

SUPPLEMENTAL MATERIALS

Supplemental Tables

TABLE S1 Oligonucleotide primers used in this study.

Primers	Sequences
Common primers ^a	
Chi-F	5'-AGAGATCTGATTACCAAAGCAAAGTCAAAAAATT-3' (Forward)
Chi-R	5'-CAG <u>CTGCAG</u> GCTAGTTTCGCTAATGACGGCATTAA-3' (Reverse)
Specific primers	
Y46A-F	5'-TCAAAAAATTGTTGGGCCTTCCTCGTGGGCGT-3' (Forward)
Y46A-R	5'-TACCCAACAATTGGACTTGCTTGGTGAATCT-3' (Reverse)
W50A-F	5'-TGGGTACTTCCTCGCGGGCGTTACGGACGTAA-3' (Forward)
W50A-R	5'-GCCGAAGGAAAGTACCCAACAATTGGACTTGAC-3' (Reverse)
R55A-F	5'-GGGCGTTACGGAGCTAATTCAAGTTGCTGACA-3' (Forward)
R55A-R	5'-GCTCCGTAAACGCCAACGAAGGAAAGTACCCAACA-3' (Reverse)
W171A-F	5'-TATTCCGTTGGTGGCGCGACTTGGCTAACCGCTT-3' (Forward)
W171A-R	5'-GCGCCACCAACGAAATAATTGTTTAAGTGAGGA-3' (Reverse)
E211A-F	5'-TAGATTTAGACTGGCATATCCGGCGTTGAAACGA-3' (Forward)
E211A-R	5'-GCCCAGTCTAAATCTACGCCATCAAACCCATATGCG-3' (Reverse)
D287A-F	5'-TAGTATTATGACATATGCTTCCACGGCGGATGGGA-3' (Forward)
D287A-R	5'-GCATATGTCATAATACTAATCCAATCGAGTATTGA-3' (Reverse)
R343A-F	5'-CGTACCCTTTACGGAGCTGGCTGGAAAAGTTGTGG-3' (Forward)
R343A-R	5'-GCTCCGTAAAAGGGTACGCCATAACTAGTTATCG-3' (Reverse)
D385A-F	5'-TGACACAGGTGTATGCTTACGGTGATTAGCAGC-3' (Forward)

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

W447A-F 5'-AAGTGGAGCAATGTTGCGGAACTAAGCGGAGATTG-3' (Forward)

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

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

S450A-R 5'-GCTAGTTCCAAAACATTGCTCCACTAACCTTTC-3' (Reverse)

^a The underlined sequences indicates the restriction enzymes *Bg*II (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

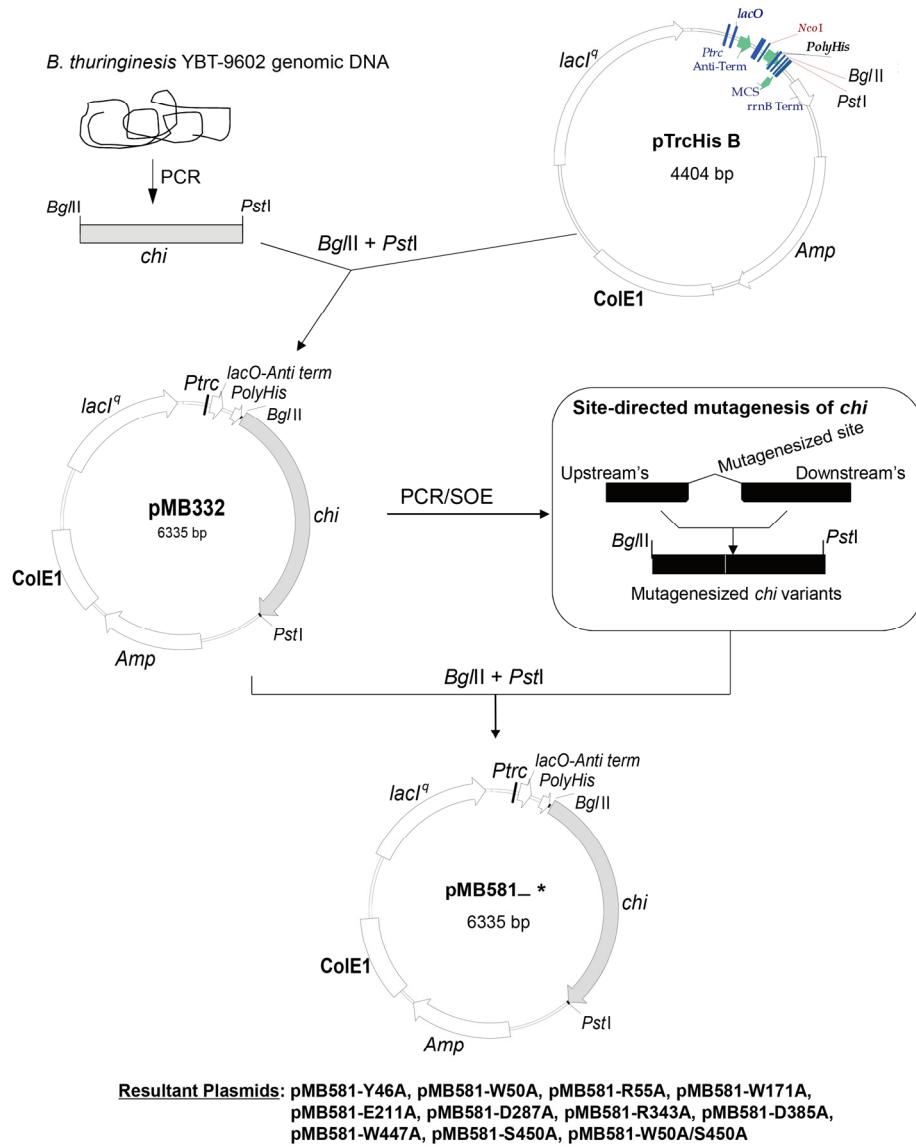


FIG S1 Schematic illustration of recombinant plasmid construction and the amino acid sites of the Chi9602 variants subjected to site-directed mutagenesis.

Consensus	10	20	30	40	50	60
Chi9602	MAMRSQKFTL	LLLSLLLFLP	LFLTNFITPN	LALADSPKQS	QKIVGYFPFW	G-VYGRN-Y
1ITX	- - - - -	- - - - -	- - - - LQP -	- ATA EAAD-S	YKIVGYYPSW	A--AYGRN-Y
4PTM	- - - - -	- - - - -	- - - - -	- - - - AHAAS	YLSVGYFNGG	GDVTAGPG-G
1FFQ	DGCTASDATE	I VVADTDGS	LAPLKEPLLE	KNKPYKQNSG	KVVGSYFVEW	G-VYGRN-F
3G6L	- - - - -	- - - - R	ATPRMEDLAS	TDLSTRATGS	I N-AVYFTNW	G-IYGRN-F
3ARO	TGCSSKSAPVE	ITIADTDGS	LKPLTMNVDP	NNKSYNTDPS	I VMGTYFVEW	G-IYGRD-Y
1HKM	- - - - -	- - - - -	- - - - -	- - - - -	AKLVCYFTNW	AQYRQGEARF
1LL4	- - - - -	- - - - -	- - - - -	- - - - GG	FRSVVFVNW	A--IYGRG-H
3CH9	FATSTIVKVA	LLLSSLCVDA	AVMWNRDTSS	TDLEARASSG	YRSVVFVNW	A--IYGRN-H
3W4R	- - - - -	- - - - MRAL	LLTTLAVLAV	GINAAEESDSR	ARVVCFNSNW	AVYRPVGVRG
4DWS	ETYSPRDVN	KEDGSQGNL	YTATRVCPM	YNHYVGDKTK	PKL SAY ITDW	CQYDARLDGG
Consensus	70	80	90	100	110	120
Chi9602	QVAD - - -	- - - - I DA	SKLTHLN YAF	ADICWNGKGHG	- - - - NPS	THPDNPNKQT
1ITX	NVAD - - -	- - - - IDP	TKVTHN YAF	ADICWNGIHG	- - - - NP	- DPGPNPVT
4PTM	DINK - - -	- - - - LDV	TQITHLNYSF	GLIYND - - -	- - - - -	- - - - EKQET
1FFQ	TVDK - - -	- - - - I PA	QNLTHLLYGF	I PICGGNGIN	- - DSLKEIEG	- SFQALQRSC
3G6L	QPAD - - -	- - - - LQA	SKLHVLYSF	MNL RVD - - -	- - - - -	- - - - 60
3ARO	TVDN - - -	- - - - MPV	DNLTHILYGF	I PICGPN - - -	- - ESVKSVGG	NSFNALQTAC
1HKM	LPKD - - -	- - - - LDP	SLCTHLIYAF	AGMTNHQ - - -	- - - - -	- - - - 44
1LL4	NPQD - - -	- - - - LKA	DQFTHILYAF	ANIRPSG - - -	- - - - -	- - - - 43
3CH9	NPQD - - -	- - - - LPV	ERLTTHVLYAF	ANVRPET - - -	- - - - -	- - - - 81
3W4R	GIED - - -	- - - - I PV	DMCTHIIYSF	IGVTEDT - - -	- - - - -	- - - - 68
4DWS	GSKEERGRG	FDLATLMQNP	ATYDRLLIFSF	LGICGDIIGNK	SKKVQEVDWG	WNAQAPSGL
Consensus	130	140	150	160	170	180
Chi9602	WNCKESGVPL	QNKEVPNGTL	VLGEPWADVT	KSYPGSGTTW	E - - - - DC	YARCNGFTEL
1ITX	WTCQNEKS -	QTINVPGNTI	V LGDPWI DTG	KTFAG - - DTW	CDK - - Q - -	- PIAGNIINQL
4PTM	NPALKDPSRL	HQIYLS P - -	- - - - -	- - - - -	- - - - -	- KVMAIDLQL
1FFQ	QGREDFKVSI	HDPFAALQKA	QK - - GVTAWD	D - - - - -	- - - - -	- PYKGNGFQL
3G6L	- - - - GTVYS	GDTYADLEKH	YSDDSWNDIG	T - - - - -	- - - - -	- NAYGCVKQL
3ARO	RGVNDYEVVI	HDPWAAYQKS	F P - QAGHEYS	T - - - - -	- - - - -	- PIKGNYAML
1HKM	- - - - L	S TTEWN	- - - - -	- - - - -	- - - - -	- DETLYQE FN
1LL4	- - - - EVYL	S DTWADTDKH	YPGDKWDEPG	N - - - - -	- - - - -	- NVYGCICKM
3CH9	- - - - GEVYM	TDSWADI EKH	YPGDSWSDTG	N - - - - -	- - - - -	- NVYGCICKL
3W4R	- - - - QQVLI	IDPELDV - - -	- - - - -	- - - - -	- - - - -	- DKNGFKNFT
4DWS	PQIGKGHI VP	LDPYGD LGTA	RNVGLPPESA	DTSIESGTFL	PYYQ - - - Q	NRAAGL LGG
Consensus	190	200	210	220	230	240
Chi9602	* : - - -	- PHLKTIIISV	GGWT - WS NRF	SDMAADEKTR	KVFAE STVAF	LRAYG - FDGV
1ITX	NKLKQTN - -	- PNLLKTIISV	GGWT - WS NRF	S DVAATAATR	EVFANS AVDF	LRKYN - FDGV
4PTM	PVLRKQN - -	- PELKVLLS V	GGWG - ARG - F	S GAAATAESR	AVFIRS SVQV	I KQYH - LDGI
1FFQ	MALKQAH - -	- PDLKILPSI	GGWT - LSDPF	F FMGDK - VKR	DRFVG SVKEF	LQTWKFFDG
3G6L	YKLK KAN - -	- RSLKIMLSI	GGWT - WSTNF	PAAASTEATR	ATFAKTAVEF	MKDWG - FDGI
3ARO	MALKQRN - -	- PDLKIPSI	GGWT - LSDPF	YDFVDK - KN	DTFVASVKKF	LKTWKFYDGV
1HKM	- GLKKMN - -	- PKLK TLLAI	GGWNFGTQKF	TDMVATANNR	QT FVNSA I RF	LRKYS - FDGL
1LL4	YLLK KNN - -	- RN LK TLLSI	GGWT - YSPNF	KTPA STEEGR	KKFADT S LKL	MKD LG - FDGI
3CH9	YLLK KQN - -	- RN LKVLLSI	GGWT - YSPNF	A PAAS T DAGR	KNFAK TAVKL	LQDLG - FDGL
3W4R	- SLRSK H - -	- PGVKFTVAV	GGWAEGGSKY	SKMVAKSTR	MAF VRSV VDF	L NKYN - FDGL
4DWS	R ELOKKAHAM	GHKL DLA FSI	GGWS - LSSYE	SALAENPDER	R VFVA SVDF	F VR F PMF SCV
Consensus	250	260	270	280	290	300
Chi9602	* : * : * : *	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
1ITX	DLDWEYPGVE	TIPGGSYRPE	- DKQNF TLL	QDVRNALNKA	GAED - - GKQY	L LTIA SGASQ
4PTM	DLDWEYPVSG	G LDGNSKRPE	- DKQNYTLL	SKI REKL DAA	GAVD - - GKKY	L LTIA SGASA
1FFQ	DLDWEYPVNG	AWGLVESQPA	- DRANFTLL	A ELHKALDKG	- - - - - K	L LTIA VGANV
3G6L	DLDWEFPGGK	G ANPNLGS PQ	- DGETYVLLM	KELRAMLDQL	SVET - - GRKY	ELTSAI SAGK
3ARO	DLDWEFPGGG	GAAADKGDPV	NDGPAYIA LM	REL RVMLDEL	EAET - - GRTY	ELTSAI GVGY
1HKM	DLDWEYP - G	SQGSPAV - -	- DKERFTTLL	QDLANA FQQE	AQTS - GKERL	L LSA AVPAQG
1LL4	DLDWEYPED	KQAND - -	- - - - FVLL	KACREALDAY	SAKHPNGKKF	L LTIA SPAGP
3CH9	DLDWEYPEND	QQAND - -	- - - - FVLL	KEVRTALDSY	SAANAGGQHF	L LTVA SPAGP
3W4R	DLDWEYPGAA	DRGGSF S - -	- DKDKFLYLV	QELRR A FIRE	GKG - - - - W	ELTAA VPLAN
4DWS	DLDWEYPGGG	GDEGNISSDK	- DGENYVLL	KELRSALDSR	FGYS - - NRK	EISIACSGVK

(To be continued)

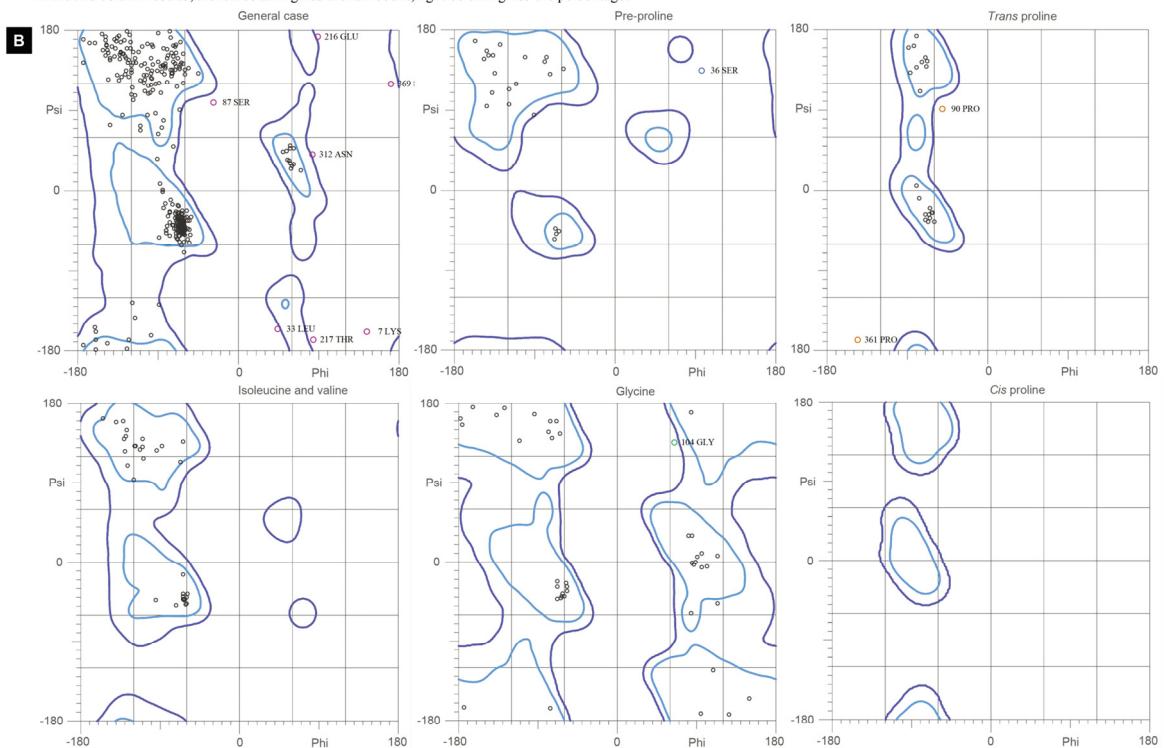
	310	320	330	340	350	360	
Consensus	· · · · · · · · · · · · · · · · · · · · · · · · ·	*	*	*	*	*	
Chi9602	R - YADHT E L K	K I S Q I LDW I S	I M T YDF H G - G	WEAT S NH NAA	L YKD P N D P A A	N T - - - - -	313
1ITX	T - YA A N T E L A	K I A A I V D W I N	I M T YDF N G - A	W Q K I S A H N A P	L N Y D P A A S A A	G V P - - - - -	275
4PTM	K S P Q E W V D V K	G I A P Y L D Y I N	L M T YD M A Y - G	T Q Y F N S N L Y D	S K Q W P T V A A A	D R - - - - -	232
1FFQ	D - K I D K V A Y N	V A Q N S M D H I F	L M S Y D F Y G - A	F D L K N L G H Q T	A L N A P A W K P -	- - - - -	297
3G6L	S - H Y N V L K L A	Q L G S V L D N I N	L M A Y D Y A G - S	W D - S V S G H Q T	N L Y P S T S N P -	- - - - -	245
3ARO	D - K I E D V D Y A	D A V Q Y M D Y I F	A M T YDF Y G - G	W N - N V P G H Q T	A L Y C G S F M R P	G Q C D G G G V D E	307
1HKM	T Y V D A G Y E V D	K I A Q N L D F V N	L M A Y D F H G - S	W E K V T G H N S P	L Y K R Q E Q S G A	A A - - - - -	218
1LL4	Q - N Y N K L K L A	E M D K Y L D F W N	L M A Y D F S G - S	W D - K V S G H M S	N V F P S T T K P -	- - - - -	227
3C19	D - K I K V L H L K	D M D Q Q L D F W N	L M A Y D Y A G - S	F S - S L S G H Q A	N V Y N D T S N P -	- - - - -	266
3W4R	F R L M E G Y H V P	E L C Q E L D A I H	V M S Y D L R G - N	W A G F A D V H S P	L Y K R P H D Q W A	Y E - - - - -	244
4DWS	A K L K K S N I D Q	L V A N G L D N I Y	L M S Y D F F G T I	W A D Y I G H H T N	L Y S P K D P G E Q	E - - - - -	341
			▲				
Consensus	· · · · · · · · · · · · · · · · · · · · · · · · ·	*	*	*	*	*	
Chi9602	- - - - - N F	Y V D G A I N V Y T	- N E G V P V D K L	V L G V P F Y G R G	- - - - -	W K S C G K - - -	350
1ITX	- - - - - D A N T F	N V A A G A Q G H L	- D A G V P A A K L	V L G V P F Y G R G	- - - - -	W D G C A Q - - -	315
4PTM	- - - - - Y	S A N F V V D N Y L	- A A G L K P A Q L	N L G I G F Y G R V	P K R A T E P G I D	W D K A D A - - -	278
1FFQ	- - - - - D T A Y	T T V N G V N A L L	- A Q G V K P G K I	V V G T A M Y G R G	- - - - -	W T G V N G Y Q N -	339
3G6L	- - - - - S S T P F	S T K A A V D A Y I	- A A G V P A S K I	I L G M P I Y G R A	- - - - -	F V G T D G - - -	285
3ARO	N G E P Y K G P A Y	T A D N G I Q L L L	- A Q G V P A N K L	V L G T A M Y G R G	- - - - -	W E G V T P D T L -	355
1HKM	- - - - - S L	N V D A A V Q Q W L	- Q K G T P A S K L	I L G M P T Y G R S	- - - - -	F T L A S S S - - -	256
1LL4	- - - - - E S T P F	S S D K A V K D Y I	- K A G V P A N K I	V L G M P L Y G R S	- - - - -	F A S T D G - - -	267
3C19	- - - - - L S T P F	N T Q T A L D L Y R	- A G G V P A N K I	V L G M P L Y G R S	- - - - -	F A N T D G - - -	306
3W4R	- - - - - K L	N V N D G L Q L W E	- D K G C P T N K L	V V G I P F Y G R S	- - - - -	F T L S S G N N - - -	283
4DWS	- - - - - L F D L	S A E A A I D Y L H	N E L G I P M E K I	H L G Y A N Y G R S	- - - - -	A V G G - - -	379
			▲				
Consensus	· · · · · · · · · · · · · · · · · · · · · · · · ·	*	*	*	*	*	
Chi9602	- - - - - E N	N G Q Y Q P C K P G	S D G K L A S K G T	W D D Y S T G D T G	V Y D Y G D I A A N	Y V N - - - - -	395
1ITX	- - - - - A G	N G Q Y Q T C T G G	S - - - - S V G T	W - - - - E A G	S F D F Y D L E A N	Y I N - - - - -	349
4PTM	- - - - - A K	N P V T Q P Y F T A	R - - - - E T A V F K	A M G L D L T K D S	Y F K Y N D I V S K	L L N D P - - -	322
1FFQ	- - - - - N I P F T G T A G	P - - - - -	- - - - - V K G T W E N G	I V D Y R Q I A G Q	F M S G E W - - -	374	
3G6L	- - - - - P	G K P Y S T I G E G	- - - - -	- - - - - S W E S G	I W D Y K V L P - -	- - - - -	309
3ARO	- - - - - T D P	N D P M T G T A T G	K - - - - - L K G	S T A Q G V W E D G	V I D Y K G I K S F	M L G A N N - - -	398
1HKM	- - - - - D	T R V G A P A T -	- - - - - G S G	T P G P F T K E G G	M L A Y Y E V C S W	K - - - - -	289
1LL4	- - - - - I	G T S F N G V G G G	- - - - -	- - - - - S W E N G	W V D Y K D M P - -	- - - - -	291
3C19	- - - - - P	G K P Y N G V G Q G	- - - - -	- - - - - S W E N G	W V D Y K A L P - -	- - - - -	330
3W4R	- - - - - N	Y N L G T Y I N K E	A - - - - - G G G	D P A P Y T N A T G	F W A Y Y E I C T E	V D - - - - -	320
4DWS	- - - - - D L T	T R Q Y T K N G P A	L G - - - - -	- - - - - T M E N G	A P E F F D I V K N	Y M D A E H S L S -	418
			▲				
Consensus	· · · · · · · · · · · · · · · · · · · · · · · · ·	*	*	*	*	*	
Chi9602	- - - - - K N G F V R Y	W N D T A K V P Y L	Y N A T T G T - - -	- F I S Y D D N E S	M K Y K T D Y I K T	K G L S G A M F W E	448
1ITX	- - - - - K N G Y T R Y	W N D T A K V P Y L	Y N A S N K R - - -	- F I S Y D D A E S	V G Y K T A Y I K S	K G L G G A M F W E	402
4PTM	- - - - - Q R R F T A H	W D S D A Q V P Y L	T M K S A E G K P L	F A I S Y E N P R S	V A L K A D Y I K S	K G L G G A M F W E	379
1FFQ	- - - - - Q Y T	Y D T A E A P Y V	F K P S T G D - - -	- L I T F D D A R S	V Q A K G K Y V L D	K Q L G G G L F S W E	423
3G6L	- - - - - K A G A T V I	T D S A A G A T Y S	Y D S S S R T - - -	- M I S Y D T P D M	V R T K V S Y A K G	L G L G G S M F W E	362
3ARO	- - - - - T G I N G F E Y G	Y D Q A E A P W V	W N R S T G E - - -	- L I T F D D H R S	V L A K G N Y A K S	L G L A G L F S W E	453
1HKM	- - - - - G A T K Q R I Q -	- - - - - D Q K V P Y I	F R D N Q - - -	- W V G F D D V E S	F K T K V S Y L K Q	K G L G G A M V W A	338
1LL4	- - - - - Q Q G A Q V T	E L E D I A A S Y S	Y D K N K R Y - - -	- L I S Y D T V K I	A G K K A E Y I T K	N G M G G G M W W E	344
3C19	- - - - - Q A G A T E H	V L P D I M A S Y S	Y D A T N K F - - -	- L I S Y D N P Q V	A N L K S G Y I K S	L G L G G A M W W D	383
3W4R	- - - - - T A D S K W T K K	W D E H G K C P Y A	Y K G T Q - - -	- W V G Y E D P R S	V E I K M N W I K E	K G Y L G A M T W A	373
4DWS	- - - - - M G K N G F V L M	T D T N A D A D F L	F S E A K G H - - -	- F I S L D T P R T	V K Q K G E Y A A K	N K L G G V F S W S	473
			▲				
Consensus	· · · · · · · · · · · · · · · · · · · · · · · · ·	*	*	*	*	*	
Chi9602	L S G D C - - R T S	P K Y S C	461				
1ITX	L S G D R - - N K T	- - - - -	410				
4PTM	Y G A D D - - N N R	- - - - -	387				
1FFQ	I D A D - - N G D	I L N S M	435				
3G6L	A S A D K - - T G S	D S - - -	372				
3ARO	I D A D - - N G D	- - - - -	460				
1HKM	L D L D D - - F A G	F S C N -	350				
1LL4	S S S D K - - T G N	E S L V G	357				
3C19	S S S D K - - T G S	D S - - -	393				
3W4R	I D M D D - - F Q G	- L C G E	385				
4DWS	G D Q D C G L L A N	A A R - -	486				
			▲				

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.

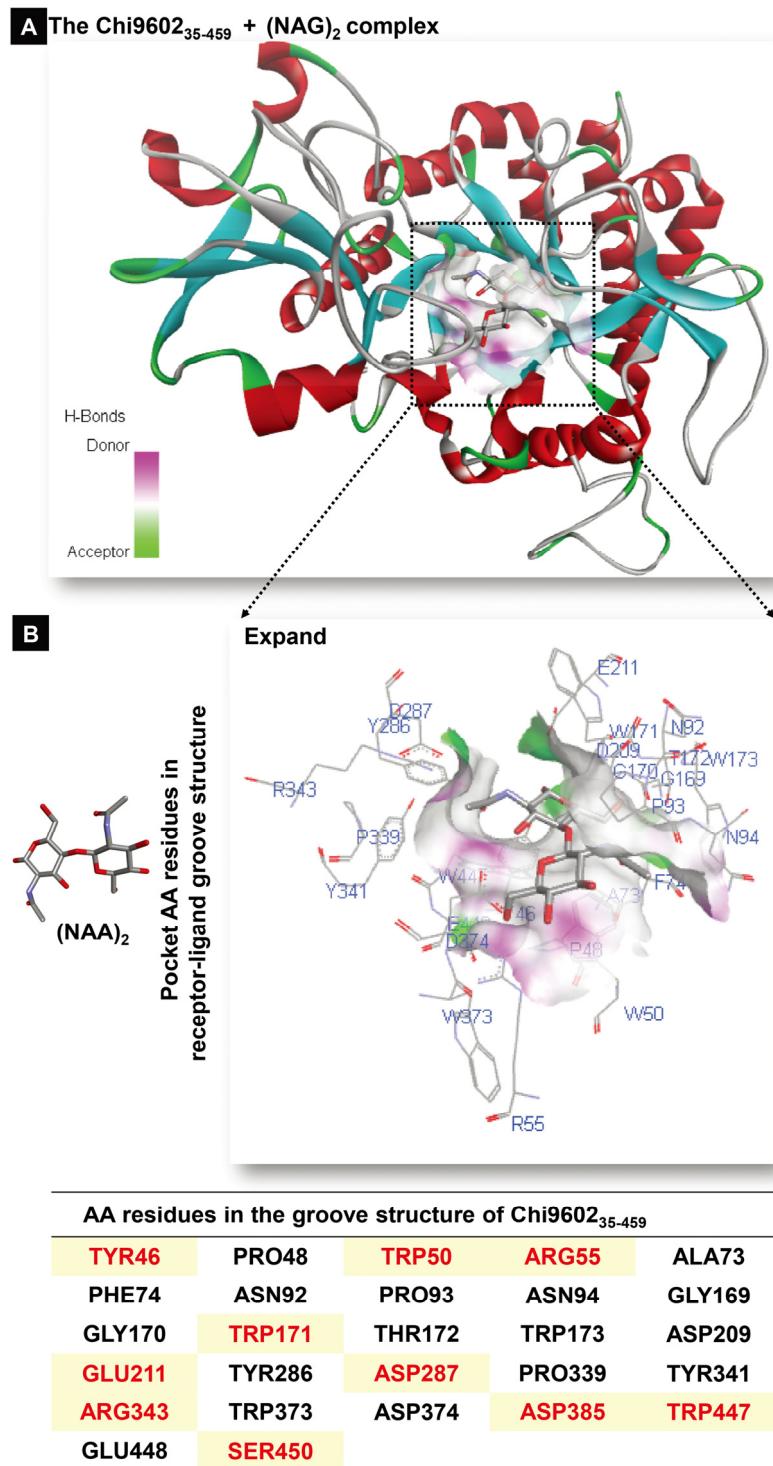


FIG S4 Ribbon model of the Chi9602₃₅₋₄₅₉ + (NAG)₂ complex (A) and an expanded view of this model showing the surrounding residues of the substrate binding groove (B). In B, the red-colored residues represent the residues selected for site-directed mutagenesis. AA, amino acid.

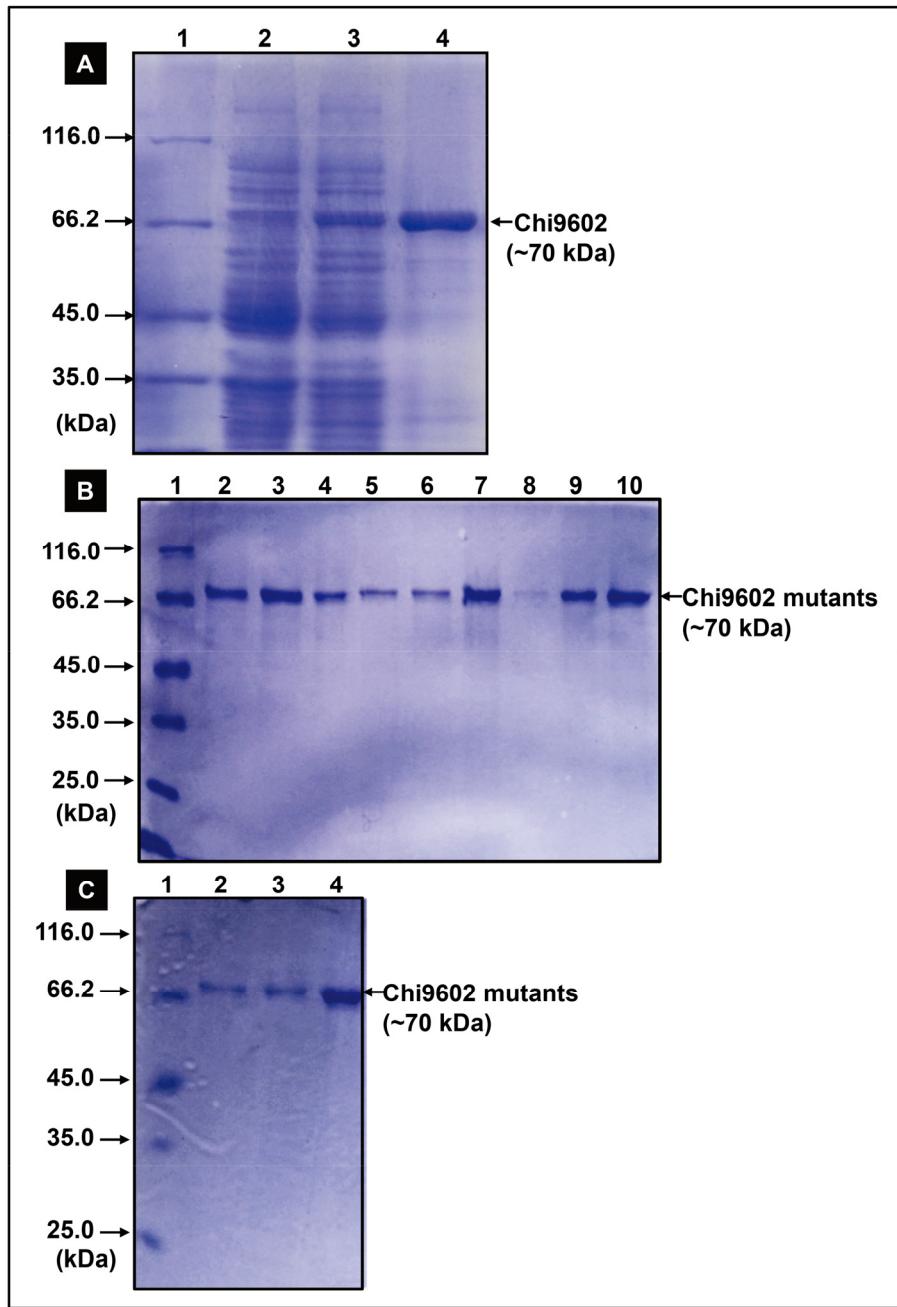


FIG S5 SDS-PAGE profiles of wild-type Chi9602 and the mutant chitinases. A, Lane 1, protein MW marker; lane 2, *E. coli* TOP10 (negative control); lane 3, *E. coli* MB332 expressing the *chi* gene; lane 4, purified Chi9602 from MB332. B, Purified mutant chitinases. Lanes 1 to 10, protein MW marker, ChiY46A,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