Supplemental Data

"The role of net charge on the catalytic domain and the influence of the cell-wall binding domain on the bactericidal activity, specificity and host-range of phage lysins"

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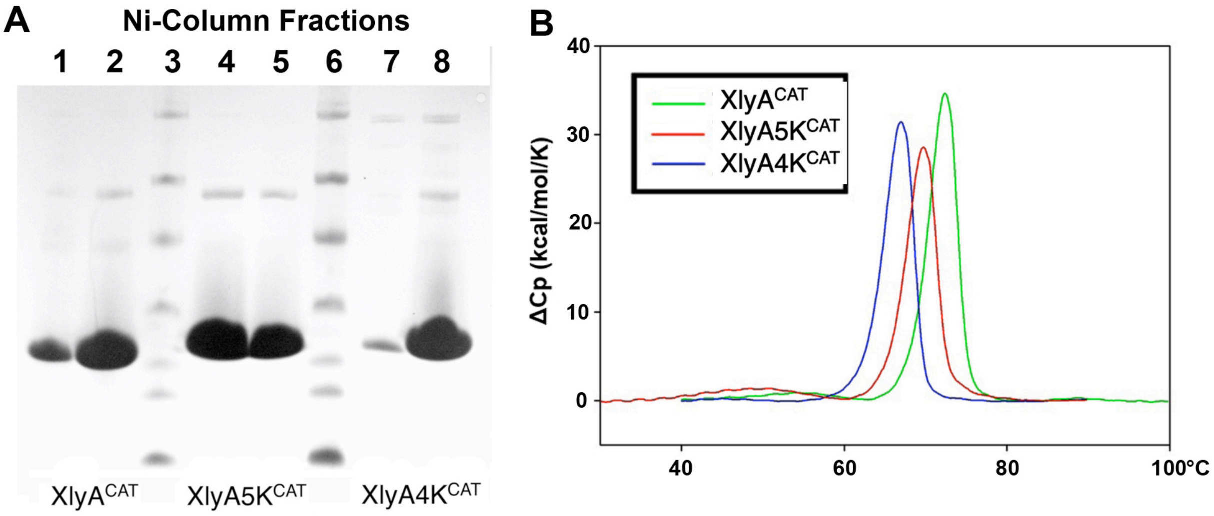
Supplemental Table I. DNA oligonucleotide sequences used for cloning and mutagenesis in this study.

Primer	Sequence (5'-3')
PlyL E90A forward	GAG TCC ATT TCT GTA GCT ATC TGT TAT TCA AAA
PlyL E90A reverse	TTT TGA ATA ACA GAT AGC TAC AGA AAT GGA CTC
XlyA Full length forward	AAA GGA GAT ATA **CAT ATG** GTT AAC ATT ATT CAA GAC TTT ATT
XlyA Full length reverse	GAA TTC **GGA TCC** TCA TCA GCT TAA TTG CGC TGC GAT CTT CTC TTT
XlyA^{cat} (aa157) reverse	GAA TTC **GGA TCC** TCA CCC AAT TCC TGC TTT GAA CGA ATC
XlyA D7K	CAT ATG GTT AAC ATT ATT CAA AAA TTT ATT CCG GTT GGC GCA AAT
XlyA T22K/L24K	CGT CCA GGC TAC GCA ATG AAA CCG AAA TAC ATT ACG GTG CAC
XlyA T63K	CAT TTT ACA GTT GAT GAT AAA GAA ATT TAT CAG CAT CTG CCT
XlyA T145K	CCG CGA AAA TTG TTA GAT AAA TGG GAT TCG TTC AAA GCA GGA
XlyA E93A forward	CGG GCT TCT ATT GGA ATT GCG ATT TGC GAA AAT GCC GAT GGC
XlyA E93A reverse	ATC GGC ATT TTC GCA AAT CGC AAT TCC AAT AGA AGC CCG GTT
PlyBa04 Full length forward	CCG CGC GGC AGC CAT ATG GGA CAT ATT ATT GAT ATT TCA
PlyBa04^{cat} (aa189) reverse	TTA GCA GCC GGA TCC TTA TGC CGA TTC TGT AAA CCA AGA TAG
PlyBa04 Full length reverse	TTA GCA GCC GGA TCC TTA TTT AAC TTC ATA CCA CCA ACC
Remove NdeI forward	GGT TTA TAT GTT GGT CAT CAC ATG TAT ACA CCT TTC GGT
Remove NdeI reverse	ACC GAA AGG TGT ATA CAT GTG ATG ACC AAC ATA TAA ACC
PlyBa04 N15D	TCA AAA TGG AAT GGC GAT ATT GAC TGG TCT ATA GCG AAA CAA
PlyBa04 G43D	TAT GTA GAT CCA CTA TAT AAA GAC TAT GTA CAA GCA ATG AAG
PlyBa04 T128D	TTA TAT GTT GGT CAT CAC ATG TAT GAT CCT TTC GGT ATG GCG AAC
PlyBa04 T160D	CCT TGC GAT ATT TGG CAA TAT GAT GAA ACG GGT AAT GTA CCT
PlyG_cat forward	CGC GGC AGC **CAT ATG** GAA ATT CAG AAA AAA CTG GTT GAT
PlyG reverse	GCA GCC **GGA TCC** AAT CGC CAC GTT ACC GTT TTT CAC TTT

Bold type indicates restriction endonuclease site. Underlining indicates mutated base.

Supplemental Fig. 1 Quality control of XlyA^{CAT} and its gain-of-function mutants. ***A***, column fractions from final purification step for wild-type and mutant protein. ***B & C***, Thermal melting profiles show only small differences in stability, as determined by Differential Scanning Calorimetry.



C

Endolysin	pI	Charge	Tm (°C)	ΔH (kcal/mol)
XlyA^{CAT}	5.8	-3	72.4	171.6
XlyA5K^{CAT}	8.0	+3	69.7	163.2
XlyA4K^{CAT}	7.3	+2	67.0	152.6
PlyL	8.8	+6		

Supplemental Fig. 2 Melting profiles of PlyL provides no evidence for significant interaction between the catalytic domain and CBD. Profile of full-length protein is not significantly different from the sum of domain profiles, as determined Differential Scanning Calorimetry.