Supplementary materials

Synergistic degradation of pyrethroids by the quorum sensing-regulated carboxylesterase of *Bacillus subtilis* BSF01

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Table S1 The calibration and spiked recoveries of six pyrethroids

Pyrethroid	Calibration	R^2	Spiked concentration (mg L^{-1})	Average recovery (%)	RSD (%)
β-cypermethrin	$y = 54.562x - 60.248$	0.9998	1	82.2	3.3
			25	87.5	2.6
			50	94.6	2.4
Cypermethrin	$y = 38.082x - 44.181$	0.9995	1	87.1	3.3
			25	85.4	0.9
			50	91.1	0.9
β-cyfluthrin	$y = 51.984x - 50.939$	0.9996	1	81.3	2.0
			25	86.2	2.2
			50	89.9	2.4
Cyfluthrin	$y = 53.241x - 38.069$	0.9999	1	89.1	5.2
			25	87.7	4.3
			50	88.5	2.9
λ-cyhalothrin	$y = 61.130x - 24.732$	0.9997	1	91.0	6.7
			25	96.2	5.3
			50	89.6	2.4
Cyhalothrin	$y = 59.435x - 28.235$	0.9998	1	79.0	2.1
			25	85.7	2.5
			50	89.3	1.2

Table S2 Results of alanine scanning mutagenisis

Mutation	Mutation Energy (kcal/mol)	Effect of mutation
:LEU64>ALA	0.64	Destabilizing
:LEU172>ALA	0.74	Destabilizing
:LYS92>ALA	0.74	Destabilizing
:LEU130>ALA	0.86	Destabilizing
:PHE161>ALA	0.92	Destabilizing

Table S3 Results of saturation mutation

Mutation	Mutation energy (kcal/mol)	Effect of mutation	VDW interaction energy (kcal/mol)	Electrostatic interaction energy (kcal/mol)
:LEU64>PRO	0.77	Destabilizing	1.44	0.08
:LYS92>TYR	1.64	Destabilizing	3.4	0.08
:LEU130>ARG	1.35	Destabilizing	2.57	0.13
:PHE161>GLY	0.97	Destabilizing	1.83	0.02
:LEU172>GLY	0.99	Destabilizing	2.02	-0.02

Table S4 Primer sequences used in this study

Primer function category and name	Primer (5'-3') [#]	Restriction enzymes
Gene Cloning		
comA-F	ATGAAAAAGATACTAGTGATTG	
comA-R	TTAAAGTACACCGTCTGATT	
CesB-F	ATGATACAAGATTCAATGC	
CesB-R	CTATTTTATCCCCCCGCAT	
Construction plasmids of protein expression		
comA-eF	CCG**CTCGAG**ATGAAAAAGATACTAGTG	*Xhol* I
comA-eR	CG**GAATTC**TTAAAGTACACCGTCTGAT	*EcoR* I
CesB-eF	CG**GGATCCA**TGATACAAGATTCAATGC	*BamH* I
CesB-eR	CC**AAGCTT**CTATTTTATCCCCCCGCAT	*Hind* III
Site-directed mutagenesis for CesB		
T7F	TAATACGACTCACTATAGGG	
T7TER	TGCTAGTTATTGCTCAGCGG	
L64P-F	CTTCACGGGGGCCcTTTCAGCTCTGC	
L64P-R	GCAGAGCTGAAAgGGCCCCCGTGAAG	
K92Y-F	GATATGATAGGAGACtacAATAAAAGTATACC	
K92Y-R	GGTATACTTTTATTgtaGTCTCCTATCATATC	
L130R-F	CTGGCCGGCTTTTCGagaGGCGGGTCCCATATC	
L130R-R	GATATGGGACCCGCCtctCGAAAAGCCGGCCAG	
F161G-F	GCGTTTATTTCAggTCATCCGGATG	
F161G-R	CATCCGGATGAccTGAAATAAACGC	
L172G-F	CTATAAATACGCTGCAGAAggTACAGGGGCAAGTGGAGC	
L172G-R	GCTCCACTTGCCCCTGTAccTTCTGCAGCGTATTTATAG	
qRT-PCR		
CesB-RT-F	AGTTTGCCGCGGTTGAAA	
CesB-RT-R	TAAGCGAAGGTGCGTCCT	
comA-RT-F	CAATCAAAACCGCTTCCGTC	
comA-RT-R	GAAATCGCAGATGCCCTTCA	
16s rDNA-RT-F (reference)	TTGCTCCCTGATGTTAGCGGC	
16s rDNA-RT-R (reference)	ACGCATCGTTGCCTTGGTGAG	

Restriction enzymes were underlined; Site-directed mutagenesis was in lower case.

Supplementary figure caption:

Fig. S1 Genetic basis of *comA* in strain BSF01. A, PCR amplification products (M: DNA marker; Lane 1: gene *comA*); and B, Nucleotide sequence and amino acid sequence of *comA* gene

Fig. S2 Genetic and transcriptional basis of *cesB* in strain BSF01. A, PCR amplification products (M: DNA marker; Lane 1: gene *comA*); and B, comparison of multiple sequence alignments among CesB and other homologous enzymes.

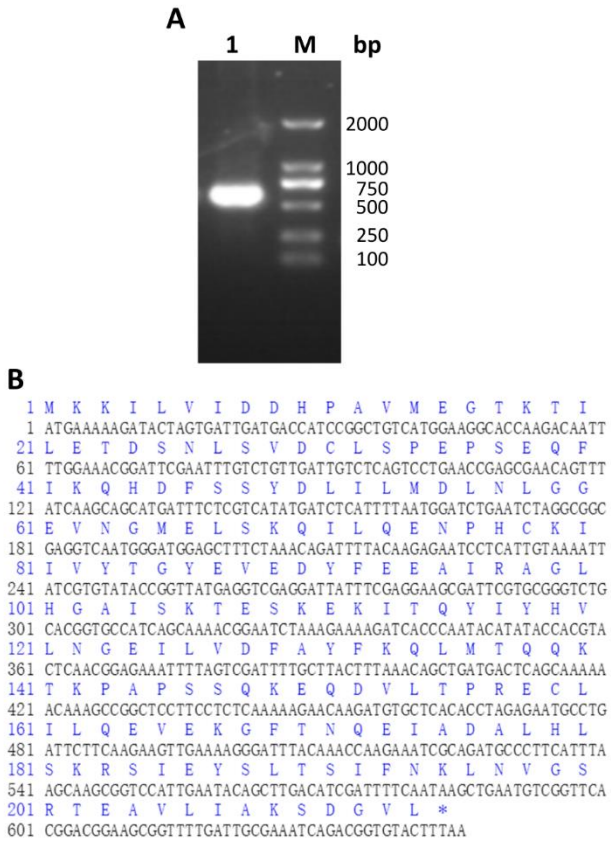

Fig. S1 Genetic basis of *comA* in strain BSF01. A, PCR amplification products (M: DNA marker; Lane 1: gene *comA*); and B, Nucleotide sequence and amino acid sequence of *comA* gene

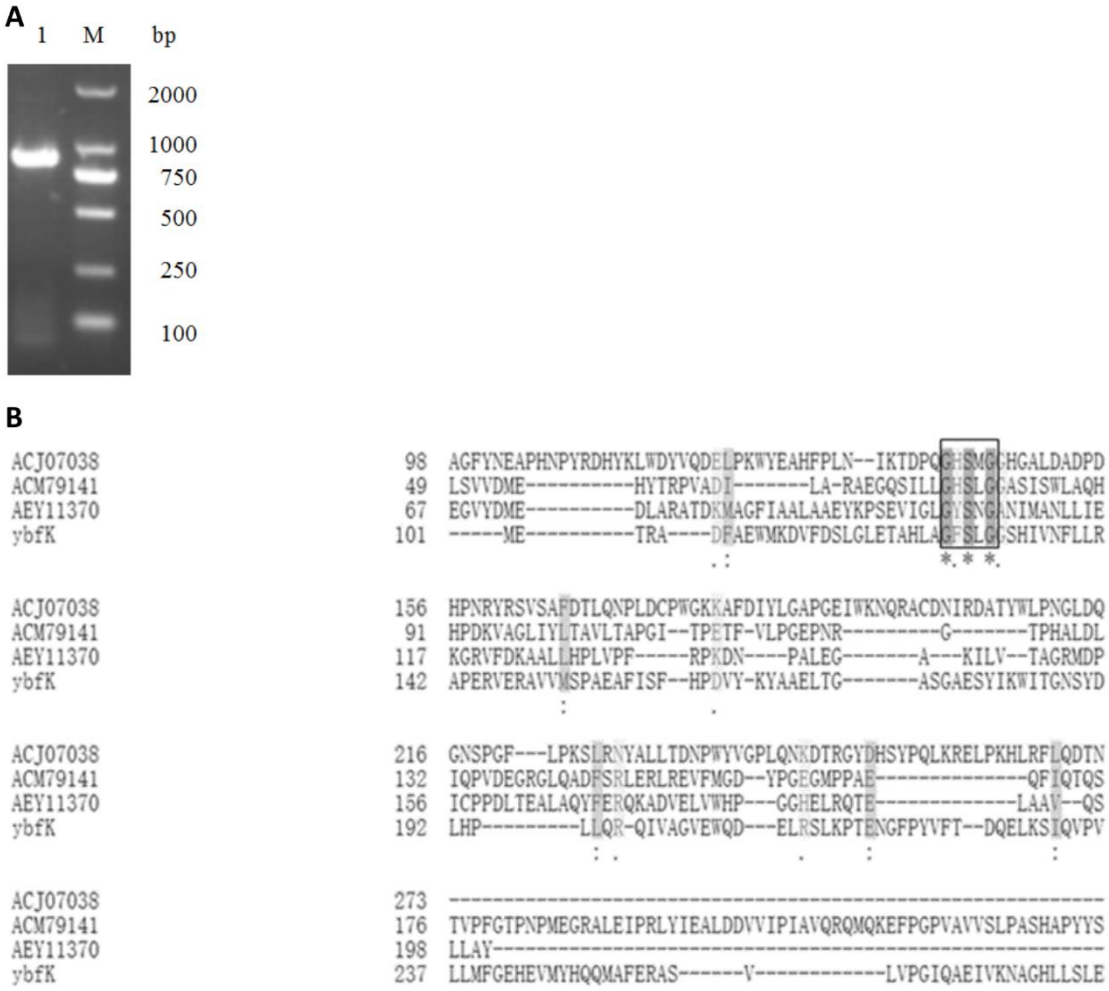

Fig. S2 Genetic and transcriptional basis of *cesB* in strain BSF01. A, PCR amplification products (M: DNA marker; Lane 1: gene *comA*); and B, comparison of multiple sequence alignments among CesB* and other homologous enzymes.

* The alternative name "ybfK" for carboxylesterase CesB was applied during analysis. Its conserved motif Gly-X-Ser-X-Gly was boxed.