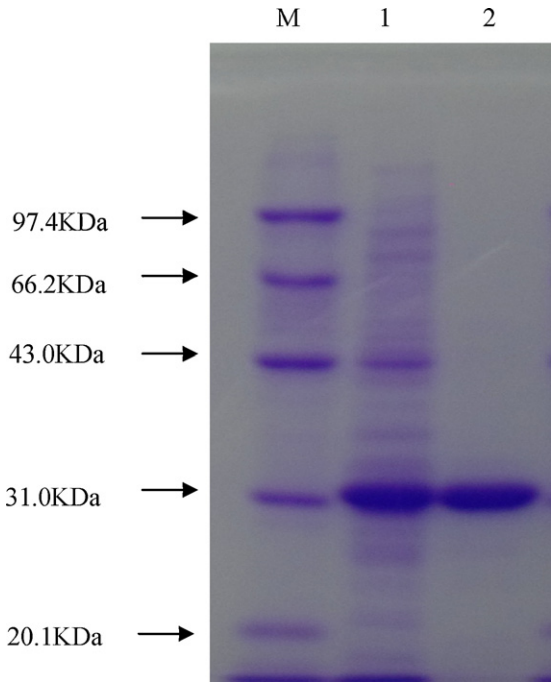

Fig. 4. SDS-PAGE analysis of the recombinant protein of PytZ. Lane M, protein marker; lane 1, total protein of *E. coli* BL21 (DE3)/pET30a-*pytZ*; lane 2, the purified protein of PytZ.

influence the enzyme activity significantly. Besides, the substrate specificity to *p*-nitrophenyl esters revealed that the PytZ was an esterase, not a lipase (data not shown). When lambda-cyhalothrin was used as substrate, the K_m and V_{max} values of the enzyme PytZ were 2.65 mmol L^{-1} and 53.19 nmol min^{-1}, respectively.

3.6. Effect of temperature and pH on the enzyme activity

Environmental factors, such as temperature and pH, have an important effect on the enzyme activity. The enzyme activity of PytZ was determined under the temperature of 15–60 °C. As shown in Fig. 6a, the optimal temperature for the enzyme activity was 35 °C. The relative activities were more than 76.5% in the temperature range of 30–45 °C Thermostability assay revealed that the enzyme was stable up to 45 °C and 56% of residual activity remained at 50 °C. The PytZ lost enzymatic activity when it was incubated at 60 °C for 2 h. The pH can also influence the enzymatic activity significantly. Each enzyme has a suitable pH range to work efficiently. PytZ showed activity in a broad pH range of 5.0–9.0. The maximal enzyme activity was observed at pH 7.5 (Fig. 6b). In this work, PytZ displayed high activity and stability over a broad range of temperature and pH value. These characteristics are important for the practical application of an enzyme, taking the complexity of the natural environment into consideration.

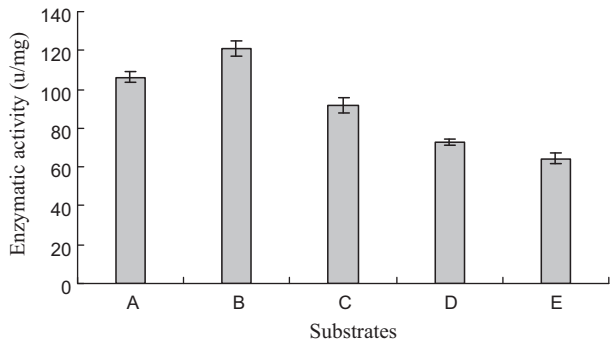

Fig. 5. Substrate specificity of purified PytZ to various pyrethroids. A, beta-cypermethrin; B, lambda-cyhalothrin; C beta-cyfluthrin; D, permethrin; and E, deltamethrin. All the determinations were done in triplicate.

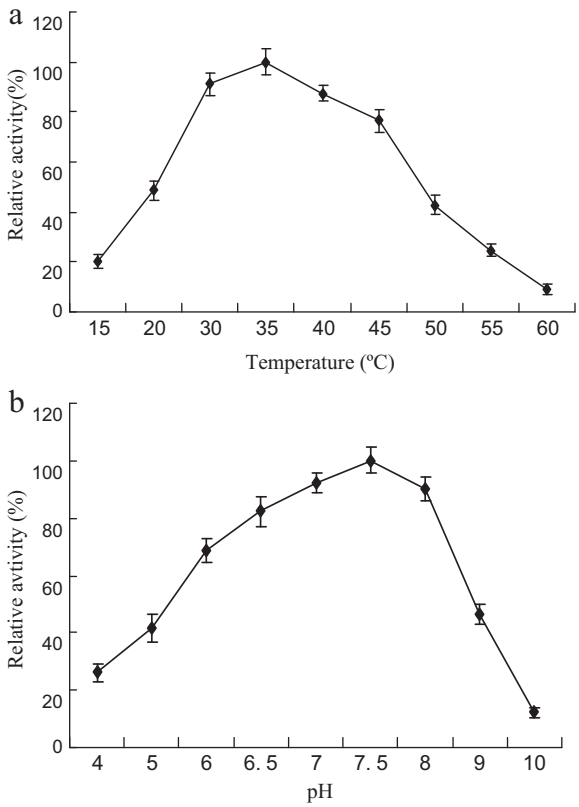

Fig. 6. Effect of temperature (a) and pH (b) on enzyme activity of purified PytZ. The highest activity at 35 °C and pH 7.5 was set as 100%, respectively. All the measurements were done in triplicate.

3.7. Effect of metal ions and chemical agents on the enzyme activity

The effect of metal ions and chemical agents on the enzyme activity is shown in Tables 2 and 3, respectively. The enzyme was strongly inhibited by metal ions of Ag^+ and Hg^{2+}, surfactant SDS, Ser protease inhibitor PMSF and His modifier DEPC (1 mM). However, M^{2+} (1 mM), Tween-20 and Tween-80 (1.0%) caused a slight increase in enzyme activity. The chelating agents EDTA and 1,10-phenanthroline (1 mM) showed little effect on the enzyme activity, suggesting that PytZ might need no cofactors. This feature was similar to the pyrethroid hydrolase from *Sphingobium* sp. JZ-1 [24]. The enzyme was strongly inhibited by Ser protease inhibitor PMSF. This indicated that the PytZ may not have a lid structure, which could

Table 2
Effect of metal ions on enzyme activity of purified PytZ. The activity of PytZ without the addition of metal ions was set as 100%.

Compounds	Concentration (mM)	Relative activity (%)
Control	0	100.0 ± 2.8
NaCl	1	102.5 ± 1.7
KCl	1	100.4 ± 3.2
$MgSO_4$	1	117.6 ± 4.3
$ZnCl_2$	1	83.6 ± 2.2
$FeSO_4$	1	105.9 ± 3.8
$AgNO_3$	1	12.9 ± 1.4
$HgCl_2$	1	10.3 ± 2.1

Table 3
Effect of chemical reagents on the enzyme activity of purified PytZ. The activity of enzyme without addition of chemical reagents was taken to be 100%.

Chemical reagents	Concentration	Relative activity (%)
Control	0	100.0 ± 2.8
Tween-20	1%	112.4 ± 3.5
Tween-80	1%	110.3 ± 4.2
SDS	1 mM	6.7 ± 1.2
PMSF	1 mM	8.2 ± 1.6
DEPC	1 mM	13.6 ± 2.9
EDTA	1 mM	94.5 ± 3.9
1,10-Phenanthroline	1 mM	96.7 ± 4.5

eliminate the inhibition effect of PMSF [39]. The function of Ser^{105} needs to be confirmed by the site-directed mutagenesis.

4. Conclusions

In the present study, a novel pyrethroid-hydrolyzing carboxylesterase gene (*pytZ*) was cloned from the genomic library of *O. anthropi* YZ-1. The *pytZ* was a new pyrethroid-degrading gene. PytZ was expressed in *E. coli* BL21 (DE3). The recombinant protein was soluble and this facilitated its purification for the function study and practical application. The purified enzyme displayed a broad substrate specificity, high enzyme activity, and favorable stability over a wide range of temperature and pH. These enzymatic properties are very important, considering the complexity of the practical environment. All the results indicated that the pyrethroid-degrading enzyme of PytZ is a very potential candidate for the detoxification of pyrethroid residues in environmental biotechnology. The further studies for its practical application, such as bioreactor and wash solutions, are ongoing.

Acknowledgement

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