

SUPPLEMENTAL MATERIALS

Supplemental Tables

TABLE S1 Oligonucleotide primers used in this study.

Primers	Sequences
Common primers ^a	
Chi-F	5'–AGAGATCTGATTCACCAAAGCAAAGTCAAAAATT–3' (Forward)
Chi-R	5'–CAGCTGCAGCTAGTTTTTCGCTAATGACGGCATTAA–3' (Reverse)
Specific primers	
Y46A-F	5'–TCAAAAATTGTTGGGGCCTTTCCTTCGTGGGGCGT–3' (Forward)
Y46A-R	5'–TACCCAACAATTTTTGACTTTGCTTTGGTGAATCT–3' (Reverse)
W50A-F	5'–TGGGTACTTTCCTTCGGCGGGCGTTTACGGACGTAA–3' (Forward)
W50A-R	5'–GCCGAAGGAAAGTACCCAACAATTTTTGACTTTGC–3' (Reverse)
R55A-F	5'–GGGGCGTTTACGGAGCTAATTATCAAGTTGCTGACA–3' (Forward)
R55A-R	5'–GCTCCGTAAACGCCCCACGAAGGAAAGTACCCAACA–3' (Reverse)
W171A-F	5'–TATTTCCGTTGGTGGCGCGACTTGGTCTAACCGCTT–3' (Forward)
W171A-R	5'–GCGCCACCAACGGAAATAATTGTTTTTAAGTGAGGA–3' (Reverse)
E211A-F	5'–TAGATTTAGACTGGGCATATCCGGGCGTTGAAACGA–3' (Forward)
E211A-R	5'–GCCCAGTCTAAATCTACGCCATCAAACCCATATGCG–3' (Reverse)
D287A-F	5'–TAGTATTATGACATATGCTTTCACGGCGGATGGGA–3' (Forward)
D287A-R	5'–GCATATGTCATAATACTAATCCAATCGAGTATTTGA–3' (Reverse)
R343A-F	5'–CGTACCCTTTTACGGAGCTGGCTGGAAAAGTTGTGG–3' (Forward)
R343A-R	5'–GCTCCGTAAAAGGGTACGCCTAATACTAGTTTATCG–3' (Reverse)
D385A-F	5'–TGACACAGGTGTCTATGCTTACGGTGATTTAGCAGC–3' (Forward)

D385A-R 5'-GCATAGACACCTGTGTCACCGGTAGAGTAATCATCC-3' (Reverse)

W447A-F 5'-AAGTGGAGCAATGTTTGC GGAACTAAGCGGAGATTG-3' (Forward)

W447A-R 5'-GCAAACATTGCTCCACTTAAACCTTTCGTCTTTATA-3' (Reverse)

S450A-F 5'-AATGTTTTGGGAACTAGCCGGAGATTGCCGTACAAG-3' (Forward)

S450A-R 5'-GCTAGTTC CCAAACATTGCTCCACTTAAACCTTTC-3' (Reverse)

^a The underlined sequences indicates the restriction enzymes *Bgl*III (AGATCT) and *Pst*I (CTGCAG) sites.

TABLE S2 Partial homologous proteins to Chi9602₃₅₋₄₅₉ by BLASTP search from PDB.

No.	PDB ID	GenBank Accession	Sources	Identity
0	–	KF671757.1	<i>Bacillus thuringiensis</i> Chi9602	100%
1	1ITX	20150306	<i>Bacillus circulans</i> chitinase A1	61%
2	4PTM	605604531	<i>Serratia proteamaculans</i> chitinase D	35%
3	1FFQ	28948336	<i>Serratia marcescens</i>	34%
4	3G6L	288965415	<i>Clonostachys rosea</i> chitinase Crchi1	33%
5	3ARO	329665965	<i>Vibrio harveyi</i> chitinase A	33%
6	1HKM	47168339	<i>Homo sapiens</i> chitinase	32%
7	1LL4	24158867	<i>Coccidioides immitis</i> chitinase 1	31%
8	3CH9	170785246	<i>Aspergillus fumigatus</i> chitinase B1	31%
9	3W4R	584579550	<i>Ostrinia furnacalis</i> chitinase	30%
10	4DWS	GI:453055980	<i>Yersinia entomophaga</i> chitinase 1	28%

Supplemental Figure Captions

FIG S1 Schematic illustration of recombinant plasmid construction and the amino acid sites of the Chi9602 variants subjected to site-directed mutagenesis.

FIG S2. Alignment analysis of partial amino acid sequences of Chi9602 and several family 18 chitinases. The blue rectangular frames indicate the specific chitinase-binding motif “SXGG” and the conserved CCD motif “DXDXE” of the family 18 chitinases. The red triangles indicate the amino acid sites that were selected for mutagenesis in this study.

FIG S3 Evaluation of the structural coordinates of the constructed Chi9602₃₅₋₄₅₇ model by Ramachandran plot analysis. A, Overall view of Ramachandran plot results for the Chi9602₃₅₋₄₅₉ model; B, Detailed Ramachandran plot analyses revealing the coordination of the residues in favored or allowed regions.

FIG S4 Ribbon model of the Chi9602₃₅₋₄₅₉ + (NAG)₂ complex (A) and an expanded view of this model showing the surrounding residues of the substrate binding groove (B). In B, the red-colored residues represent the residues selected for site-directed mutagenesis. AA, amino acid.

FIG S5 SDS-PAGE profiles of wild-type Chi9602 and the mutant chitinases. A, Lane 1, protein MW marker; lane 2, *E. coli* TOP10 (negative control); lane 3, *E. coli* MB332 expressing the *chi* gene; lane 4, purified Chi9602 from MB332. B, Purified mutant chitinases. Lanes 1 to 10, protein MW marker, ChiY46A, ChiW50A, ChiR55A, ChiW171A, ChiE211A, ChiD287A, ChiR343A, ChiD385A, Chi9602

(control), respectively. C, Purified mutant chitinases (continued). Lanes 1 to 4, protein MW marker, ChiW447A, ChiS450A, Chi9602 (control), respectively.

SUPPLEMENTARY FIGURES

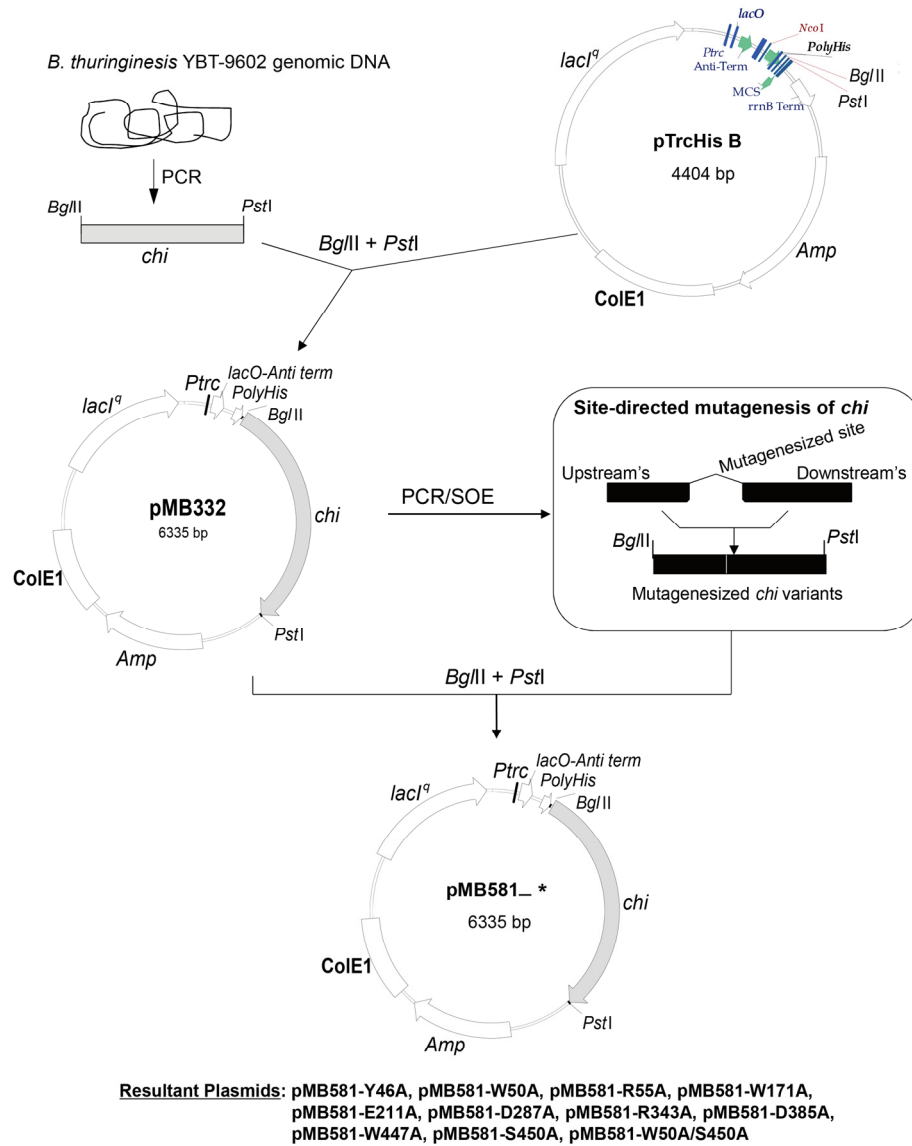


FIG S1 Schematic illustration of recombinant plasmid construction and the amino acid sites of the Chi9602 variants subjected to site-directed mutagenesis.

	10	20	30	40	50	60								
Consensus								
Chi9602	MAMRSQKFTL	LLLSLLLFLP	LFLT NFITPN	LALADSPKQS	QKIVGYFPSW	G--VYGRN-Y	57							
1ITX	-----	-----	-----LQP-	-ATAEAAD-S	YKIVGYFPPSW	A--AYGRN-Y	28							
4PTM	-----	-----	-----	-----AHAAS	YLSVGYFNGG	GDVTAGPG-G	24							
1FFQ	DGCTASDATE	IVVADTDGSH	LAPLKEPLLE	KNKPYKQNSG	KVVGSYFVEW	G--VYGRN-F	57							
3G6L	-----	-----R	ATPRMEDLAS	TDLSTRATGS	IN-AVYFTNW	G--IYGRN-F	37							
3ARO	TGCSKSAPE	ITIAADTDGSH	LKPLTMNVDP	NNKSYNTDPS	I VMGYFVVEW	G--IYGRD-Y	57							
1HKM	-----	-----	-----	-----	AKLVCYFTNW	AQYRQGEARF	20							
1LL4	-----	-----	-----	-----GG	FRSVVYFVNW	A--IYGRG-H	19							
3CH9	FATSTIVKVA	LLSSSLCVD	AVMWRDTS	TDLEARASSG	YRSVVYFVNW	A--IYGRN-H	57							
3W4R	-----	-----MRA	LTTLAVLAV	GINAAESDSR	ARVVCYFSNW	AVYRPGVGRY	44							
4DWS	ETYSRPDVNF	KEDGSQGNLS	YTATRVCA	PMYNHVYGD	KTKPKLSAYITD	WCQYDARLDGG	60							
	70	80	90	100	110	120								
Consensus								
Chi9602	QVAD-----	-----IDA	SKLTHLN	YAFAD	CWNGKHG	-----NPS	THPDNPNKQT	97						
1ITX	NVAD-----	-----IDP	TKVTHIN	YAFAD	CWNGIHG	-----NP-	-DPSGPNPVT	66						
4PTM	DINK-----	-----LDV	TQITHLN	YSFGL	IYND	-----	-----EKQET	52						
1FFQ	TVDK-----	-----IPA	QNLTHL	LYGFIP	CGNGIN	-----DSLKEIEG	-SFQALQRS	101						
3G6L	QPAD-----	-----LQA	SKILHV	LYSFMN	LRVD	-----	-----	60						
3ARO	TVDN-----	-----MPV	DNLTHI	LYGFIP	CGPN	-----ESVKS	SVGGNSFNALQ	99						
1HKM	LPKD-----	-----LDP	SLCTHL	LYAFAG	M TNHQ	-----	-----	44						
1LL4	NPQD-----	-----LKA	DQFTHI	LYAFAN	RP	SG	-----	43						
3CH9	NPQD-----	-----LKV	ERLTHV	LYAFAN	RP	ET	-----	81						
3W4R	GIED-----	-----IPV	DMCTHI	LYSFI	GV	TED	-----	68						
4DWS	GSKEEERGRG	FDLATLMQNP	ATYDRLI	IFSE	LGICG	DIGNK	SKKVQEVWDG	WNAQAPSLGL	120					
	130	140	150	160	170	180								
Consensus								
Chi9602	WNCKESGVPL	QNKEVPNGTL	VLGEPWADVT	KSYPGSGTTW	E-----DCDK	YARCGNFGEL	152							
1ITX	WTCQNEKS--	QTINVPNGTI	VLGDPWIDTG	KT FAG--DTW	D-----Q---	-PIAGNINQL	113							
4PTM	NPALKDPSRL	HQIYLSP--	-----	-----	-----	-KVMADLQLL	78							
1FFQ	QGREDFKVS	IHDPPAALQKA	QK--GVTAWD	D-----	-----	-PYKGNFGQL	139							
3G6L	-----GTVYS	GDTYADLEKH	YSDDSWNDIG	T-----	-----	-NAYGCVKQL	95							
3ARO	RGVNDYEVVI	HDPWAA YQKS	FP-QAGHEYS	T-----	-----	-PIKGNYAML	138							
1HKM	-----L	STTEWN--	-----	-----	-----	-DETLYQEFN	60							
1LL4	-----EYVL	SDTWADTDKH	YPGDKWDEPG	N-----	-----	-NVYGCIKQM	77							
3CH9	-----GEVYM	TDSWADIEKH	YPGDSWSDTG	N-----	-----	-NVYGCIKQL	116							
3W4R	-----QQVLI	IDPELVD--	-----	-----	-----	-DKNGFKNFT	89							
4DWS	PQIGKGHIVP	LDPYGD LGTA	RNVGLPPE	SA DTSIESG	TFLPYYQ-----	Q NRAAGLLGLL	175							
	190	200	210	220	230	240								
Consensus								
Chi9602	KRLKAKY---	-PHLKTII	SVGGWT	-WSNRF	SDMAADEKTR	KVFAESTVAF	LRAYG-FDGV	206						
1ITX	NKLLKQTN---	-PNLKTII	SVGGWT	-WSNRF	SDVAATAATR	EVFAANS	AVDFLRKYH-FDGV	167						
4PTM	PVLRKQKQ---	-PELKVLL	SVGGWG	-ARG-F	SGAAATAESR	AVFIRSVQVQ	IQQYH-LDGI	131						
1FFQ	MALKKQAH---	-PDLKILPS	I GGWT	-LSDPF	FFMGDK-VKR	DRFVGSVKEF	LQTKWFFDGV	193						
3G6L	YKLLKKAN---	-RSLKIMLS	I GGWT	-LSDPF	PAAASTAATR	ATFAKTAVEF	MKDWG-FDGI	149						
3ARO	MALKKQRN---	-PDLKIIPS	I GGWT	-LSDPF	YDFVDK-KNR	DTFVASVKKF	LKTWKFYDGI	192						
1HKM	-GLKKMN---	-PKLKTLL	AI GGWN	NFGTQKF	TDMVATANNR	QTFVNSAIRF	LRKYS-FDGI	114						
1LL4	YLLKKQNN---	-RNLKTLL	SI GGWT	-YSPNF	KTPASTEGR	KKFADTSLKL	MKDLG-FDGI	131						
3CH9	YLLKKQNN---	-RNLKVL	SI GGWT	-YSPNF	APAASTDAGR	KNFAKTAVKL	LQDLG-FDGI	170						
3W4R	-SLRSKH---	-PGVKFTV	AV GGWA	EGGSKY	SKMVAAKSTR	MAFVRSVVDF	LNKYN-FDGI	143						
4DWS	RELQKKAHAM	GHLDLA	FSI GGWS	-LSSYF	SALAE NPDER	RVFVASVVDF	FVRFPMFSCV	234						
	250	260	270	280	290	300								
Consensus	*: * * * * *								
Chi9602	DL DWEYYP	GVETIP	GGSYRPE	-DKQNF	TL L QDVR	NALNKA	GAED--GKQY	LLTIA	SGASQ	263				
1ITX	DL DWEYYP	VSGLD	GN SKRPE	-DKQNY	TL L SKI	REKLDA	GAVD--GKKY	LLTIA	SGASA	224				
4PTM	DL DWEYYP	VNGAW	GLVESQPA	-DRANF	TL L AEL	HKALDKG	-----K	LLTIA	AVGANV	181				
1FFQ	DL DWEYYP	GGKGAN	PNLGSPPQ	-DGETY	VL L K	REL RAMLDQL	SVET--GRKY	ELTSA	ISAGK	250				
3G6L	DV DWEYYP	ASETD	ANN--	-----	MV L L	QVRVQELDSY	SATYANGYHF	QLSIA	APAGP	199				
3ARO	DI DWEYYP	GGGGA	ADKGD	PVNDGP	YIALM	REL RVMLDEL	EAET--GRTY	ELTSA	IGVGY	250				
1HKM	DL DWEYYP	-G S	QSPAV--	-DKER	F T L M	QDLANA	FQEEAQT	S-GKERL	LLSA	AVPAGQ	167			
1LL4	DI DWEYYP	PEDEK	QAND--	-----	FV L L	KACREALD	YSAKHP	NGK	LLTIA	SPAGP	181			
3CH9	DI DWEYYP	ENDQ	QAND--	-----	FV L L	KEVRTAL	SDYSAA	NA	GGQHF	LLTVA	SPAGP	220		
3W4R	DL DWEYYP	GAA DR	GSFS--	-DKDK	F L Y L V	QELRR	AFIRE	GKG--	-----	W ELTAA	VPLAN	193		
4DWS	DI DWEYYP	GGG G	DEGNI	SSDK	-DG	ENYV	LLI KEL	RSAL	DSRFGYS	-----	NRK ELISA	C	SGVK	290

(To be continued)

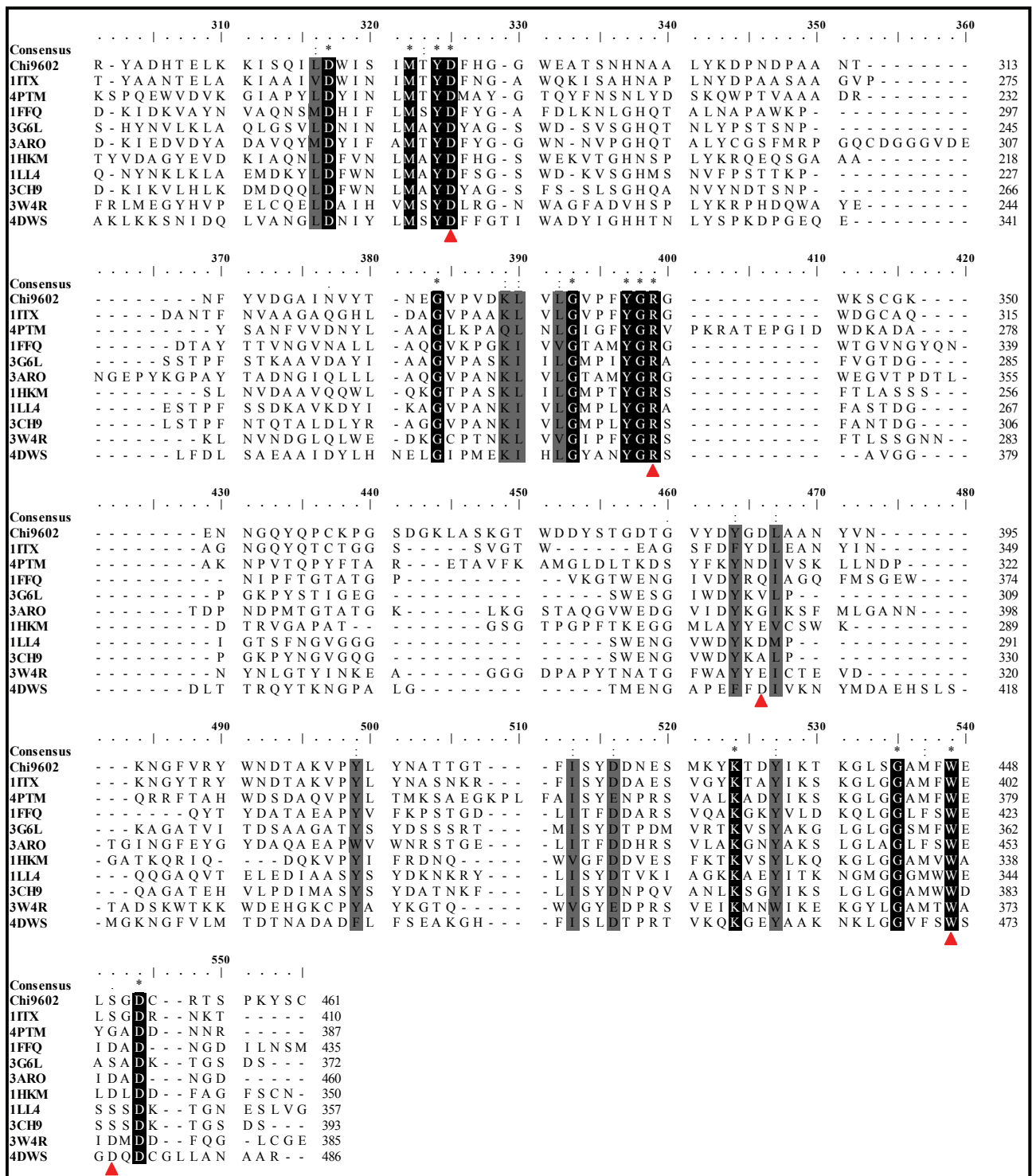
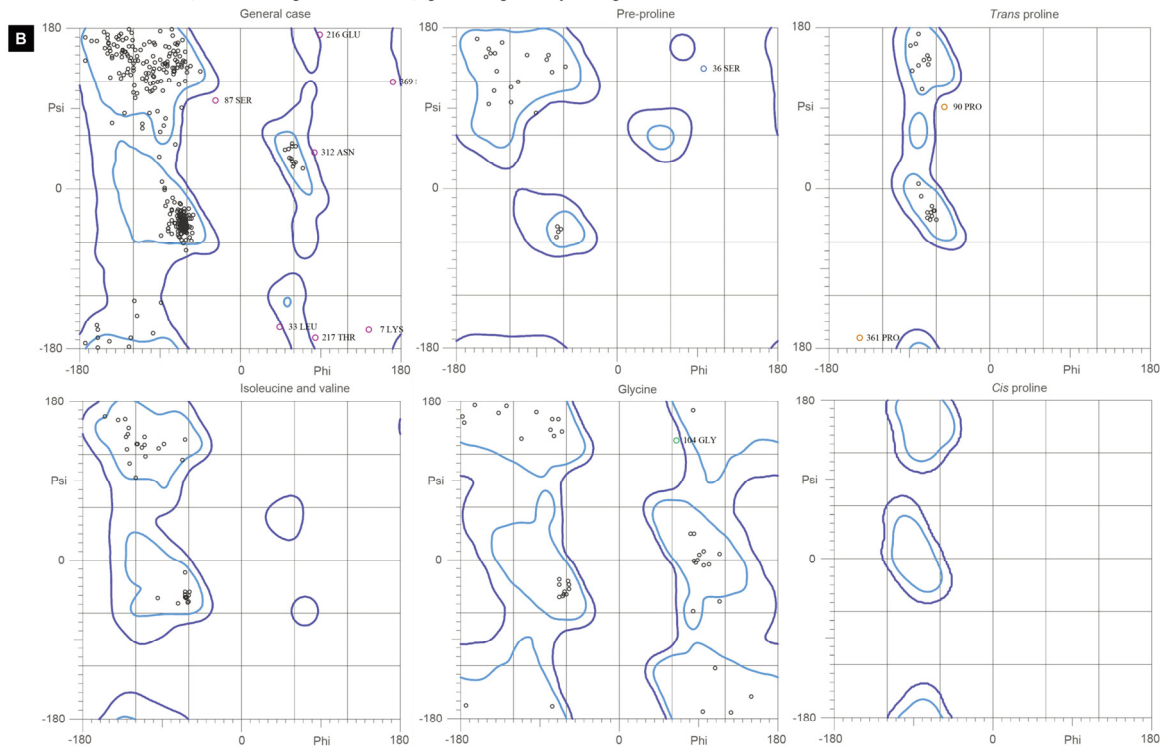


FIG S2. Alignment analysis of partial amino acid sequences of Chi9602 and several family 18 chitinases. The blue rectangular frames indicate the specific chitinase-binding motif “SXGG” and the conserved CCD motif “DXDXE” of the family 18 chitinases. The red triangles indicate the amino acid sites that were selected for mutagenesis in this study.

A	All-Atom Contacts	Clashscore, all atoms:	74.41	0 th percentile* (N=1784, all resolutions)
		Clashscore is the number of serious steric overlaps (> 0.4 Å) per 1000 atoms.		
Protein Geometry	Poor rotamers	6	1.57%	Goal: <1%
	Ramachandran outliers	11	2.41%	Goal: <0.05%
	Ramachandran favored	426	93.42%	Goal: >98%
	MolProbity score [^]	2.92		26 th percentile* (N=27675, 0Å - 99Å)
	Cβ deviations >0.25Å	5	1.20%	Goal: 0
	Bad backbone bonds:	3 / 3721	0.08%	Goal: 0%
	Bad backbone angles:	70 / 5058	1.38%	Goal: <0.1%

In the two column results, the left column gives the raw count, right column gives the percentage.



Ramachandran results:

93.4% (426/456) of all residues were in favored regions.

97.6% (445/456) of all residues were in allowed regions.

There were 8 outliers (phi, psi):

7 Lys (144.4, -159.5); 33 Leu (44.7, -156.7); 36 Ser (98.0, 135.0); 87 Ser (-28.4, 99.5); 90 Pro (-51.6, 92.2); 104 Gly (65.4, 136.4); 216 Glu (89.7, 173.7); 217 Thr (84.3, -168.9); 312 Asn (83.1, 41.4); 361 Pro (-146.8, -168.3); 369 Ser (171.8, 120.1)

FIG S3 Evaluation of the structural coordinates of the constructed Chi9602₃₅₋₄₅₇ model by Ramachandran plot analysis. A, Overall view of Ramachandran plot results for the Chi9602₃₅₋₄₅₉ model; B, Detailed Ramachandran plot analyses revealing the coordination of the residues in favored or allowed regions.

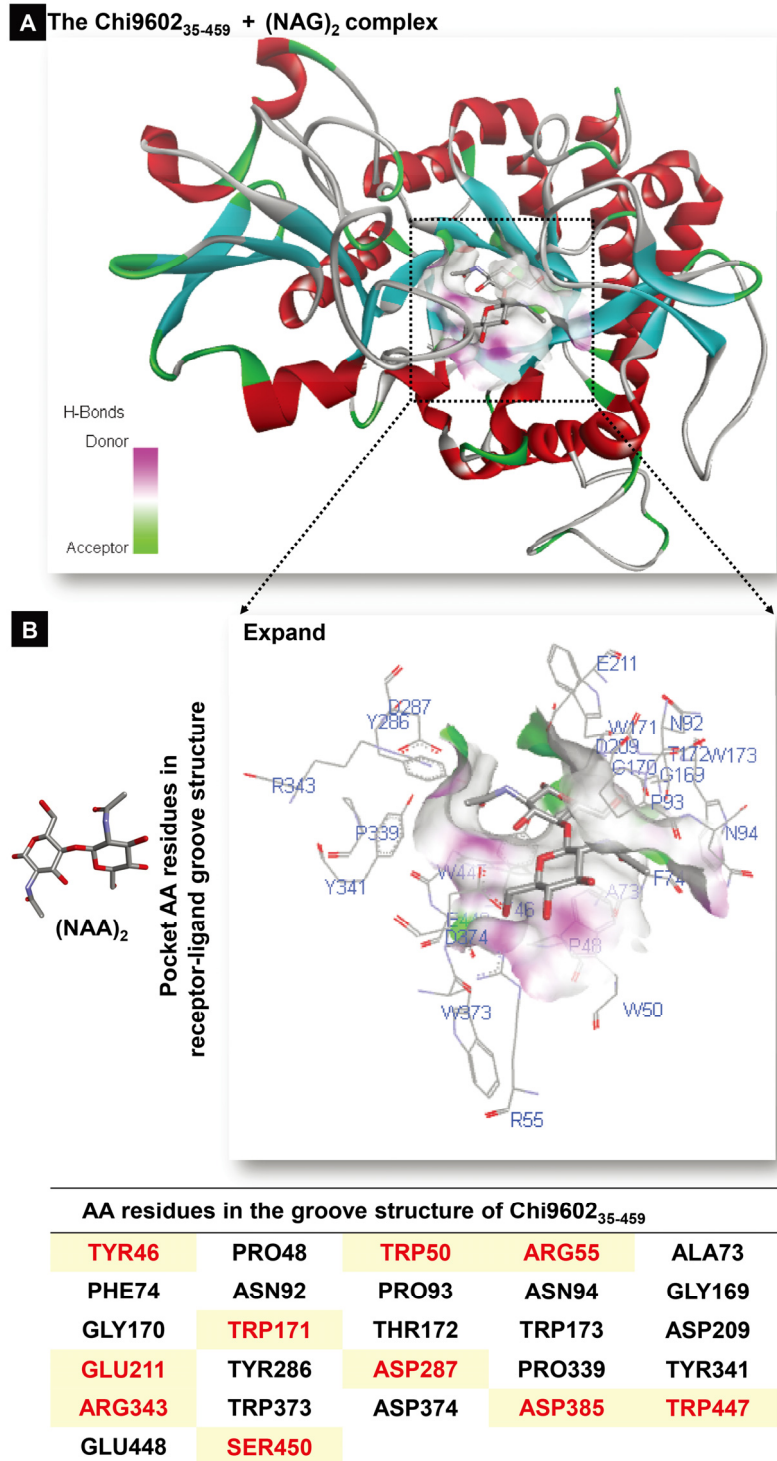


FIG S4 Ribbon model of the Chi9602₃₅₋₄₅₉ + (NAG)₂ complex (A) and an expanded view of this model showing the surrounding residues of the substrate binding groove (B). In B, the red-colored residues represent the residues selected for site-directed mutagenesis. AA, amino acid.

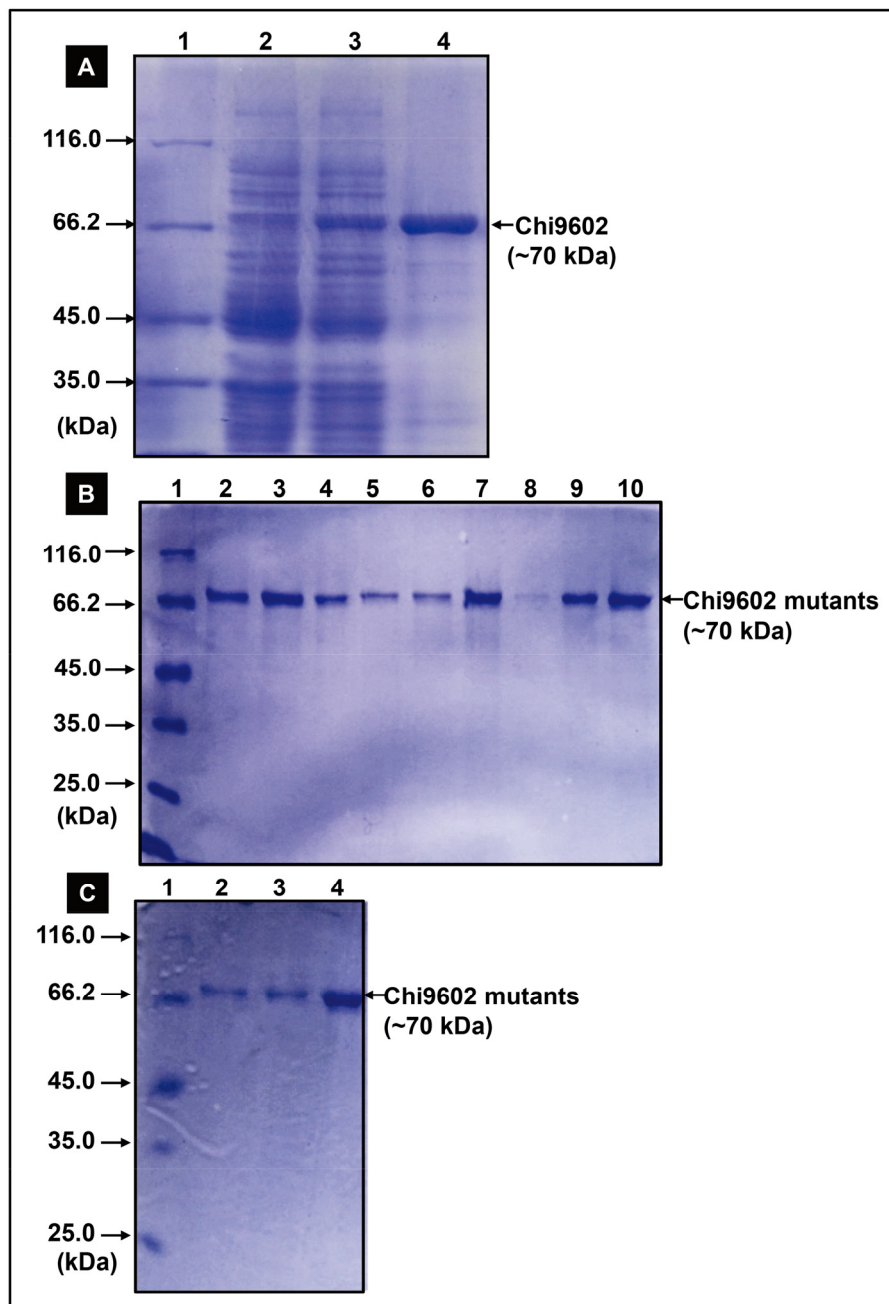


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