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Title: Discovery of novel *S. aureus* autolysins and molecular engineering to enhance bacteriolytic activity

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Table S1 List of primers used for cloning.

Construct Name	5' Primer*	3' Primer*
LytH _{Full}	ATAGCACCATGGCGGAAGATAGTGGGAACAT CACG	TCGAATTCGGATCCTTACGCAGAAAAATAAA TTTTAAGGCC
LytH _{MurNAc}	TGCATAGCACCATGGCGACAAATTTAGATAT TGTCGC	TGCATAGCACCATGGCGACAAATTTAGATAT TGTCGC
LytO _{Full}	ATCGTCGATCCATGGCGCAAGCAAATAAAC	TAGTCAGTCGGATCCTTAACTGATTTCTCCC CATAAG
LytO _{PGRP-SH3}	ATCGTCGATCCATGGCGACAGCTAAGCCAC	TAGTCAGTCGGATCCTTAACTGATTTCTCCC CATAAG
LytO _{CHAP}	ATCGTCGATCCATGGCGCAAGCAAATAAAC	TAGTCAGTCGGATCCTTATTCTTTTTTAGGT GCTTG
LytO _{CHAP-PGRP}	ATCGTCGATCCATGGCGCAAGCAAATAAAC	TAGTCAGTCGGATCCTTAAAGCGCTTGAC
SsaALP _{Full}	ATCGTCGATCCATGGCGTCTACACAACATAC	TAGTCAGTCGGATCCTTAAATGGATGAATGCA TAG
SsaALP _{CHAP}	ATCGTCGATCCATGGCGTCAGGTGGTAC	TAGTCAGTCGGATCCTTAAATGGATGAATGCA TAG
LDP _{Full}	ATTCATAATCCATGGCGCAACAACATGGC	CGGAGCTCGAATTCGGATCCTTAGTGGATGT AATTATA
LDP _{CHAP}	ATATTCATAATCCATGGCGGCATACAAACCT GC	CGGAGCTCGAATTCGGATCCTTAGTGGATGT AATTATA
PH _{Full}	TGCATAGCACCATGGCGTTGATAACAAAAAA CCAAGC	TCGAATTCGGATCCCTAAATCGTGCTAAACT TACCAAAAC
PH _{MurNAc-SH3}	TGCATAGCACCATGGCGGATAAAGTAAGTGT TGG	TCGAATTCGGATCCCTAAATCGTGCTAAACT TACCAAAAC
PH _{CHAP-MurNAc}	TGCATAGCACCATGGCGTTGATAACAAAAAA CCAAGC	GCTCGAATTCGGATCCCTAATTACCAGCTAC C
PH _{CHAP}	TGCATAGCACCATGGCGTTGATAACAAAAAA CCAAGC	GCTCGAATTCGGATCCCTAATCTGGGAAATT TAATC
PH _{MurNAc}	TGCATAGCACCATGGCGGATAAAGTAAGTGT TGG	GCTCGAATTCGGATCCCTAATTACCAGCTAC C
LBD _{Vector}	ATCTGATAACATATGCCATGGAATCATGGAT CCGCAGGATATGG	TTTGTACATAAGCTTTCACCTTTATAG
LytH-LBD	ATAGCACCATGGCGGAAGATAGTGGGAACAT CACG	TCGAATTCGGATCCCGCAGAAAAATAAATTT TAAGGCC
LytO-LBD	ATAGCACCATGGCGGAAGATAGTGGGAACAT CACG	TAGTCAGTCGGATCCTGATCGTGGCGCTG
SsaALP-LBD	ATCGTCGATCCATGGCGTCAGGTGGTAC	TAGTCAGTCGGATCCATGGATGAATGCATAG
LDP-LBD	ATATTCATAATCCATGGCGGCATACAAACCT GC	CGGAGCTCGAATTCGGATCCGTGGATGTAAT TATA
PH-LBD	TGCATAGCACCATGGCGTTGATAACAAAAAA CCAAGC	GCTCGAATTCGGATCCATCTGGGAAATTTAA TC

*All primers are written in the 5' to 3' direction.

Table S2 Biochemical information and purification notes for each autolysin construct.

Construct Name	Molecular Mass* (kD)	Theoretical pI*	Extinction Coefficient* (M ⁻¹ cm ⁻¹)	Soluble?	Typical Purified Yield (mg L ⁻¹)	Purification Notes
LytH _{Full}	29.3	6.18	38390	Y	2	Ni Only
LytH _{MurNAc}	22.5	6.35	18910	Y	2	Ni Only
LytO _{Full}	55.1	8.63	130750	Y	1	
LytO _{PGRP-SH3}	35.9	9.11	69330	Y	7	300mM NaCl
LytO _{CHAP}	19.3	6.02	61420	Y	4	Ni only
LytO _{CHAP-PGRP}	43.1	8.65	89840	Y	4	---
SsaALP _{Full}	26.9	6.22	44350	Y	1.5	Ni resin turns bright purple upon binding of protein
SsaALP _{CHAP}	15.2	6.47	34380	Y	3	---
LDP _{Full}	29.1	9.98	34840	Y	1.5	350 mM NaCl elution
LDP _{CHAP}	15.6	9.68	27390	Y	0.5	---
PH _{Full}	54.9	9.24	87670	Y	1	300 mM NaCl elution
PH _{MurNAc-SH3}	37.5	9.49	49280	N	0	---
PH _{CHAP-MurNAc}	42.8	8.56	67270	N	0	---
PH _{CHAP}	18.8	6.48	38390	N	0	---
PH _{MurNAc}	25.4	9.02	28880	N	0	---
LytH-LBD	34.5	8.92	49850	Y	1	---
LytA-LBD	31.3	8.75	92360	Y	2	---
SsaALP-LBD	27.2	9.16	65320	Y	0.6	---
LDP-LBD	27.6	9.82	58330	N	0	---
PH-LBD	30.8	9.06	69330	Y	0.1	Ni Only

*Calculated by ExPASy ProtParam tool

Table S3 All results from the SMART Domain Search

SMART Query	UniProt Accession Number	Submitted Name
LysM	Q2G0D4	Secretory antigen SsaA-like protein
	Q2G278	LysM Domain Protein
	Q9ZNI1	Probable Cell Wall Hydrolase LytN
	P02976	Immunoglobulin G-binding protein A
	Q2FYF1	Elastin-binding protein EbpS
	Q2G0U9	N-acetylmuramoyl-L-alanine amidase sle1
SH3	Q2FWV4	Peptidoglycan hydrolase, putative
	Q2FX77	Autolysin
	Q2FXU3	Probable cell wall amidase LytH
	Q2FYD8	Petidoglycan hydrolase, putative

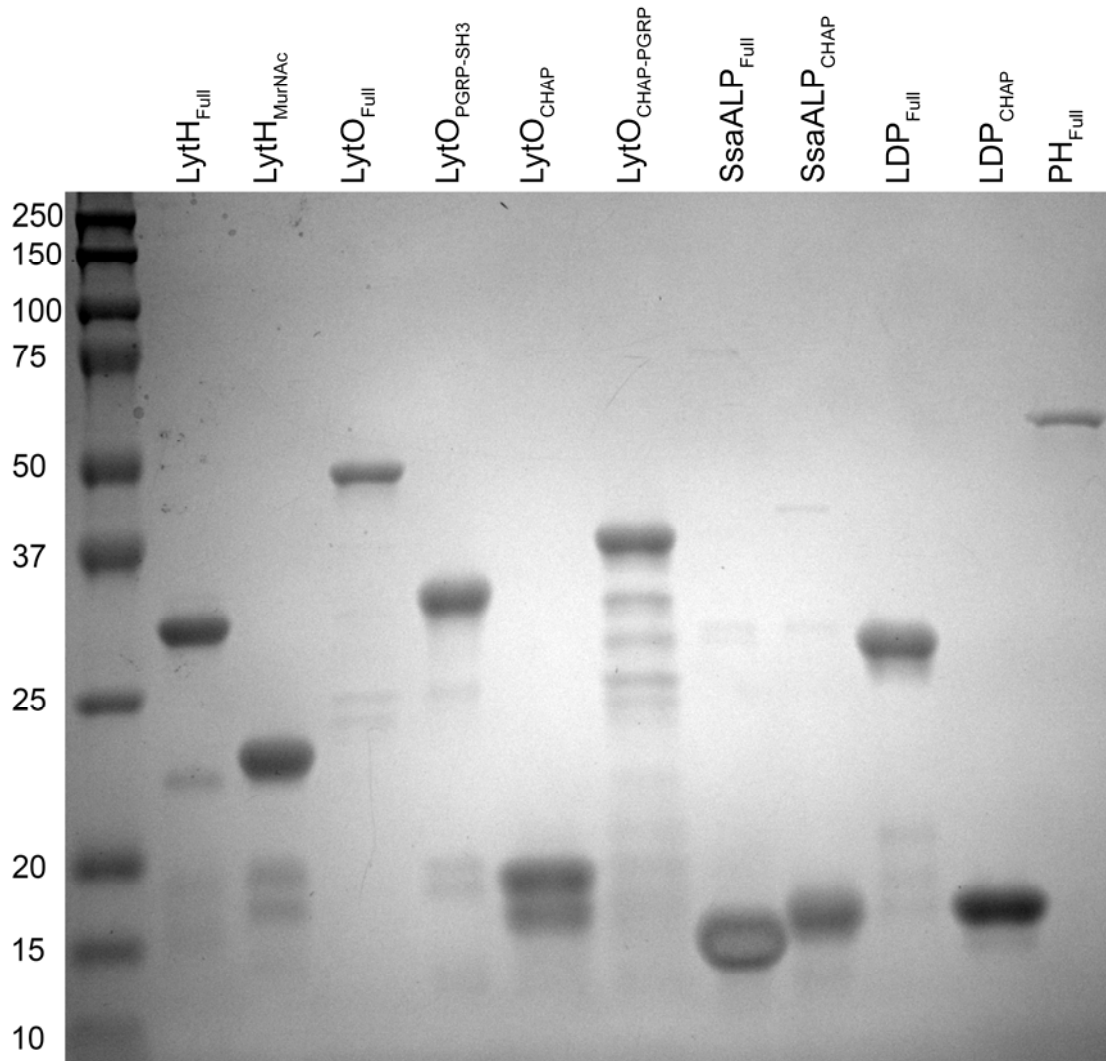


Figure S1 Representative SDS-PAGE gel of purified autolysins. The numbers on the left represent the molecular mass in kDa of the Precision Plus Protein Kaleidoscope ladder, and lanes are labeled by construct name.

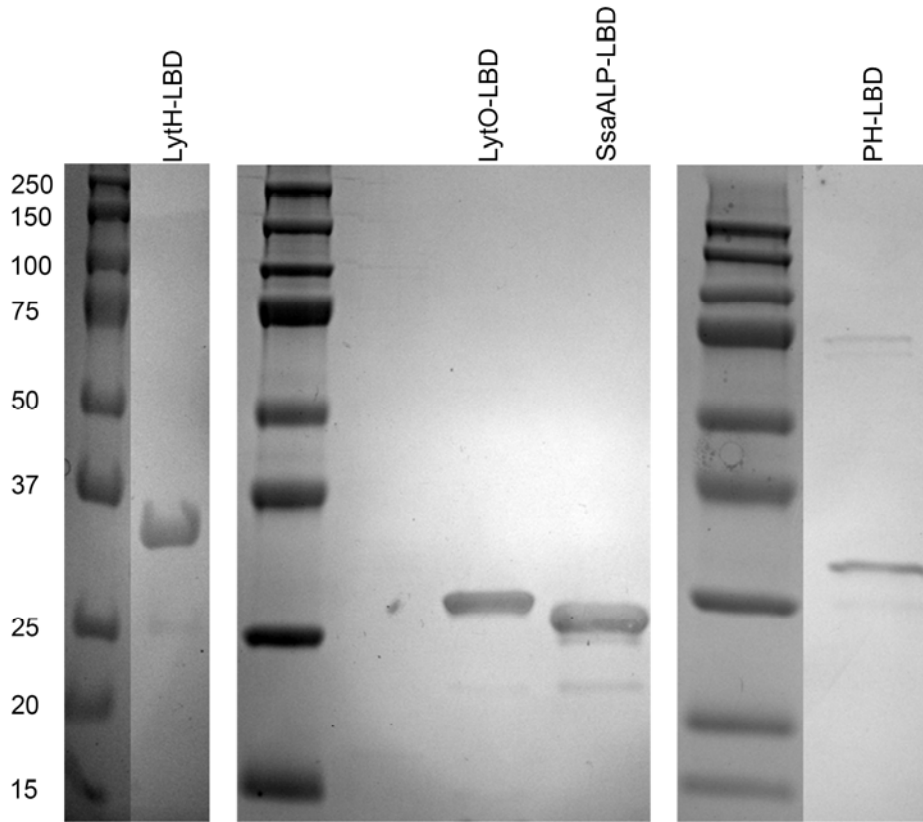


Figure S2 Representative SDS-PAGE gel depicting purification of chimeric enzymes. Numbers on the left denote the molecular mass in kDa of the Precision Plus Protein Kaleidoscope ladder seen on the left side of each panel. Lanes are labeled by construct name.

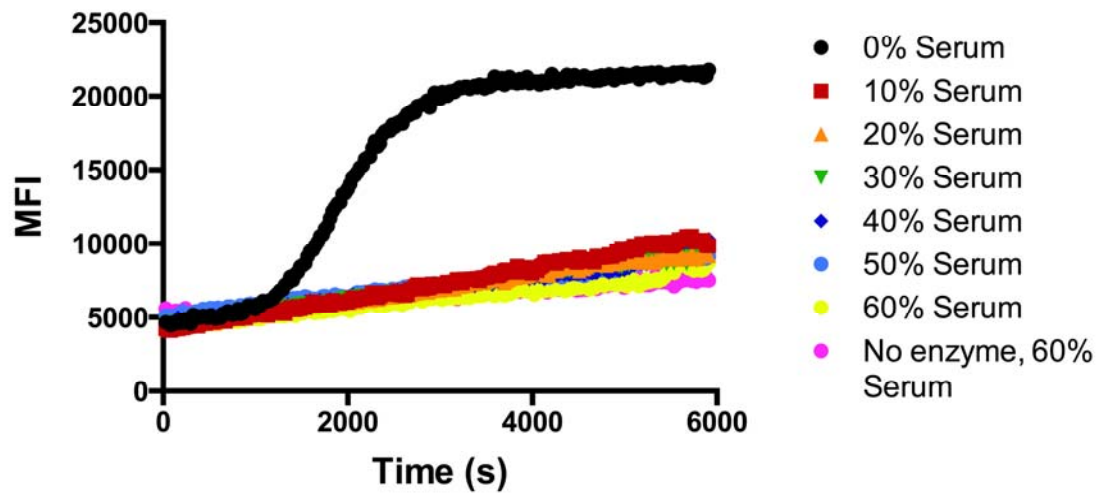


Figure S3 Representative kinetic curves of SsaALP-LBD in the presence of human serum.