

**Cloning and characterization of a pyrethroid pesticide
decomposing esterase gene, *Est3385*, from
Rhodopseudomonas palustris PSB-S**

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Table S1 Characteristics of pyrethroids degrading proteins originated from bacteria

Protein symbol	Molecule weight (kDa)	Optimal conditions		stability		substrate sepcificity	Inhibited metal ions	<i>Km</i> ($\mu\text{mol}\cdot\text{ml}^{-1}$)	The conserved Pentapeptide motif	Esterase family	Reference
		Temperature ($^{\circ}\text{C}$)	pH	Temperature ($^{\circ}\text{C}$)	pH						
pytH	31	35	7.5	30-60	5.5-9.0	permethrin	$\text{Ag}^+, \text{Ni}^{2+}, \text{Cu}^{2+}, \text{Hg}^{2+}, \text{Zn}^{2+}$	0.062 \pm 0.002	+	/	Wang et al., 2009
estI	67.5	35	7.0	30-50	6.0-8.0	permethrin	$\text{Hg}^{2+}, \text{Ag}^+$	14 \pm 1.08	+	/	Choi et al., 2004
estP	73	40	7.0	40-50	5.5-9.0	permethrin	$\text{Ag}^+, \text{Hg}^{2+}$	0.16 \pm 0.005	-	/	Wu et al., 2006
EstPS1	68	60	8.0	40-70	6.0-9.0	fenpropathrin		12.8	/	/	Cai et al., 2017
pytY	41.7	35	7.5	30-45	6.5-8.0	cyhalothrin	$\text{Ag}^+, \text{Hg}^{2+}$	234	+	VI	Ruan et al., 2013
pytZ	25	35	7.5	30-45	6.5-8.0	cyhalothrin	$\text{Ag}^+, \text{Hg}^{2+}$	265	+	VI	Zhai et al., 2012
E4	30.8	55	6.5	<55	4.5-8.5	cypermethrin	/	/	+	V	Fan et al., 2012
pye3	31.5	40	7.0	<45	5.5-9.0	Permethrin	$\text{Ag}^+, \text{Hg}^{2+}$	0.18	+	I	Li et al., 2008
EstSt7	/	80	9.0	80-90	8.0-10.0	/	/	/	/	/	Wen et al., 2013
Est3385	33.94	35	6.0	15-65	5.0-8.0	fenpropathrin	$\text{Fe}^{3+}, \text{Cu}^{2+}, \text{Mg}^{2+}$	0.734	-	I	This study

+, existing; -, no existing; /, data not being available.

Fig. S1. Multi alignment of the sequences of pyrethroid-pesticides degrading genes. The conserved “seed” nucleotide sequences were shown as underlined ones.

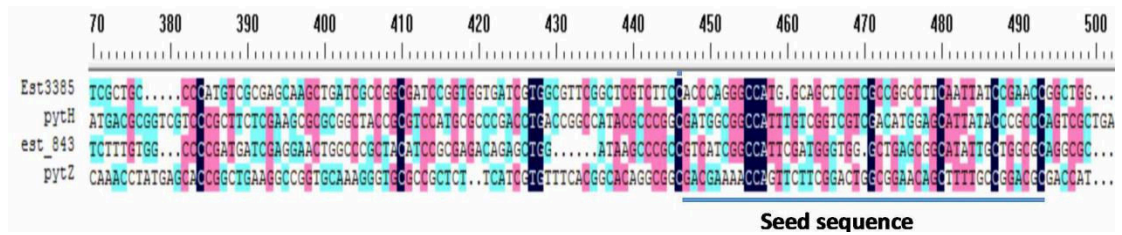


Fig. S2. Multi alignment of the amino-acid sequences of Est3385 with selected esterases. Four high identity contigs were shown with red overlines (H1~H4).

Est3385	0
Moraxella_sp._CAA37220.1_MFI	3
Streptomyces_albus_AAA53485.1_MHSTPRTG	8
Consensus	H1	
Est3385	VGRRAAGTAAALLVVTCANVGAAPSAQAQTAQAAPSSAFAAV	40
Moraxella_sp._CAA37220.1_	MIRKSEPAKAIIVIGALVFSIPTLAEVTLSETIVSSIKS.	42
Streptomyces_albus_AAA53485.1_	ITCAFGSRRLAASAAVAVVGLITLSTPGAQADN...	45
Consensus	1 v	
Est3385	QPSGSGVDDLAQSAQPERNLGRAVDKMKCVARQAGDIFS	80
Moraxella_sp._CAA37220.1_	.EATVSSTKKALPATFSDCIADSKITAVALSDFTRNGPFS	81
Streptomyces_albus_AAA53485.1_PYERGPAFTRASIEAPRFPYVVSQISVSSIVV	77
Consensus		
Est3385	FVFCLPFKGCVRPICSLPHVASKLIAE.....DFV	110
Moraxella_sp._CAA37220.1_	IRTKRISRQSAKGFGGTIHYPTNASE..CGLLGAIAVVP	119
Streptomyces_albus_AAA53485.1_	SGFGGGTIYYPTSTGCGTFGAVVVTFE.....	104
Consensus	H2 g g	
Est3385	VIVAFGSSSTQGHGSSSPAFNYENRLAAQLRRQVETAEIS	150
Moraxella_sp._CAA37220.1_	GYVSYENSIKWWGERLASWGEVVIINTNSIYDSDSRAA	159
Streptomyces_albus_AAA53485.1_	.FATSSMAWLGERLASQGEVVFITDITLTLESDSRGR	143
Consensus	H3 s H4 p	
Est3385	VINRGKGGEDAPEMLARLKSSVLDLKPDIWVCFETNAIL	190
Moraxella_sp._CAA37220.1_	QLNAALINMIADDIVGSMITP..KRLGATGWSMGGGALK	197
Streptomyces_albus_AAA53485.1_	QMLAALVLTTERSARTRITG..TRLGVIGHSMGGGTLE	181
Consensus	g	
Est3385	RDL.....DFACTAKVVEEGISAQAAGADIVLVDP	221
Moraxella_sp._CAA37220.1_	LAT.....ERSIVRAIMPLAFYHDRSYGEVKIPI	226
Streptomyces_albus_AAA53485.1_	AAK.....SRPSLRKAATPTEWNLKTIWFEVITP	210
Consensus		
Est3385	CYAPFVNER.....AENAGRMMKLLNKVAETRHVGLFPR	255
Moraxella_sp._CAA37220.1_	IVIACEED.....RIAETKRYANAFYKNAIGPK	254
Streptomyces_albus_AAA53485.1_	TLVVGATG.....DIVAFVATHAKFFYSLSLPSST	239
Consensus		
Est3385	FEVMRDWHERQSEVIDNFITPDGLHMNDG.....YAC	288
Moraxella_sp._CAA37220.1_	MKVVEVNGSHFCPSYRFNEILLSKFGIARMCRYIN....	289
Streptomyces_albus_AAA53485.1_	DRAYLELNATHFAPNLSNTIIAKYSVSKLR.....	271
Consensus	w	
Est3385	FAQLLG.....EDIIRSVG...QIKLGIH	309
Moraxella_sp._CAA37220.1_NDIRFDKFLCANENYS	305
Streptomyces_albus_AAA53485.1_FIDEDIRVEQFLCPLFV	288
Consensus		
Est3385	VPSEVHTIREM.....	320
Moraxella_sp._CAA37220.1_	KSPRISAPDYKDCF.....	319
Streptomyces_albus_AAA53485.1_	PDRDIEEIRGTCPLGG.....	304
Consensus	y	

Fig. S3. Expression, purification and western blotting of recombinant protein Est3385. M: protein marker. Lane 1: soluble recombinant Est3385 protein in the supernatant of *E. coli* Rosetta/pGEX-3x-3385. Lane 2: purified recombinant Est3385 protein. 3: no protein control.

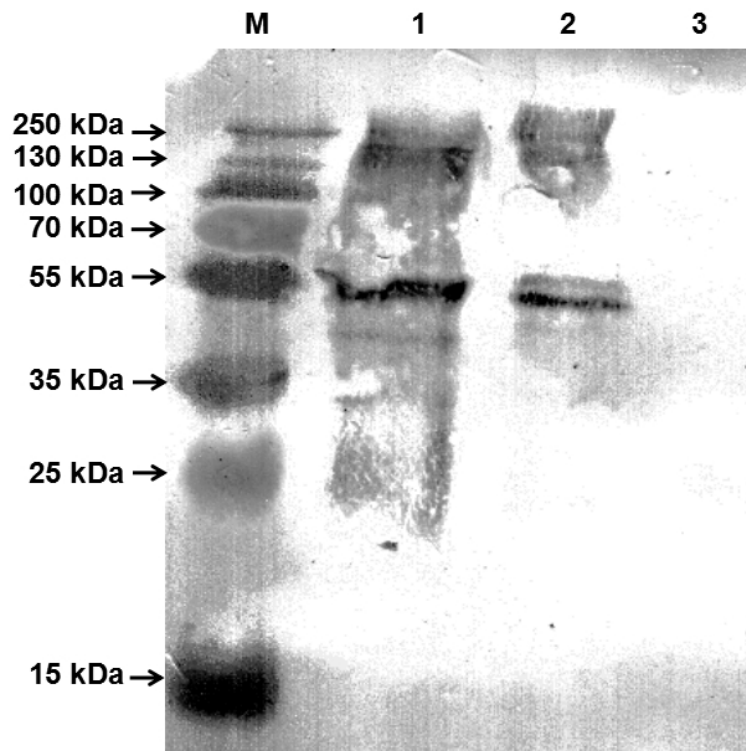


Fig. S4. Quantification of fenpropathrin residue by gas-chromatography (GC). (Reaction mixture including $100 \mu\text{g}\cdot\text{ml}^{-1}$ of fenpropathrin within PBS buffer. **Est3385**: reaction mixture adding $1 \mu\text{g}\cdot\text{ml}^{-1}$ Est3385; **CK1 (GST)**: reaction mixture adding $1 \mu\text{g}\cdot\text{ml}^{-1}$ glutathione S-transferase; **CK2 (No protein)**: reaction mixture adding equivalent volume of PBS buffer.)

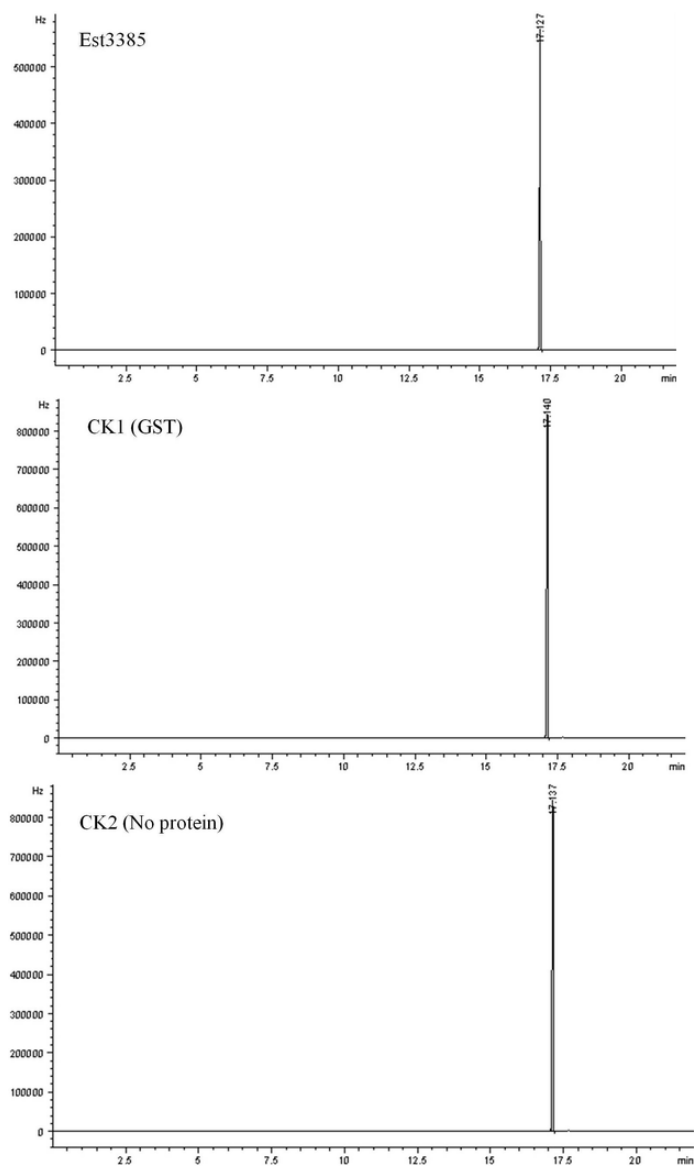


Fig. S5. Degradation of ρ -nitrophenyl esters and short-chain fatty acids by Est3385. The substrate activity was set as 100% when ρ -nitrophenyl acetate and Formic acid was used as the substrate. (Reaction mixture including $20 \mu\text{g}\cdot\text{ml}^{-1}$ ρ -nitrophenyl esters and fatty acids, $1 \mu\text{g}\cdot\text{ml}^{-1}$ Est3385. A: Degrading efficiency of ρ -nitrophenyl esters by Est3385; B: Degrading efficiency of short-chain fatty acids.)

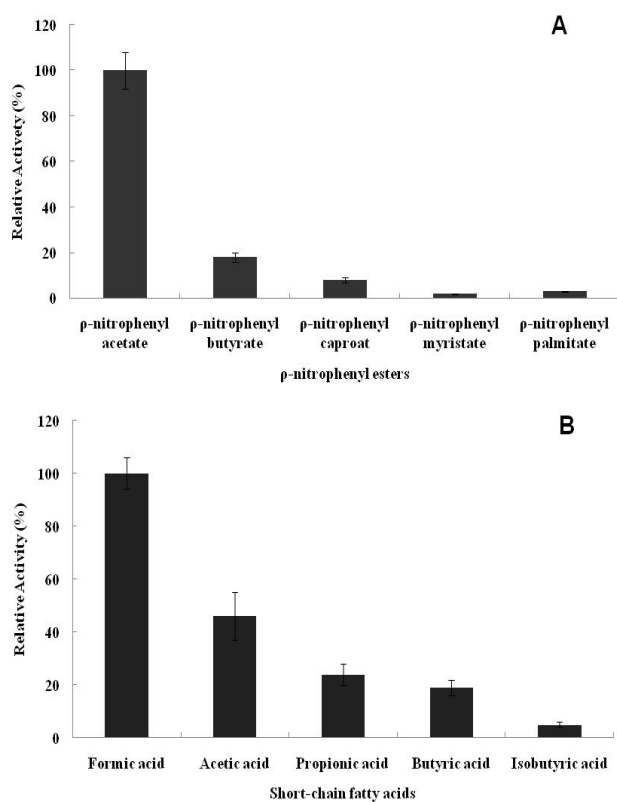


Fig. S6. The protein structure of Est3385. A: 3D structure of protein Est3385; B: Amino acid residues Ser117-119; C: Amino acid residues Ser125-128.

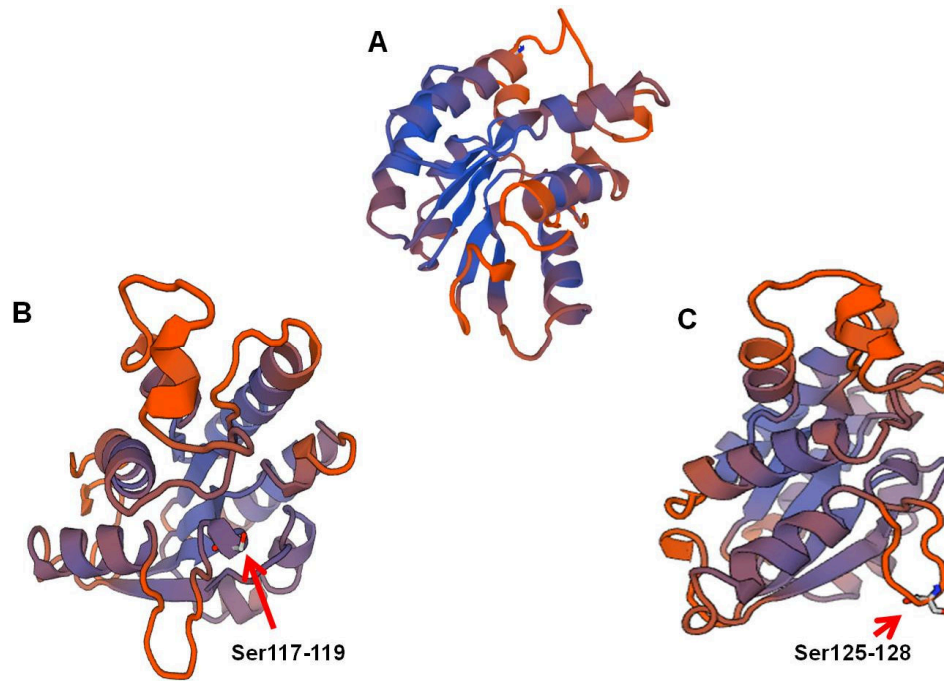


Fig. S7. The kinetic constant of protein Est3385 degrading fenpropathrin (Michaelis-Menten equation). [V]: degrading velocity ($U \cdot \mu\text{g}^{-1}$); [S]: fenpropathrin initial concentration ($\mu\text{g} \cdot \mu\text{l}^{-1}$).

