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Cloning of a Novel Pyrethroid-Hydrolyzing Carboxylesterase Gene from *Sphingobium* sp. Strain JZ-1 and Characterization of the Gene Product[▽]

Bao-zhan Wang, Peng Guo, Bao-jian Hang, Lian Li, Jian He,* and Shun-peng Li

Key Laboratory of Microbiological Engineering of Agricultural Environment, Ministry of Agriculture, Life Sciences College of Nanjing Agricultural University, Nanjing, Jiangsu 210095, People's Republic of China

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A novel esterase gene, *pytH*, encoding a pyrethroid-hydrolyzing carboxylesterase was cloned from *Sphingobium* sp. strain JZ-1. The gene contained an open reading frame of 840 bp. Sequence identity searches revealed that the deduced enzyme shared the highest similarity with many α/β-hydrolase fold proteins (20 to 24% identities). PytH was expressed in *Escherichia coli* BL21 and purified using Ni-nitrilotriacetic acid affinity chromatography. It was a monomeric structure with a molecular mass of approximately 31 kDa and a pI of 4.85. PytH was able to transform *p*-nitrophenyl esters of short-chain fatty acids and a wide range of pyrethroid pesticides, and isomer selectivity was not observed. No cofactors were required for enzyme activity.

Pyrethroid pesticides are now the major class of insecticides used for insect control in agriculture and households as a replacement for more toxic organophosphorus pesticides, and their usage is continuing to grow (10). Although pyrethroid pesticides generally have lower acute oral mammalian toxicity than organophosphate insecticides, exposure to very high levels of pyrethroid pesticides might cause endocrine disruption, lymph node and spleen damage, and carcinogenesis (6, 12). In addition, most pyrethroid pesticides possess acute toxicity to some nontarget organisms, such as bees, fish, and aquatic invertebrates, often at concentrations of less than 0.5 μg/kg (19, 22). Great concerns have been raised about the persistence and degradation of pyrethroid pesticides in the environment.

In general, pyrethroid pesticides are degraded by both abiotic and biotic pathways, including photooxidation, chemical oxidation, and biodegradation. Microorganisms play the most important role in degradation of pyrethroids in soils and sediments. Many pyrethroid-degrading microorganisms have been isolated from soils (13, 16, 24, 27).

The major routes of pyrethroid metabolism in pyrethroid-resistant insects and pyrethroid-degrading microorganisms include oxidation by cytochrome P450s and ester hydrolysis by carboxylesterases (9). Carboxylesterases are a family of enzymes that are important in the hydrolysis of a large number of endogenous and xenobiotic ester-containing compounds, such as carbamates, organophosphorus pesticides, and pyrethroids. Carboxylesterases from *Bacillus cereus* SM3 (17), *Aspergillus niger* ZD11 (13), *Nephotettix cincticeps* (2), and mouse liver microsomes (23) hydrolyzing the carboxyl ester linkage of the pyrethroids were purified to homogeneity and characterized. Genes encoding the pyrethroid-hydrolyzing carboxylesterases from mouse liver microsomes and *Klebsiella* sp. strain ZD112 were cloned and functionally expressed (23, 27).

Pyrethroids differ from many other pesticides in that they contain one to three chiral centers; the chirality may arise from the acid moiety, the alcohol moiety, or both (Fig. 1). A pyrethroid compound therefore consists of two to eight isomers. Isomers of a chiral compound often differ from each other in biological properties. Isomer selectivity has been widely observed in insecticidal activity for the isomers of a pyrethroid compound. Recently, studies have shown that biodegradation of pyrethroids also exhibits significant isomer selectivity (15, 23).

In this study, we described the isolation and identification of the pyrethroid-degrading *Sphingobium* sp. strain JZ-1, the cloning and expression of the gene *pytH* encoding a novel pyrethroid-hydrolyzing carboxylesterase, and the characterization of the purified enzyme.

MATERIALS AND METHODS

Chemicals. Fenpropathrin, cypermethrin, fenvalerate, deltamethrin, permethrin, cyhalothrin, bifenthrin, and 2,2-dimethyl-3-(2,2-dichlorovinyl)-cyclopropanecarboxylic acid were obtained from Yangnong Chemical Group Co., Ltd., Jiangsu, China. 3-Phenoxybenzaldehyde, *p*-chloromercuribenzoic acid (pCMB), diethyl pyrocarbonate (DEPC), iodoacetamide, and phenylmethylsulfonyl fluoride (PMSF) were purchased from Sigma. *cis*- and *trans*-permethrin and *cis*- and *trans*-cypermethrin were purchased from the Agro-Environment Protection Institute of MOA, China. All chemicals were analytical grade and ≥98% pure. The stock solutions of different pyrethroids (40 mM) were prepared in methanol and sterilized by membrane filtration (pore size, 0.22 μm).

Isolation and identification of pyrethroid-degrading bacteria. To isolate pyrethroid-degrading bacteria, a conventional enrichment culture was carried out in 10-fold-diluted LB medium supplemented with 0.4 mM cypermethrin as an additional carbon source. The sludge sample used as the initial inoculant was collected from a pyrethroid-manufacturing wastewater treatment facility. After five rounds of transfer, the enrichment culture was spread on LB agar plates supplemented with 0.4 mM cypermethrin. A colony producing a visible transparent halo by degradation of cypermethrin was picked out and purified. The ability to degrade pyrethroids was determined by gas chromatography (GC) analysis. The isolate was characterized and identified by morphological, physiological, and biochemical characteristics and 16S rRNA gene analysis as described by Wittich et al. (26).

* Corresponding author. Mailing address: College of Life Sciences, Nanjing Agricultural University, 6 Tongwei Road, Nanjing, Jiangsu 210095, China. Phone: 86 2584396685. Fax: 86 2584396314. E-mail: hejian@njau.edu.cn.

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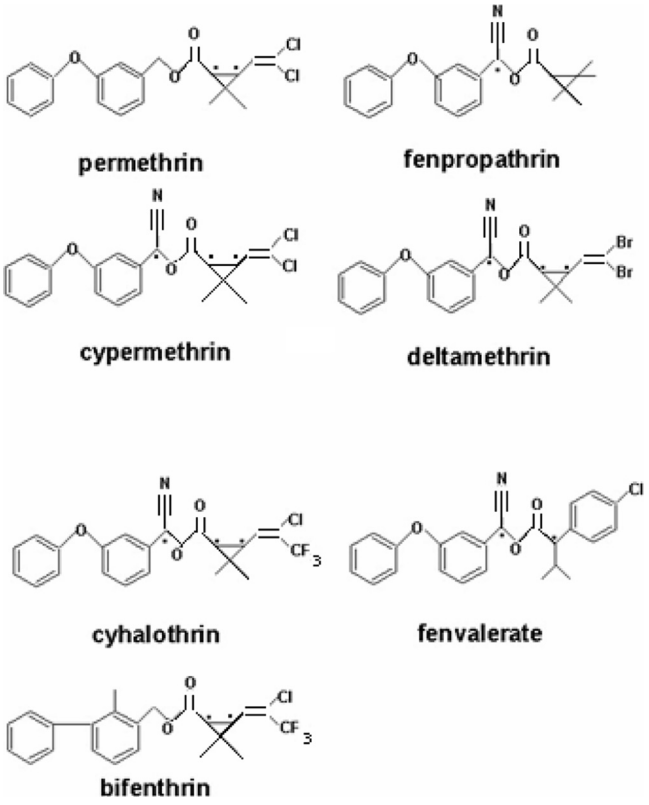

FIG. 1. Molecular structures of pyrethroids tested. Chiral centers are indicated by black dots.

Cloning of the pyrethroid-hydrolyzing carboxylesterase gene and data analysis. DNA manipulation was carried out as described by Sambrook et al. (20). To construct a size-fractionated genomic library, genomic DNA of *Sphingobium* sp. strain JZ-1 was subjected to partial digestion with Sau3AI. Fractions containing approximately 2- to 5-kb DNA fragments were pooled, ligated into the BamHI site of the plasmid pUC118 (Takara), and used to transform *Escherichia coli* DH5α. The library was plated onto LB agar containing 100 μg/ml ampicillin and 0.4 mM cypermethrin and incubated at 37°C for approximately 24 h. Colonies that produced clear transparent halos by degradation of cypermethrin were screened and further tested by GC analysis for the ability to degrade cypermethrin.

Nucleotide and deduced amino acid sequence analyses were performed using Omiga software. BlastN and BlastP were used for the nucleotide sequence and deduced amino acid identity searches (www.ncbi.nlm.nih.gov/Blast), respectively.

Gene expression and purification of the recombinant pyrethroid-hydrolyzing carboxylesterase. The open reading frame (ORF) without its translation stop codon was amplified by PCR, inserted into the NdeI-XhoI site of pET29a(+), and used to transform *E. coli* BL21(DE3). Optimal production of the fusion protein was obtained when mid-log-phase cells (optical density at 600 nm of 0.6) were induced with 1.0 mM isopropyl-β-D-thiogalactopyranoside for 12 h at 30°C. Harvested cells were washed and disrupted by sonication. Cell debris was removed by centrifugation. The supernatant was loaded onto a His-Bind resin (Novagen). The target protein was eluted with 100 mM imidazole, after elution of the nontarget proteins with 25 mM imidazole. The enzyme was dialyzed against 50 mM phosphate-buffered saline (pH 7.5) for 24 h and concentrated with an Amicon ultrafiltration tube. The protein concentration was quantified by the Bradford method using bovine serum albumin as the standard (1).

Determination of the molecular mass and pI. The molecular mass of the denatured protein was determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) according to the Laemmli method (11). The molecular mass of the native protein was determined by gel filtration (27). The pI was estimated by PAGE with 6.25% Ampholine (pH 3.5 to 10) in a gel rod (0.5 by 1.0 cm) using a kit for isoelectric focusing calibration (Pharmacia LKB).

Enzyme assay. Enzymatic activities toward *p*-nitrophenyl esters and various pyrethroids were determined. All assays were performed in 50 mM sodium phosphate buffer (pH 7.5) at 37°C. No more than 10% of the substrate was hydrolyzed during the assay. Hydrolytic activity for *p*-nitrophenyl esters was assayed according to the method described by Wu et al. (27). Hydrolytic activity for various pyrethroids was determined according to the method of Stok et al. with some modifications (23). Briefly, 1 μl of stock solution was added to 3 ml of the preincubated enzyme solution (1 to 5 μg/ml). The enzyme mixture was incubated for 30 to 60 s, depending on the substrate. One activity unit was defined as the amount of enzyme required to catalyze the formation or hydrolysis of 1 μmol of product or substrate per min.

For kinetic studies, stock solutions of each substrate were appropriately diluted in methanol into at least five different concentrations around the K_m values. Kinetic values were obtained from Lineweaver-Burk plots against various substrate concentrations.

Chemical analysis. For pyrethroid determination, the solution mixture was extracted with dichloromethane; the organic layer was dried and redissolved in *n*-hexane. The pyrethroid was detected by GC analysis. The GC conditions were as follows: electron capture detector, SPB-5 capillary column, injector/interface temperature of 260°C, oven temperature of 240°C, detector temperature of 300°C, and N_2 carrier gas at 1 ml/min.

Separation and quantification of the fenpropathrin and fenvalerate isomers were carried out by high-pressure liquid chromatography (HPLC) with a Chiralcel OJ-H column. The UV wavelength for detection was 230 nm. For fenvalerate, the mobile phase was *n*-hexane–1,2-dichloroethane–ethanol (92:6:2 [vol/vol/vol]) at a flow rate of 0.6 ml/min at 40°C. For fenpropathrin, the mobile phase was *n*-hexane–isopropanol (95:5 [vol/vol]) at a flow rate of 0.4 ml/min at 25°C.

For identification of the metabolites of cypermethrin, the solution mixture was extracted with dichloromethane. The organic layer was dried and redissolved in methanol. Separation and identification of the metabolites were carried out by reverse-phase HPLC. The sample was eluted isocratically with acetonitrile-water (60:40 [vol/vol], acidified to pH 3 with methanoic acid) and detected by measuring the absorption at 270 nm. The metabolites were further confirmed by tandem mass spectrometry.

Effect of temperature and pH on enzyme activity. The pH range of the enzyme was determined by incubating the enzyme with cypermethrin as the substrate for 30 s at pH values between 4 and 10. For pH stability determination, the sample was incubated in 50 mM phosphate buffers, pH 4 to 10, at 37°C for 4 h. As a nontreatment control, the same operation was performed but without enzyme addition. The remaining activity was assayed as described above. For determination of the thermostability, the enzyme was preincubated in a water bath at different temperatures for 1 h (18, 25), and then the residual activity was determined.

Effect of metal ions and chemical agents on enzyme activity. The effects of potential inhibitors or activators on the enzyme were determined by addition of various metal salts and chemical agents to the reaction mixture, which was preincubated for 30 min at 37°C. Pyrethroid-hydrolyzing activity was assayed as described above and expressed as a percentage of the activity obtained in the absence of the added compound.

Nucleotide sequence accession numbers. The nucleotide sequences of the 16S rRNA and *pytH* genes of *Sphingobium* sp. strain JZ-1 were deposited in the GenBank database under accession numbers FJ686047 and FJ688006, respectively.

RESULTS

Isolation and identification of the pyrethroid-degrading strain. The enrichment procedure generated a pure culture designated JZ-1. The strain was gram negative, aerobic, rod shaped (0.5 to 0.6 μm by 1.1 to 1.2 μm), and nonsporulating. Urease, Voges-Proskauer reaction, and nitrate reduction were negative, and oxidase and catalase were positive. The DNA G+C content was 63.3 ± 0.5 mol%. The 16S rRNA gene sequence of strain JZ-1 showed 98.5% similarity to that of *Sphingobium cloacae* JCM 10874^T and 95.0 to 97.2% similarities to other typical strains of the genus *Sphingobium*. Based on the results of morphological and physiological characterization and 16S rRNA gene sequence analysis, strain JZ-1 was identified as a *Sphingobium* sp. strain.

Sphingobium sp. strain JZ-1 was capable of degrading a wide range of pyrethroids, including permethrin, fenpropathrin, cyper-

TABLE 1. Kinetic constants determined for the purified recombinant PytH

Substrate	Mean ± SD			
	Sp act (μmol/min/mg)	k_{cat} (s^{-1})	K_m (μM)	k_{cat}/K_m ($\mu M^{-1} \cdot s^{-1}$)
trans-Permethrin	5.82 ± 0.29	3.03 ± 0.15	0.062 ± 0.002	48.90 ± 2.44
cis-Permethrin	5.76 ± 0.24	3.00 ± 0.13	0.065 ± 0.003	46.15 ± 1.92
Fenpropathrin	5.02 ± 0.23	2.61 ± 0.12	0.106 ± 0.005	24.67 ± 1.13
trans-Cypermethrin	4.86 ± 0.22	2.53 ± 0.11	0.110 ± 0.005	23.01 ± 1.04
cis-Cypermethrin	4.95 ± 0.23	2.57 ± 0.12	0.108 ± 0.004	23.87 ± 1.11
Cyhalothrin	2.46 ± 0.13	1.28 ± 0.07	0.348 ± 0.017	3.68 ± 0.20
Fenvalerate	1.75 ± 0.08	0.91 ± 0.04	0.585 ± 0.026	1.56 ± 0.07
Deltamethrin	1.52 ± 0.06	0.79 ± 0.03	0.788 ± 0.038	1.00 ± 0.04
Bifenthrin	0.84 ± 0.03	0.44 ± 0.02	1.586 ± 0.072	0.27 ± 0.01
p-Nitrophenyl acetate	351 ± 18	183 ± 9	124 ± 5	1.47 ± 0.07
p-Nitrophenyl butyrate	226 ± 10	118 ± 5	176 ± 8	0.67 ± 0.03
p-Nitrophenyl caproate	95 ± 4	49 ± 2	325 ± 15	0.15 ± 0.01
p-Nitrophenyl caprylate	0	0	NM[a]	NM
p-Nitrophenyl palmitate	0	0	NM	NM

[a] NM, not measurable; K_m and k_{cat}/K_m could not be calculated due to specific activity data not being available.

methrin, fenvalerate, deltamethrin, cyhalothrin, and bifenthrin. Permethrin was the preferred substrate, and bifenthrin was the most persistent. To our knowledge, this strain is the first reported strain in the genus *Sphingobium* that is capable of degrading pyrethroid.

Cloning and sequence analysis of the pyrethroid-hydrolyzing carboxylesterase gene. A positive clone that produced a transparent halo around the colony was screened from approximately 38,000 transformants. The inserted fragment in the transformant was 1,560 bp and contained three complete ORFs. The three ORFs were then subcloned into the linear vector pMD18-T and used to transform *E. coli* DH5α. One ORF was confirmed to be the target gene encoding the pyrethroid-hydrolyzing carboxylesterase and was designated *pytH*.

Sequence analysis indicated that the ORF consisted of 840 bp encoding a protein of 280 amino acids. A putative ribosomal binding site (AGGAAG) was located at 8 bp upstream of the start codon, ATG. The G+C content was 66.55%. No potential signal sequence was found. The deduced protein was compared with the known enzymes available from the Protein Data Bank (NCBI database). PytH showed the highest similarity with some α/β-hydrolase fold proteins, e.g., a hydroxynitrile lyase from *Hevea brasiliensis* (24% identity) (4), the carboxylesterase BioH from *E. coli* (23% identity) (21), a (−) γ-lactamase from an *Aureobacterium* sp. (23% identity) (14), and a hydrolase from a *Janthinobacterium* sp. (20% identity) (7).

Gene expression and purification of the recombinant PytH. The recombinant PytH was produced in *E. coli* BL21(DE3) and purified from the crude extract using Ni-nitrilotriacetic acid affinity chromatography. The purified enzyme gave a single band on SDS-PAGE. The molecular mass of the denatured enzyme was approximately 31 kDa, which was in good agreement with the molecular mass deduced from the amino acid sequence (30,835 Da). The molecular mass of the native enzyme was also approximately 31 kDa. Hence, it was assumed that PytH was a monomer. The pI value was 4.85.

PytH catalyzed the hydrolysis of cypermethrin to equimolar amounts of cyano-3-phenoxybenzyl alcohol and 2,2-dimethyl-3-(2,2-dichlorovinyl)-cyclopropanecarboxylic acid. Cyano-3-phenoxybenzyl alcohol was unstable and quickly transformed spontaneously to 3-phenoxybenzaldehyde. Similar hydrolysis by many other pyrethroid-hydrolyzing carboxylesterases was also reported (13, 17, 27).

Effect of temperature and pH on enzyme activity. The optimal pH was observed to be approximately 7.5. The enzyme was very stable at pH 5.5 to 9.0, retaining more than 80% of the original activity after preincubation at that pH range for 4 h. The enzyme was fairly stable up to 50°C, had 55% residual activity at 60°C, and was completely inactivated at 70°C.

Effect of metal ions and chemical agents on enzyme activity. The enzyme was strongly inhabited by many metal ions (Ag^+, Ni^{2+}, Cu^{2+}, Hg^{2+}, and Zn^{2+}) (0.5 mM), the surfactants SDS and Tween 80 (10 mM), the Ser protease inhibitor PMSF and the His modifier DEPC (0.5 mM), and the thiol reagent pCMB and iodoacetamide (0.5 mM), while Triton X-100 (10 mM) showed only slight inhibition (20 to 30% inhibition), and the chelating agents EDTA and 1,10-phenanthroline (10 mM) had little effect on the enzyme activity (less than 10% inhibition).

Kinetic analysis of the enzyme. The substrate specificities of the enzyme were tested with various pyrethroids as the substrates (Table 1). PytH was capable of hydrolyzing all of the pyrethroids tested, with the hydrolysis rates descending as follows: permethrin > fenpropathrin > cypermethrin > cyhalothrin > fenvalerate > deltamethrin > bifenthrin. The substrate specificities toward *p*-nitrophenyl esters of various fatty acids were also tested (Table 1). PytH showed the highest activity with *p*-nitrophenyl acetate, and the activities decreased with the increase of the aliphatic chain length. No lipolytic activity was observed with esters containing an aliphatic chain length longer than six carbon atoms, indicating that PytH was an esterase and not a lipase, considering that lipases prefer substrates with relatively long aliphatic chains. Although *p*-nitrophenyl acetate had an extremely high k_{cat} value (183 s^{-1}), the K_m value for this substrate was 78- to 2,000-fold higher than those of the pyrethroids.

Isomer selectivity of PytH. Commercial fenpropathrin and fenvalerate (the pure stereoisomers of the two pyrethroids were not available in the market), *trans*- and *cis*-permethrin, and *trans*- and *cis*-cypermethrin were used to study the isomer

TABLE 2. SF change during the hydrolysis of fenpropathrin and fenvalerate

Sample[a]	SF					
	Fenpropathrin		Fenvalerate			
	α*R*-	α*S*-	α*S*,2*R*-	α*R*,2*S*-	α*S*,2*S*-	α*R*,2*R*-
A	0.484	0.516	0.272	0.273	0.228	0.227
B	0.482	0.518	0.270	0.275	0.229	0.226
C	0.481	0.519	0.269	0.276	0.232	0.223

[a] A, at the initial point of the reaction, no substrate was hydrolyzed; B and C, approximately 50% and 80% of the substrate were hydrolyzed, respectively.

selectivity of PytH. Fenpropathrin and fenvalerate consist of two (α*R*- and α*S*-) and four (α*S*,2*R*-, α*R*,2*R*-, α*S*,2*S*-, and α*R*,2*S*-) stereoisomers, respectively (Fig. 1). The stereoisomers in the enzyme solution were separated and quantified by using chiral HPLC. Isomer selectivity in hydrolysis was evaluated by studying the changes in the stereoisomer fraction (SF), which was calculated as the fraction of a given stereoisomer over the total chemical concentration during hydrolysis (15). The observed SF values for each isomer remained very stable during the whole process of hydrolysis of both fenpropathrin and fenvalerate (Table 2), indicating that the stereoisomers of fenpropathrin or fenvalerate were hydrolyzed at an approximately equal rate. Also, the specific activities for *trans*- and *cis*-permethrin (or cypermethrin) were found to be relatively similar (Table 1), suggesting that the *cis-trans* isomerism of the cyclopropyl does not have a major influence on PytH activities. These results indicated that PytH lacked isomer selectivity for pyrethroid pesticides.

DISCUSSION

In previous studies, some chromogenic substrates (27) or fluorescent substrates (23) similar in molecular structure to pyrethroids were successfully employed as reporters to monitor pyrethroid-hydrolyzing activity. However, the major shortcoming of those mimic strategies was that it was time-consuming to ensure the distinction of the pyrethroid-hydrolyzing esterase from other nonspecific esterases. Another disadvantage was that it might fail to identify esterases that specifically recognized the carboxylester linkage of pyrethroids. For example, Maloney et al. and Dowd et al. found that the pyrethroid-hydrolyzing enzyme was distinct from 3-nitrophenyl acetate esterase (5, 17). In recent research, we found that the pyrethroid-degrading strain and cell extract produced a visible transparent halo of pyrethroid degradation on agar plates supplemented with 0.4 mM pyrethroid. In this study, we successfully used this characteristic as a reporter to monitor the pyrethroid-hydrolyzing activity. Compared to mimic strategies, this method is simple, rapid, reliable, and low-cost.

The molecular mass of PytH was approximately 31 kDa, smaller than any of the reported pyrethroid-hydrolyzing enzymes, such as permethrinase (61 kDa) from *B. cereus* SM3 (17), pyrethroid hydrolase (56 kDa) from *A. niger* ZD11 (13), EstP (73 kDa) from *Klebsiella* sp. strain ZD112 (27), pyrethroid-hydrolyzing carboxylesterase (60 kDa) from mouse liver microsomes (23), and carboxylesterase E3 (58.6 kDa) from *N. cincticeps* Uhler (2). PytH possessed a relatively high catalytic efficiency, with the catalytic efficiency values (k_{cat}/K_m) for permethrin and cypermethrin being approximately 4.7- to 82.1-fold and 17.5- to 34.3-fold higher than those of reported pyrethroid-hydrolyzing enzymes, respectively.

Database searches revealed that PytH shared no similarity with any reported pyrethroid-hydrolyzing enzyme; however, it showed 20 to 24% identities to some α/β-hydrolase fold proteins. The α/β-hydrolase fold proteins are ubiquitous enzymes that share a common tertiary fold. In all known cases, the catalytic centers of α/β-hydrolase fold proteins are typically composed of Ser-His-Asp/Glu. Additionally, the highly conserved pentapeptide GXSXG forms a tight turn in a β-strand–turn–α-helix motif (3). Sequence alignment of PytH with other α/β-hydrolases fold proteins indicated that the enzyme contained the same catalytic triad, consisting of Ser78, Asp202, and His230, and a structural motif, GHSLG (residues 76 to 80).

PytH apparently had no requirement for metal ions, since the chelating agents EDTA and 1,10-phenanthroline had little effect on the enzyme activity. The enzyme was completely inhibited by the Ser protease inhibitor PMSF and the His modifier DEPC, suggesting the involvement of a Ser and a His at the active site of the enzyme. It was interesting that the enzyme, which contained only one Cys residue at position 18, was completely inactivated by the thiol reagent pCMB and iodoacetamide. A similar result was reported for the α-galactosidase from *Thermus* sp. strain T2, which also had only one Cys residue (8).

PytH was able to degrade a wide range of pyrethroid pesticides, and hydrolysis efficiencies were dependent on the pyrethroid molecular structure (Fig. 1). The catalytic efficiency value (k_{cat}/K_m) of permethrin was 2.0-fold higher than that of cypermethrin, indicating that the additional stereocenter introduced by the α-cyano group caused a relative reduction in the hydrolysis rate, due to either steric hindrance of hydrolysis or stabilization of the ester bond. The catalytic efficiencies of cypermethrin and fenpropathrin were found to be relatively similar, suggesting that the replacement of methyl with dichloroethylene on chrysanthemic acid had no significant influence on the hydrolysis efficiency. Deltamethrin, cyhalothrin, and fenvalerate were hydrolyzed much slower than cypermethrin, suggesting that the substitutions of chloroyl with bromovinyl, fluoro, or chloridben on the chrysanthemic acid significantly reduced the catalytic efficiencies. In comparison to other pyrethroids, the catalytic efficiency toward bifenthrin decreased 4- to 190-fold, indicating that replacement of the 3-phenoxybenzyl with a biphenyl greatly hindered the enzyme-substrate interaction.

Studies so far had shown that the biodegradation of pyrethroids was commonly isomer selective. For example, there was a preference in both bacterial and mammalian pyrethroid-hydrolyzing carboxylesterases for *trans*-permethrin over *cis*-permethrin (17, 23), while the carboxylesterase from *N. cincticeps* Uhler preferred *cis*-permethrin over *trans*-permethrin (2). It is interesting that PytH possessed approximately equal activity toward the isomers of each pyrethroid tested in this work. Since most commercial pyrethroids are mixtures of isomers and many isomers may persist in the environment due to isomer-selective degradation, PytH seems to be a good candi-

date to eliminate the pyrethroid residues in soil, sediment, and agricultural products.

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AUTHOR'S CORRECTION

Cloning of a Novel Pyrethroid-Hydrolyzing Carboxylesterase Gene from *Sphingobium* sp. Strain JZ-1 and Characterization of the Gene Product

Bao-zhan Wang,† Peng Guo,† Bao-jian Hang, Lian Li, Jian He,* and Shun-peng Li

Key Laboratory of Microbiological Engineering of Agricultural Environment, Ministry of Agriculture, Life Sciences College of Nanjing Agricultural University, Nanjing, Jiangsu 210095, People's Republic of China

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Page 5496, column 1, bottom: The following footnote should appear directly below the correspondent footnote:

†Bao-zhan Wang and Peng Guo contributed equally to this work.