

Supplementary Materials

Table S1. Bacterial strains and plasmids used in this study.

Strains and Plasmids	Relevant characteristics	References
Strains		
Strains used for heterologous expression and cloning		
<i>Escherichia coli</i> JM109	<i>recA1. endA1, gyrA96, thi. hsdR17, supE44, relA1, l-, A(iac-proAB), [F', traD36, proAB, iacI^qZAM15]</i>	Promega, Madison, United States
<i>Escherichia coli</i> Rosetta	<i>[F', traD36, ΔompP, proA⁺B⁺, lacIq, Δ(lacZ)M15]ΔompT, endA1, recA1, gyrA96 (Nal^r), thi-1, hsdR17 (r_k⁻, m_k⁺), e14⁻ (McrA⁻), relA1, supE44, Δ(lac-proAB), Δ(rhaBAD)::T7 RNA polymerase</i>	Promega, Madison, United States
Strains used as targets for antimicrobial activity tests		
<i>Escherichia coli</i> ATCC 8739		[29](5)
<i>Listeria innocua</i> CIP 80.11		[64](62)
<i>Proteus vulgaris</i> ATCC 33420		(67)
<i>Salmonella enterica</i> Serotype Newport ATCC 6962		(52)
<i>Pseudomonas aeruginosa</i> ATCC 27853		(19)
Plasmids		
pT7-6his-030	<i>pT7, His-tag, Tev-site, orf030, Amp^R, LacI, LacO</i>	(5)
pET-32b(+)	<i>pT7, His tag, Trx-tag, S-tag, Amp^R, LacI, LacO, MCS</i>	Merck Millipore, Burlington, United States
pET-32b-030	pET-32b(+) derivative carrying a 333 pb DNA fragment from pT7-6his-030 with <i>orf030</i> gene	In this study
pET32b-Nter_030	pET-32b(+) derivative carrying a 117 pb DNA fragment from pT7-6his-030 with <i>orf030</i> gene fragment	In this study
pET32b-Cter_030	pET-32b(+) derivative carrying a 216 pb DNA fragment from pT7-6his-030 with <i>orf030</i> gene fragment	In this study
pET32b-Nter-H1_030	pET-32b(+) derivative carrying a 81 pb DNA fragment from pT7-6his-030 with <i>orf030</i> gene fragment	In this study

pT7: T7 promoter, *Amp^R*: ampicillin resistant, *LacI* : lacI coding sequence, *LacO* : lac Operator

Table S2. Sequences of oligonucleotide primers used in this study.

Name	Sequence 5' → 3'	Use
F-BamHI-030	AAAAAGGATCCATGACAGACAAACGTGAACTT	Amplification of <i>orf030</i> up-stream fragment
R-030-HindIII	AAAAAAGCTTCTAATGCCCCAAACAATGG	Amplification of <i>orf030</i> down-stream fragment
R-Nter_030-HindIII	AAAAAAGCTTCTAATCAACCTTTTGGCGTTGGT	Amplification of <i>orf030_nter</i> down-stream fragment
F-BamHI-Cter_030	AAAAAGGATCCATGGAAGGTGATGACGAAAAGGC	Amplification of <i>orf030_cter</i> up-stream fragment
R-Nter-H1_030-HindIII	AAAAAAGCTTTTACGTTGGATTAGCGTATGCC	Amplification of <i>orf030_nter-h1</i> down-stream fragment
F-030_mutE6G	ACAGACAAACGTGGAACCTTTAAT	to make mutations in the pT7-6his-030 plasmid in order to replace the Glu 6 to Gly in lacticaseicin 30
R-030_mutE6G	ATTAAAGTTCACGTTTGTCTGT	
F-030_mutT7P	AAACGTGAACCTTTAATGTCTG	to make mutations in the pT7-6his-030 plasmid in order to replace the Thr 7 to Pro in lacticaseicin 30
R-030_mutT7P	CGACATTAAAGGTTTACGTTT	
F-030_mutE32G	GCGCTTATCGGAACCAACG	to make mutations in the pT7-6his-030 plasmid in order to replace the Glu 32 to Gly in lacticaseicin 30
R-030_mutE32G	CGTTGGTTCCGATAAGCGC	
F-030_mutT33P	GCTTATCGAACCCAACGC	to make mutations in the pT7-6his-030 plasmid in order to replace the Thr 33 to Pro in lacticaseicin 30
R-030_mutT33P	GCGTTGGGTTTCGATAAGC	
F-030_mutT52P	CGGCTGTCCCTCAGTT	to make mutations in the pT7-6his-030 plasmid in order to replace the Thr 52 to Pro in lacticaseicin 30
R-030_mutT52P	AACTGAGGGACAGCCG	
F-030_mutD57G	GTTGTCCACGGCATTTC	to make mutations in the pT7-6his-030 plasmid in order to replace the Asp 57 to Gly in lacticaseicin 30
R-030_mutD57G	GGAAATGCCGTGGGACAAC	
F-030_mutA77P	CTGGTGCCCGTCTTCA	to make mutations in the pT7-6his-030 plasmid in order to replace the Ala 77 to Pro in lacticaseicin 30
R-030_mutA77P	TGAAGACGGGCACCAG	
F-030_mutY78S	CCGTCTTCAATTCCATCAAAAA	to make mutations in the pT7-6his-030 plasmid in order to replace the Tyr 78 to Ser in lacticaseicin 30
R-030_mutY78S	TTTTTGATGGAATTGAAGACGG	
F-030_mutY93S	GCACGTTCTCGCGCGCAA	to make mutations in the pT7-6his-030 plasmid in order to replace the Tyr 93 to Ser in lacticaseicin 30
R-030_mutY93S	TTGCGCGCGAGAACGTGC	
F-030_mutA97P	GCGCAACCCTTGGC	to make mutations in the pT7-6his-030 plasmid in order to replace the Ala 97 to Pro in lacticaseicin 30
R-030_mutA97P	GCCAAGGGTTGCGC	
T7	TAATACGACTCACTATAGGGGA	Cloning verification in pET-32(+) plasmid
T7 term	GCTAGTTATTGCTCAGCGG	

Supplementary Figure

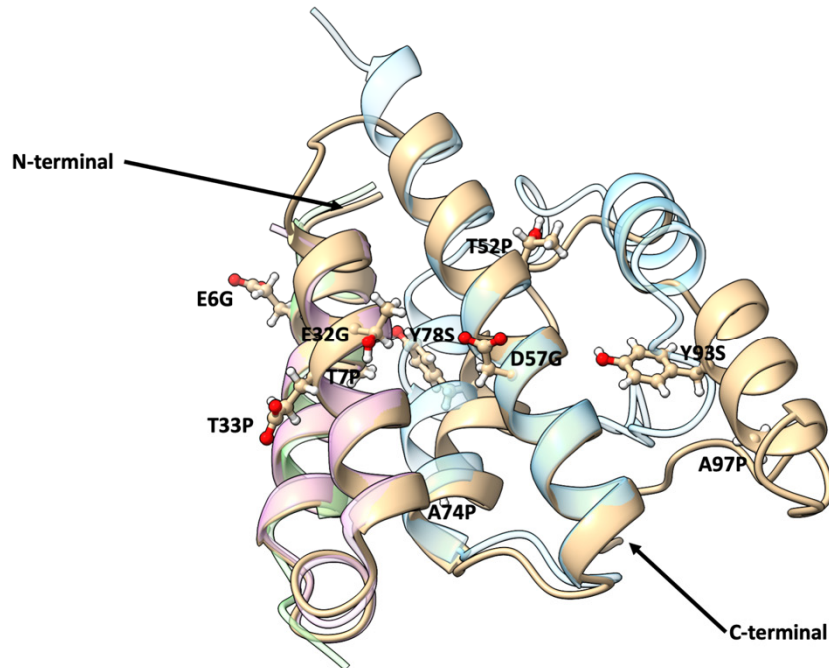


Figure S1. Structural alignment of the predicted structures of native lacticaseicin 30 (brown) and its truncated derivatives N-ter-lacticaseicin 30 (transparent red, RMSD between 37 pruned atom pairs 0.381 angstroms; across all 39 pairs: 1.535 angstroms), N-ter-H1- lacticaseicin 30 (transparent green, RMSD between 20 pruned atom pairs 0.797 angstroms; across all 20 pairs: 0.797 angstroms) and C-ter-lacticaseicin 30 (transparent blue, RMSD between 31 pruned atom pairs 0.888 angstroms; across all 73 pairs 8.364 angstroms). Substituted amino acids in the variant peptides are visible as balls and sticks in their position in the native peptide.