

Supplementary Materials

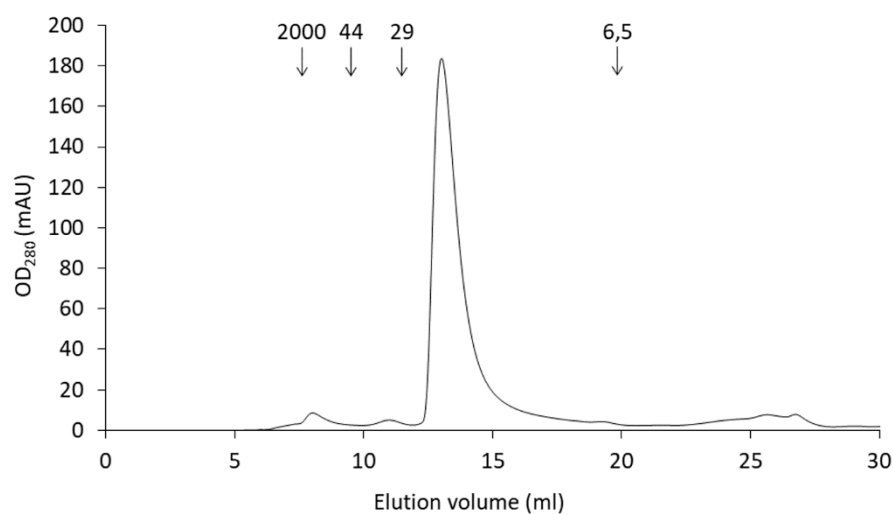


Figure S1. Size-exclusion chromatography analysis of LysC. Chromatography was carried out on a Superdex 75 10/300 GL column equilibrated with 50 mM NaH₂PO₄, 300 mM NaCl, pH 8.0 at a flow rate of 0.8 mL/min. LysC was eluted in a single peak at 13.02 mL using UV detection at 280 nm (mAU, milli-absorbance units). The positions of molecular weight standards are marked as arrows: Blue Dextran 2000 (M_r = 2,000,000); Ovalbumin (M_r = 44,000); Carbonic Anhydrase (M_r = 29,000); Ribonuclease A (M_r = 6,500).

Table S1. PCR primers used in this study.

Primer Name	Oligonucleotide Sequences (5'→3')	Annealing Temperature
Cloning of <i>lysC</i> gene		
lysC-nde-f	ACGACC <u>CATATG</u> AAAAATCTGCTGCG	50 °C
lysC-bam-h-r	GAGCGGATCCTTATTTACGTTCA	50 °C
lysCΔ2-23	ACGACCATATGCGGAAAAATTGTGGA	56 °C
Site-directed mutagenesis of <i>lysC</i> gene		
H50A-F	CTATAACTTTAATCCGAACATGATCGTGTATGCTCACACCGTGGATAAT	Codon change: CAT → GCT
H50A-R	ATTATCCACGGTGTGAGCATACACGATCATGTTCCGATTAAAGTTATAG	CAT → GCT
H51A-F	AACTTTAATCCGAACATGATCGTGTATCATGCCACCGTGGATAATAAC	CAC → GCC
H51A-R	GTTATTATCCACGGTGGCATGATACACGATCATGTTCCGATTAAAGTT	CAC → GCC
T52A-F	CATGATCGTGTATCATCACGCCGTGGATAATAACATGAC	ACC → GCC
T52A-R	GTCATGTTATTATCCACGGCGTGTATGATACACGATCATG	ACC → GCC
Y76A-F	CAGCGTGGTTGGAGCGGTATTGGTGCTCATTTCTATATTCGTAAA	TAT → GCT
Y76A-R	TTTACGAATATAGAAATGAGCACCAATACCGCTCCAACCACGCTG	TAT → GCT
H147A-F	TATCACCGATCTGAAACGCGCTAAAGATGTTTCGTCAGACC	CAT → GCT
H147A-R	GGTCTGACGAACATCTTTAGCGCGTTTCAGATCGGTGATA	CAT → GCT
T153A-F	CATAAAGATGTTTCGTCAGGCCGAATGTCCGGGTAATA	ACC → GCC
T153A-R	TATTACCCGGACATTCCGGCTGACGAACATCTTTATG	ACC → GCC
C155A-F	CCATAAAGATGTTTCGTCAGACCGAAGCTCCGGGTAATAACT	TGT → GCT
C155A-R	AGTTATTACCCGGAGCTTCGGTCTGACGAACATCTTTATGG	TGT → GCT

Primers for cloning of *lysC* gene were design with use of Primer3plus software. Primers for site-directed mutagenesis of *lysC* gene were design with use of QuikChange Primer Design Program (<http://www.genomics.agilent.com>). Underlined sequences indicate cleavage sites for restriction enzymes NdeI and BamHI, respectively.

Table S2. Physicochemical properties of *N*-terminal region of LysC.

	Intestinalin
Amino acid	30
Molecular weight	3678
	Hydrophobic amino acid - I: 3, V: 3, L: 3, F: 1, C: 0, M: 0, A: 0, W: 0
Amino acid composition	The number of G and P - G: 0, P: 1, Negatively charged amino acid - E: 1, D: 1, Positively charged amino acid - K: 5, R: 6, H: 0 Other amino acid - T: 1, S: 2, Y: 0, Q: 0, N: 3
Hydrophobic ratio	33%
Net charge	+9
Protein-binding potential (Boman index)	3.91 kcal/mol

Table S3. Antimicrobial peptide prediction of the *N*-terminal region of LysC.

Peptide Sequence	KNLLRRIRRKLRNKFSSRSDVIKTPKIVEVN	
	Class	AMP probability
Support Vector Machine (SVM) classifier	AMP	0.979
Random Forest Classifier	AMP	0.733
Artificial Neural Network (ANN) classifier	AMP	
Discriminant Analysis classifier	AMP	0.995

CAMP is available at <http://www.bicnirrh.res.in/antimicrobial/>; AMP—antimicrobial peptide.



© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).