

Supplementary Information

1. Supplementary Materials and Methods

Semi-preparative HPLC separation of commercialized nisin A

Purification was performed using the UltiMate3000 Semi-preparative HPLC (Thermo Fisher). The system was equipped with a UV detector and UltiMate 3000 Fraction Collector. A Fisher Hypersil Gold column (250 × 10 mm, i.d., 5 μm) was employed for peptide separation at 35 °C. The injection volume was 100 μL. The sampler tray temperature was 10 °C. The column flow rate was 2 mL/min, and detection was set at 215 nm. The column was used under the following conditions: mobile phase A was H₂O (0.1% formic acid); mobile phase B was acetonitrile (ACN). The gradient program was (time, B%) 0 min, 5% B; 1 min, 5% B; 30 min, 90% B; 35 min, 90% B; 36 min, 5% B; 50 min, 5% B. All chromatographic peaks were collected during the preliminary experiment, and all fractions were detected by LC-MS-MS. The desired fractions were then collected.

LC-MS analysis of nisin Z and targeted proteomics analysis

The original strain, J1-004, and the engineered *L. lactis* cells collected from the fermentation broth were analyzed by LC-MS for nisin Z and by a targeted proteomics approach according to a previously reported method (1). Briefly, *L. lactis* cells collected from the fermentation broth were pelleted by centrifugation 8,000 × g for 10 min at 4 °C, collected, and washed three times with wash buffer (100 mM NaCl, 25 mM Tris-HCl, pH 7.5). The wet cell pellet was suspended in an equal volume (1 g wet cell weight/1 mL of buffer) of lysis buffer (8 M urea, 2 M thiourea, 75 mM NaCl, 4% (w/v) 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate (CHAPS), 50 mM Tris-HCl (pH 8.0) and 1 complete EDTA-Free protease inhibitor cocktail tablet (Roche, Indianapolis, IN, USA) per 10 mL of buffer). The suspended sample was vortexed for 30 s twice and disrupted by sonication (2). The supernatant from the lysed cells was collected by centrifugation (13,000 × g for 45 min at 4 °C). Proteins from the cell lysates were measured using a noninterference protein assay kit (Sangon Biotech, Shanghai, China) and adjusted to 2 μg/μL using lysis buffer. First, 50 μL of the supernatant (100 μg of total protein) was mixed with an equal volume of 100 mM ammonium bicarbonate buffer (pH 8.0). Next, the sample was reduced at 30 °C for 1 h by the addition of 3 mM tris (2-carboxyethyl)-phosphine (TCEP) and alkylated by the addition of 15 mM iodoacetamide (IAA). The samples were incubated in dark conditions at 30 °C for an additional 1 h. The sample was diluted with ammonium bicarbonate buffer to reduce the urea concentration to 1 M. Trypsin was added to the mixture (trypsin/total protein 1:50, w/w) and incubated at 37 °C for 14 h. The detergent and salt in the digested peptide sample were removed by passage through a Pierce Detergent Removal Spin Column (Thermo Fisher Scientific) and a SepPak C18 cartridge (Waters Corp.), respectively. The purified peptides were freeze-dried and stored at -80 °C for subsequent liquid chromatography-tandem mass spectrometry (LC-MS-MS) analysis.

The peptide samples were analyzed using a hybrid quadrupole-time-of-flight (TOF) liquid chromatography (LC) tandem mass (MS/MS) spectrometer (TripleTOF 5600+, AB Sciex, Foster City, CA, USA) equipped with a nanospray ion source. Peptides were first loaded onto a C18 trap column (5 μm, 5 × 0.3 mm; Agilent Technologies, Santa Clara, CA, USA) and then eluted into a C18 analytical column (75 μm × 150 mm, 3 μm

particle size, 100 Å pore size; Eksigent, Dublin, CA, USA). Mobile phase A (3% DMSO, 97% H₂O, 0.1% formic acid) and mobile phase B (3% DMSO, 97% ACN, 0.1% formic acid) were used to establish a 100-min gradient as follows: 0 min of 5% B, 65 min of 5–23% B, 20 min of 23–52% B, 1 min of 52–80% B, maintenance at 80% B for 4 min, 0.1 min of 80–5% B, and a final step of 5% B for 10 min. A constant flow rate was set at 300 nL/min. MS scans were conducted from 350 amu-1500 amu, with a 250-ms time span. For the MS/MS analysis, each scan cycle consisted of one full-scan mass spectrum (with m/z ranging from 350-1500 and charge states from 2-5) followed by 40 MS/MS events. The threshold count was set to 120 to activate MS/MS accumulation and former target ion exclusion was set to 18 s. Raw data obtained by TripleTOF 5600+ were analyzed using ProteinPilot 5.0 (AB SCIEX) against the designated proteome database.

2. Supplementary Figures

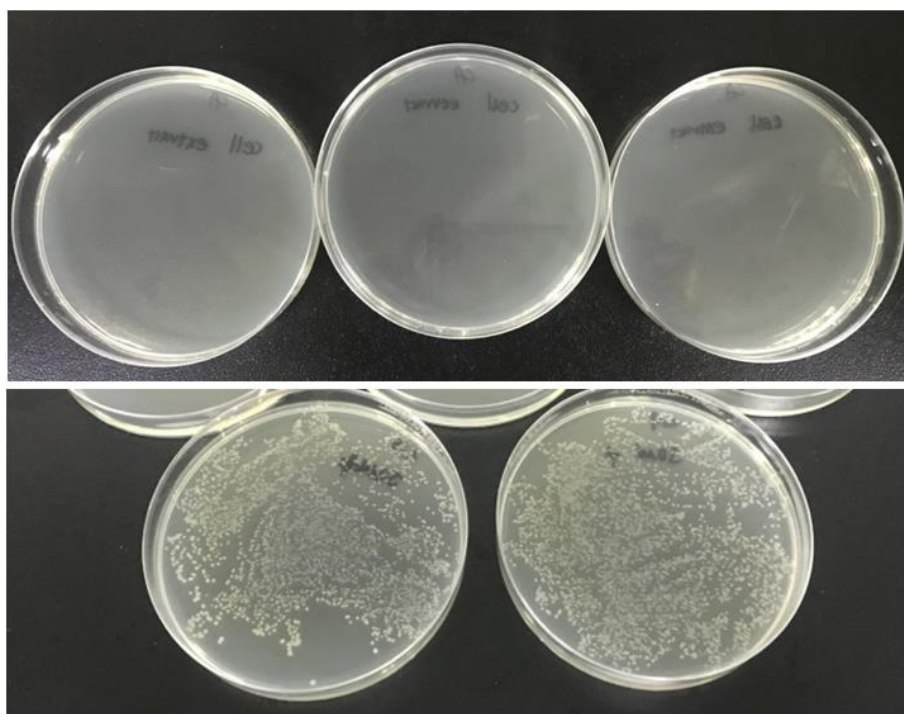


Fig. S1 | Cytoplasmic extract control plates

All LB agar (no antibiotic) plates were incubated at 37 °C for 16 h and 27 % v/v of the cell extract was used for each CFPS reaction. **Top:** Approximately 50 µL of *E. coli* cell extracts was plated on each of the three plates. No *E. coli* colonies grew on either plate. **Bottom:** Approximately 50 µL of *E. coli* (5.0×10^5 CFU mL⁻¹) cells were added to the plate as a positive control.

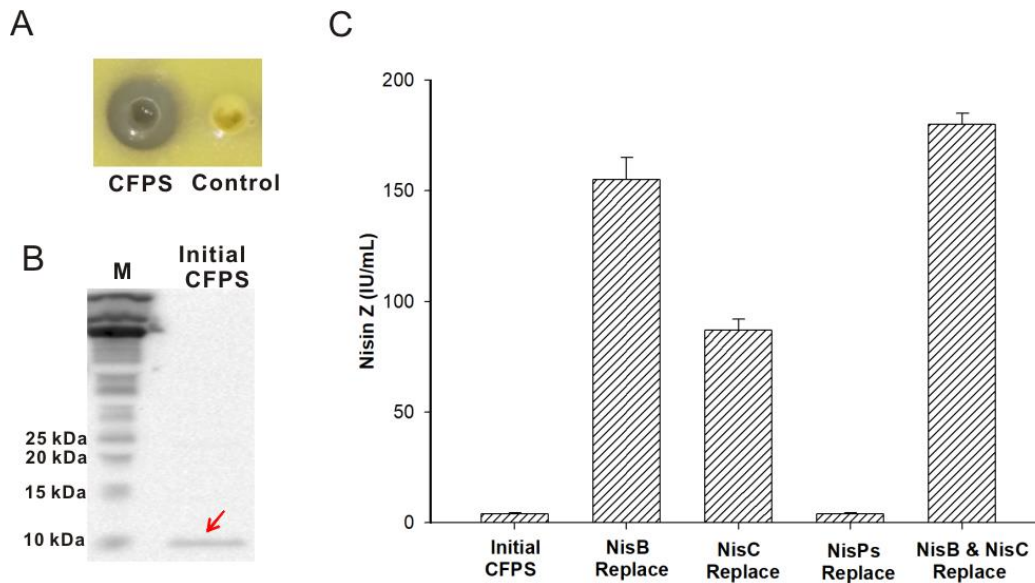


Fig. S2 | Analysis of the nisin CFPS system

(A) Validation of antimicrobial activity by the agar diffusion assay. An obvious zone of inhibition was detected on the *M. luteus* plate using the nisin CFPS reaction mixture (CFPS). Control: CFPS reaction without pJL1-*nisZ*, pET28a-*nisB*, pET28a-*nisC*, and pET28a-*nisP*. (B) Western blot analysis of expressed proteins (NisZ, NisB, NisC, and NisP) involved in Nisin CFPS. Red arrow indicates the partially modified Nisin precursor with an N-terminal His6-tag. A primary anti-His6 tag mouse monoclonal antibody was diluted 1:2,000 in blocking buffer (2% nonfat milk in TBST) before use. Horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG was used as the secondary antibody, and the SuperSignal West Pico Plus Chemiluminescent Substrate was used to visualize proteins via chemiluminescence. (C) Replacement of poorly expressed protein encoding plasmids with purified enzymes in CFPS. The final concentrations of purified proteins were fixed at 500 nM.

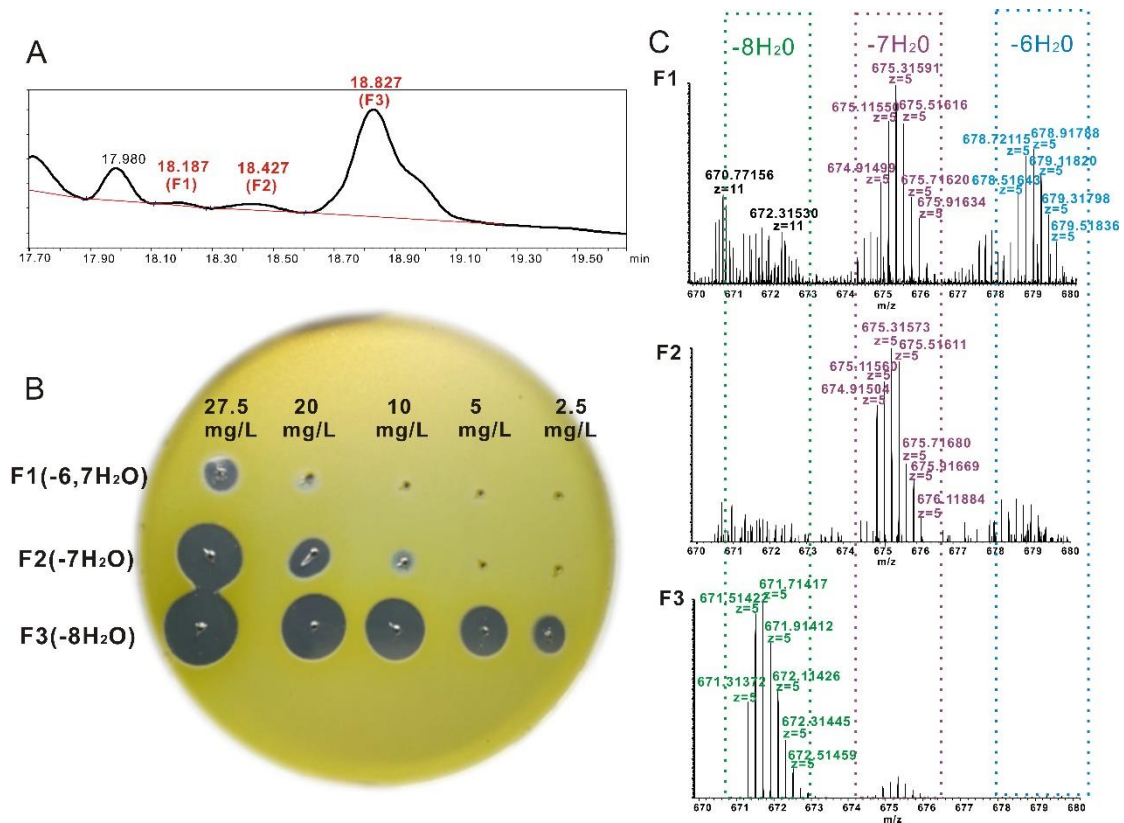


Fig. S3 | Identification of the most efficient nisin A component

(A) Separation of different components of nisin A standard by semi-preparative HPLC. F1, F2, and F3 were collected. (B) Antibacterial activity assay of different components using the agar diffusion method. The concentrations of F1, F2, and F3 were set to 2.5 mg/L, 5 mg/L, 10 mg/L, 20 mg/L, and 27.5 mg/L, and 2 μ L of each sample was used for the antibacterial activity test. (C) High-resolution mass spectrometry of different components. The F1 component consists of dehydrated (-6, -7H₂O) nisin A, the F2 component consists of dehydrated (-7 H₂O) nisin A, and the F3 component consists of dehydrated (-8 H₂O) nisin A.

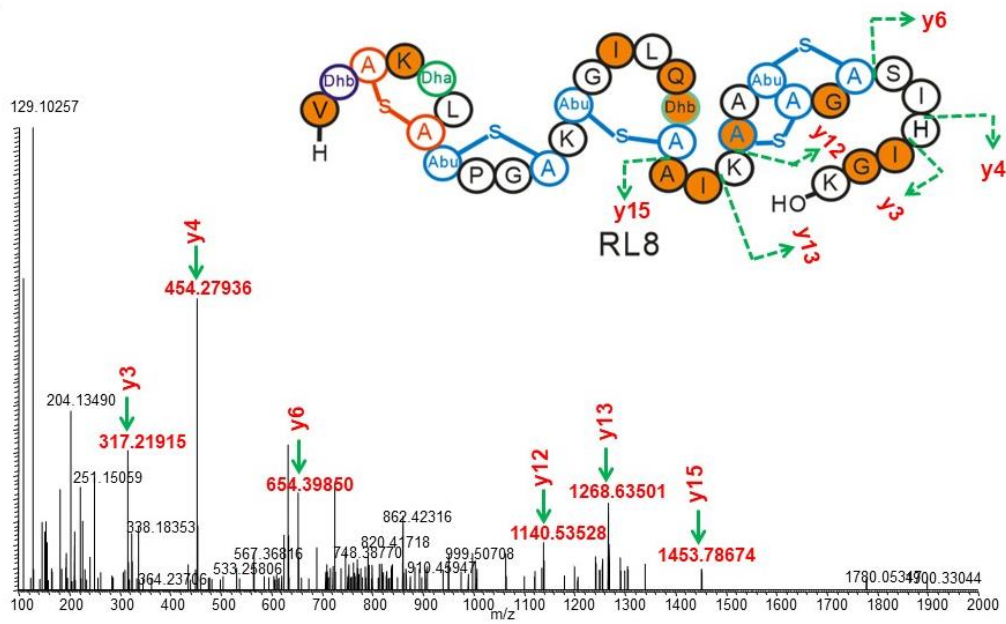
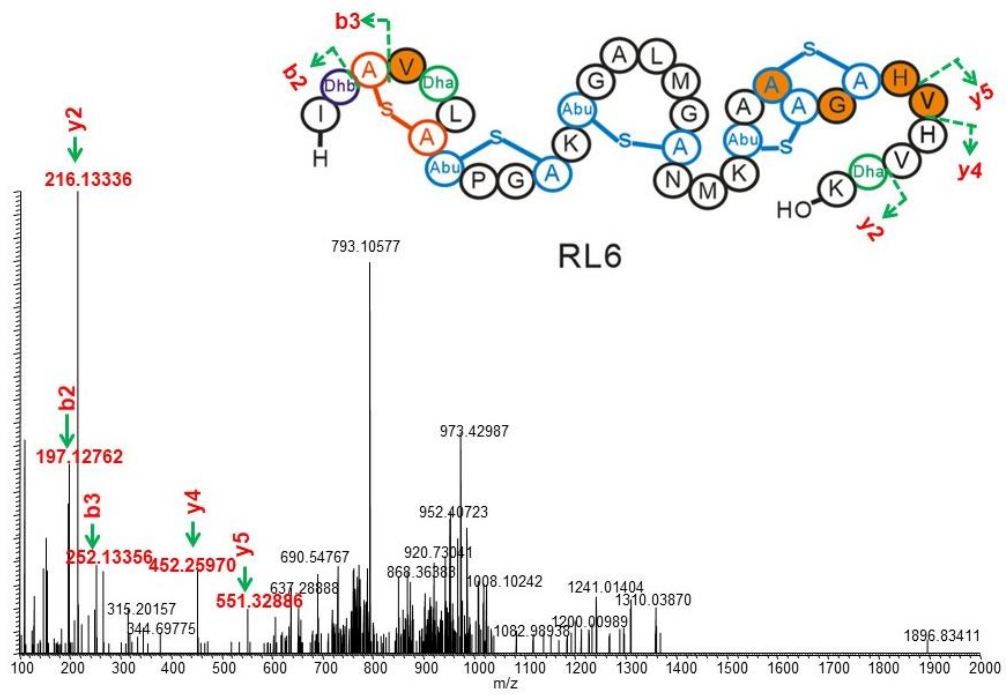


Fig. S4. Continued on next page.

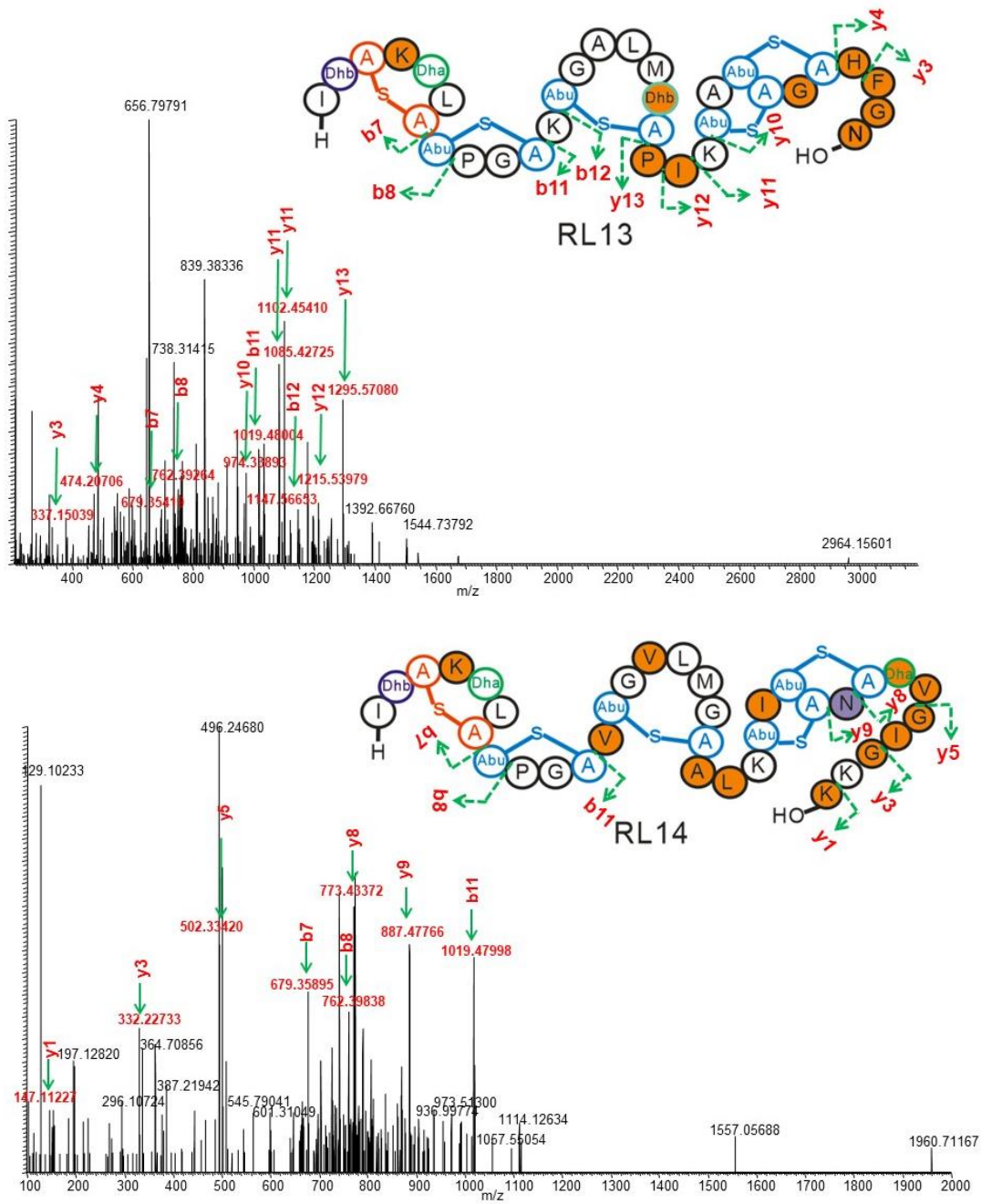


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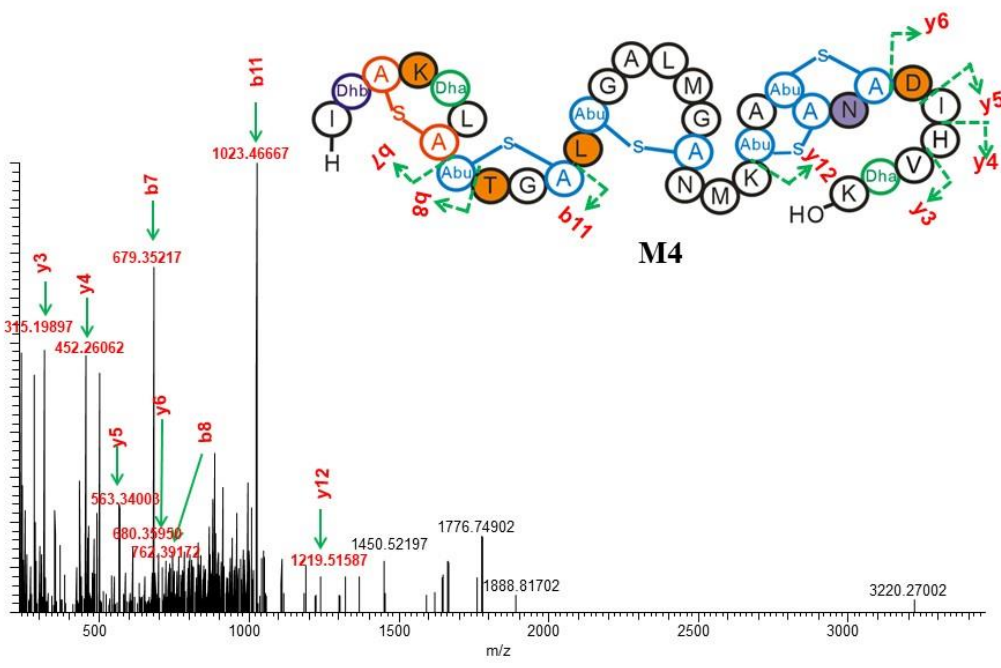
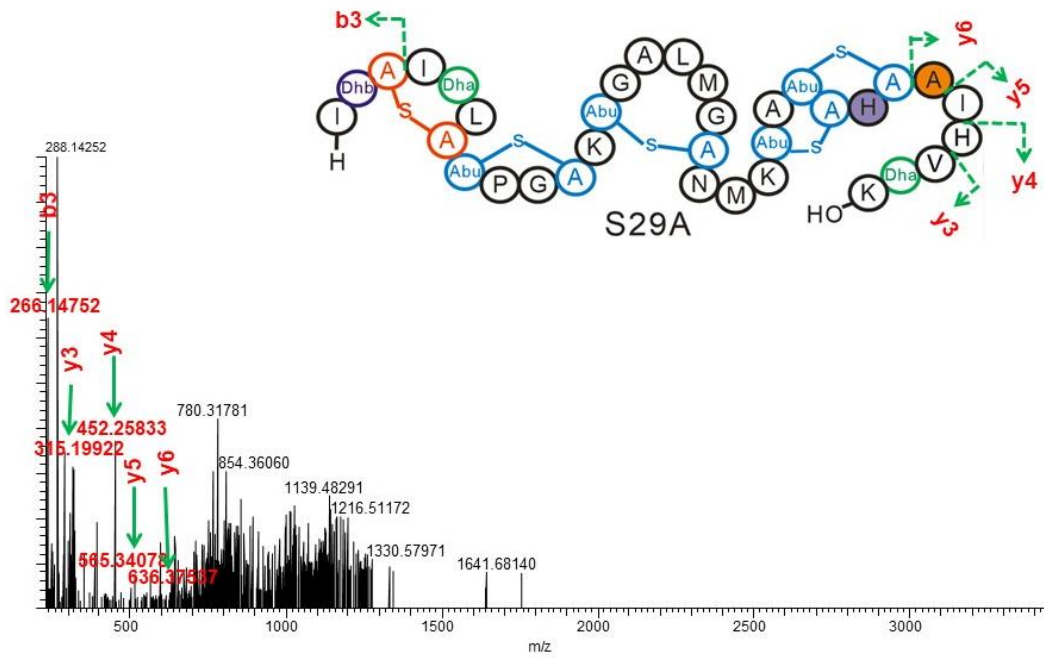


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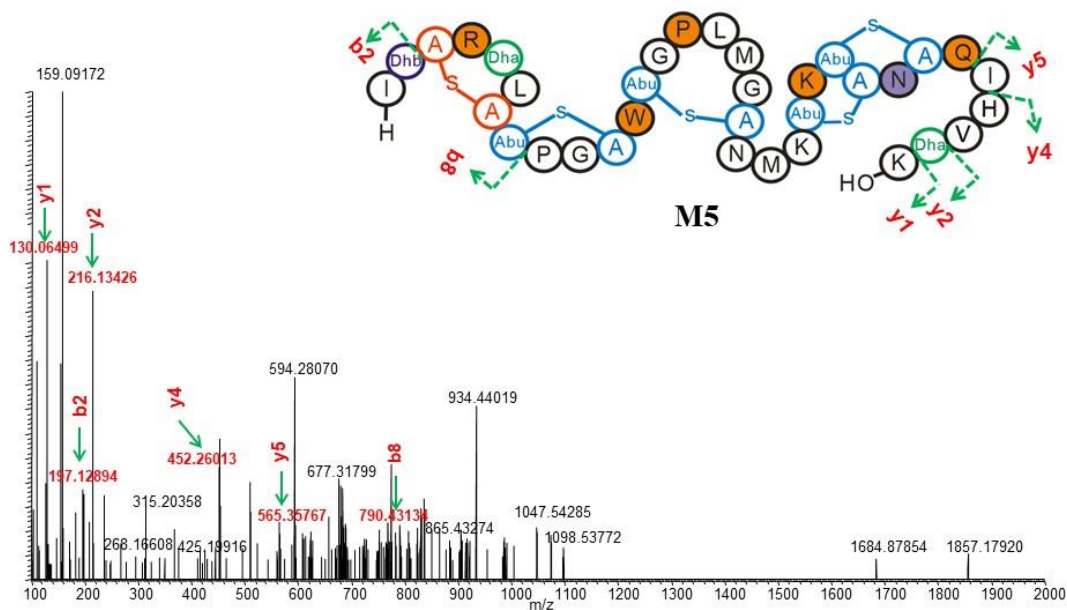


Fig. S4 | Structure of nisin analogs and nisin mutants determined by high-resolution mass spectrometry

Brown circle markers represent the amino acid residues that are different from nisin residues. Dha: dehydrolalanine; Dhb: dehydrobutyrine; Abu: 2-aminobutyric acid.

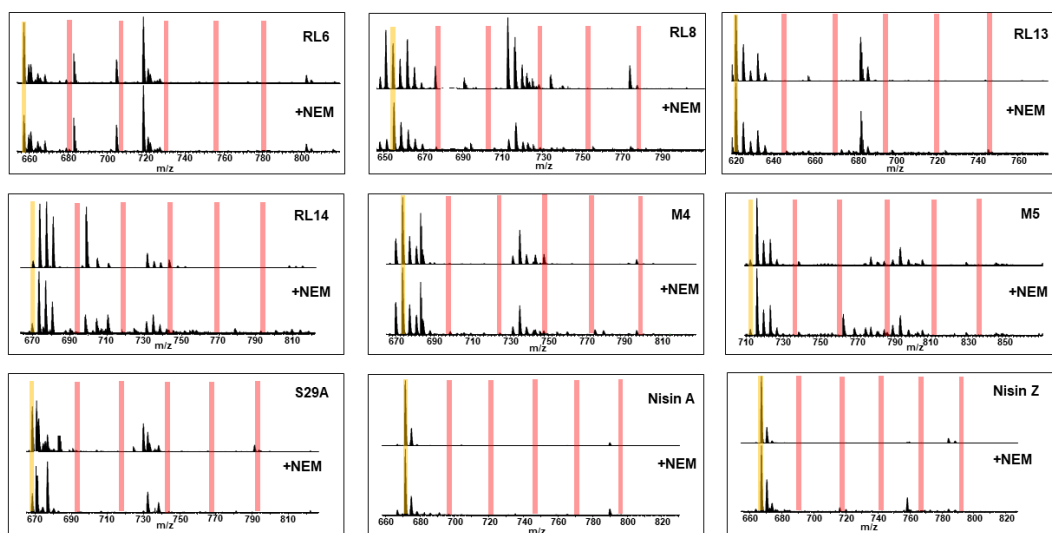


Fig. S5 | Summary of the LC-MS analysis of lanthipeptides studied in this research

All ions are in the +5-charge state. Purified modified precursor peptides were digested with trypsin to form mature RL6, RL8, RL13, RL14, M4, M5, and S29A and treated with NEM. Commercialized nisin A and nisin Z were also treated with NEM. The eight-fold dehydrated core peptides are highlighted in yellow.

Alkylated core peptides with 1–5 NEM adducts are highlighted in red. All five thioester rings were formed in mature lanthipeptides.

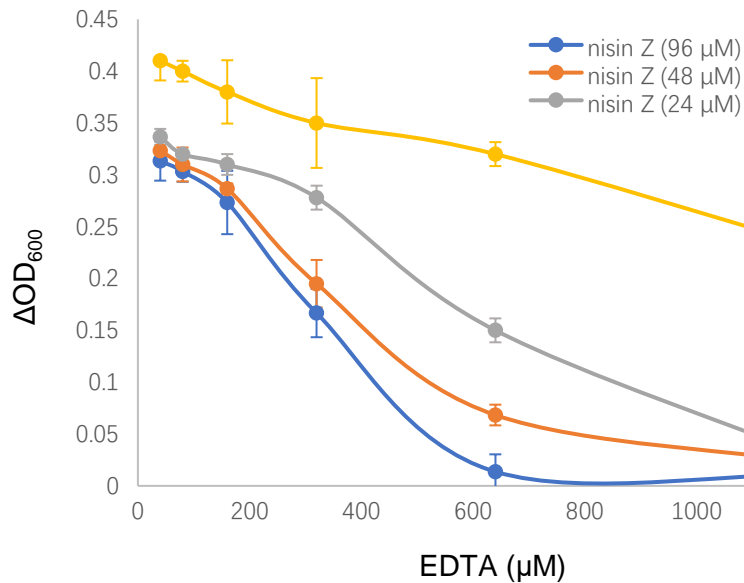


Fig. S6 | OD₆₀₀ of *E. coli* DH5α in LB medium with different concentrations of EDTA and Nisin after 18 h of co-culture

ΔOD₆₀₀ indicates the difference between readings with different concentrations of blank media. Previous studies have reported that nisin has a significant inhibitory effect on *E. coli* (3-5), however, uncommon *E. coli* strains were used. In the reported 20-fold nisin concentration condition, DH5α did not exhibit complete inhibition. To enable comparisons with previous studies, EDTA was added to the *E. coli* culture to increase the sensitivity of *E. coli* to nisin. After 18 h, *E. coli* DH5α growth was only slightly inhibited following addition of 320 μM EDTA, however, the inhibitory effects of various concentrations of nisin on *E. coli* were obvious. Error bars are based on three independent replicates.

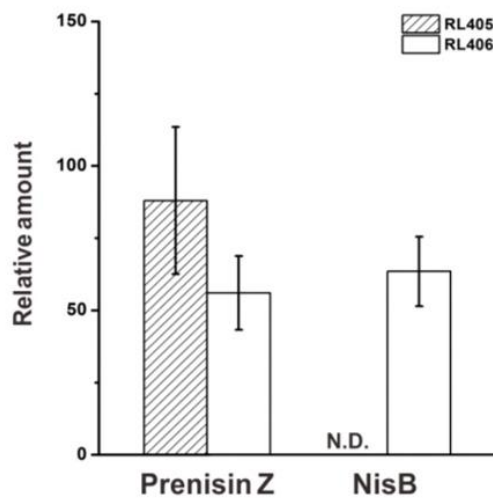


Fig. S7 | Proteomic analysis of targeted proteins involved in nisin biosynthesis in engineered strains

Relative amount: number of identified specific peptides with 95% confidence; N.D.: Not detected. Error bars are based on three independent replicates.

2. Supplementary Tables

Table S1. Strains used in this research

Strains	Describe	Source
DH10 β	Plasmid construction	Our Lab Preserve
XL1-Blue	Plasmid construction	Our Lab Preserve
BL21(DE3)	Protein/ mLANAs overexpression.	Our Lab Preserve
BL21Rosseta(DE3)	NisPs overexpression.	Our Lab Preserve
<i>Micrococcus luteus</i>	Indicator strain for nisin antibacterial activity test	NCIB 8166
BL21(DE3)/pYZ95/pYX126	Expression of sumo-tagged mRL6	This study
BL21(DE3)/pYZ96/pYX126	Expression of sumo-tagged mRL8	This study
BL21(DE3)/pYX122/pYX125	Expression of sumo-tagged mRL13	This study
BL21(DE3)/pYX123/pYX125	Expression of sumo-tagged mRL14	This study
BL21(DE3)/pYZ92/pYX126	Expression of sumo-tagged mM4	This study
BL21(DE3)/pYZ93/pYX126	Expression of sumo-tagged mM5	This study
BL21(DE3)/pYZ97/pYX126	Expression of sumo-tagged S29A	This study
<i>Enterococcus faecalis</i>	Clinical standard strain for antibacterial testing	ATCC 29212
<i>Staphylococcus aureus</i>	Clinical standard strain for antibacterial testing	ATCC 25923
Methicillin-resistant <i>Staphylococcus aureus</i> (MRSA)	Clinical isolation of antibiotic test resistant strains	Renmin hospital of Wuhan University
<i>Lactococcus lactis</i> J1-004	Nisin Z industrial producing strain J1-004/pRL415, Overexpress <i>nisZ</i> in J1-004	J1 Biotech. Co.
RL405	J1-004/pRL423, Overexpress <i>nisZ</i> and <i>nisB</i> in J1-004	This study
RL406	Expression of His6-tagged mRL6 (without <i>nisB</i> overexpressed) in <i>E. coli</i>	This study
BL21(DE3)/ pYX106	overexpress His6-tagged mRL6 (with <i>nisB</i> overexpressed) in <i>E. coli</i>	This study

Table S2 MIC values of several lanthipeptides against microorganisms

MIC* mg/L(μ M)	<i>M. luteus</i>	<i>E. faecalis</i>	<i>S. aureus</i>	MRSA
Nisin A	0.06 (0.018)	250 (74.53)	125 (37.27)	125 (37.27)
Nisin Z	0.12 (0.036)	250 (75.05)	250 (75.05)	125 (37.52)
RL6	0.12 (0.037)	250 (76.17)	>250 (>76.17)	>250 (>76.17)
RL8	0.24 (0.073)	250 (76.52)	>250 (>76.52)	>250 (>76.52)
RL13	0.24 (0.077)	250 (80.8)	>250 (>80.8)	>250 (>80.8)
RL14	0.015 (0.0045)	62.5 (18.69)	250 (74.75)	250 (74.75)

Note: * Quantification of lanthipeptides using the eight-fold dehydrated molecules

pYX105/106-R	5'-CTCGACTCGAGTCATTTCTCTTCCCTCCTTTC -3'
pYX122-F	5'-GATATGGATCCAATGAGTACAAAAGATTTTAAAC-3'
pYX122-R	5'-AGCTCGAATTCTTAGTTGCCGAAGTGGCAAC-3'
pYX123-F	5'-GATATGGATCCAATGAGTACAAAAGATTTTAAAC-3'
pYX123-R	5'-AGCTCGAATTCTTATTCTTGCCAATACCCAC-3'
Primers of plasmids for nisin overexpression in <i>L. lactis</i>	
pRL415-F	5'- GTAGCTTTTTAAATATGGGTCGATCTAATATCTTGATTTTCTAG TTCCTG-3'
pRL415-R	5'- TCCAAGTTAAATCTTTGTACTCATTTTGAGTGCCTCCTTAT AATTTATT-3'
pRL415-VF	5'- AATAAATTATAAGGAGGCACTCAAATGAGTACAAAAGATTT TAACTTGGA-3'
pRL415-VR	5'- CAGGAAGTAGAAAATCAAGATATTAGATCGACCCATATTTAA AAAGCTAC-3'
pRL423-F	5'- GTAGTATTCACGTAAGCAAATAACCAAATCAAAGGATAGTAT TTTGTTAG-3'
pRL423-R	5'- CTTGCATGCCTGCAGGTCGACTCTAGTCATTTTCATGTATTCTT CCGAAAC-3'
pRL423-VF	5'- GTTTCGGAAGAATACATGAAATGACTAGAGTCGACCTGCAG GCATGCAAG-3'
pRL423-VR	5'- CTAACAAAATACTATCCTTTGATTTGGTTATTTGCTTACGTGA ATACTAC-3'

Note: For plasmid construction via restriction enzyme digestion and ligation, complementary sequences were designed using the Primer Premier 5 software. Suitable restriction sites and protective bases were introduced. For plasmid construction via the Gibson cloning method, complementary sequences were designed using the Primer Premier 5 software and were flanked by the homologous sequence. The restriction sites used for cloning are underlined.

Table S4. Plasmids for protein purification

Plasmids	Replica tion origin	Overexpressed genes	Resistance	Reference
Plasmids for CFPS biosynthesis				
pJL1- <i>nisZ</i>	pBR322	PT7: N-terminal His6-tagged <i>nisZ</i>	Kan	This study
pET28a- <i>nisB</i>	pBR322	PT7: N-terminal His6-tagged <i>nisB</i>	Kan	This study
pET28a- <i>nisC</i>	pBR322	PT7: N-terminal His6-tagged <i>nisC</i>	Kan	This study
pET28a- <i>nisP</i>	pBR322	PT7: N-terminal His6-tagged <i>nisP</i>	Kan	This study
pET28a- <i>nisPs</i>	pBR322	PT7: N-terminal His6-tagged <i>nisPs</i>	Kan	This study
pRL1	pBR322	PT7: N-terminal His6-tagged precursor peptide gene of RL1	Kan	This study
pRL2	pBR322	PT7: N-terminal His6-tagged precursor peptide gene of RL2	Kan	This study
pRL3	pBR322	PT7: N-terminal His6-tagged precursor peptide gene of RL3	Kan	This study

pRL4	pBR322	PT7: N-terminal His6-tagged precursor peptide gene of RL4	Kan	This study
pRL5	pBR322	PT7: N-terminal His6-tagged precursor peptide gene of RL5	Kan	This study
pRL6	pBR322	PT7: N-terminal His6-tagged precursor peptide gene of RL6	Kan	This study
pRL7	pBR322	PT7: N-terminal His6-tagged precursor peptide gene of RL7	Kan	This study
pRL8	pBR322	PT7: N-terminal His6-tagged precursor peptide gene of RL8	Kan	This study
pRL9	pBR322	PT7: N-terminal His6-tagged precursor peptide gene of RL9	Kan	This study
pRL10	pBR322	PT7: N-terminal His6-tagged precursor peptide gene of RL10	Kan	This study
pRL11	pBR322	PT7: N-terminal His6-tagged precursor peptide gene of RL11	Kan	This study
pRL12	pBR322	PT7: N-terminal His6-tagged precursor peptide gene of RL12	Kan	This study
pRL13	pBR322	PT7: N-terminal His6-tagged precursor peptide gene of RL13	Kan	This study
pRL14	pBR322	PT7: N-terminal His6-tagged precursor peptide gene of RL14	Kan	This study
pRL15	pBR322	PT7: N-terminal His6-tagged precursor peptide gene of RL15	Kan	This study
pRL16	pBR322	PT7: N-terminal His6-tagged precursor peptide gene of RL16	Kan	This study
pRL17	pBR322	PT7: N-terminal His6-tagged precursor peptide gene of RL17	Kan	This study
pRL18	pBR322	PT7: N-terminal His6-tagged precursor peptide gene of RL18	Kan	This study
pJL1-bagelicin	pBR322	PT7: N-terminal His6-tagged precursor peptide gene of bagelicin	Kan	This study

Plasmids for *mlanA* overexpression in *E. coli*

pYZ82	RSF	PT7: <i>nisB</i>	Kan	This study
pYZ85	RSF	PT7: <i>nisB</i> and N-terminal His6-tagged precursor peptide gene of RL6	Kan	This study
pYZ86	RSF	PT7: <i>nisB</i> and N-terminal His6-tagged precursor peptide gene of RL8	Kan	This study
pYZ87	RSF	PT7: <i>nisB</i> and N-terminal His6-tagged precursor peptide gene of S29A	Kan	This study
pYZ89	RSF	PT7: <i>nisB</i> and N-terminal His6-tagged precursor peptide gene of M5	Kan	This study
pYZ90	RSF	PT7: <i>nisB</i> and N-terminal His6-tagged precursor peptide gene of M4	Kan	This study
pYZ91	RSF	PT7: <i>nisB</i> and N-terminal His6-tagged NisZ	Kan	This study
pYZ92	RSF	PT7: <i>nisB</i> and N-terminal Sumo-tagged precursor peptide gene of M4	Kan	This study
pYZ93	RSF	PT7: <i>nisB</i> and N-terminal Sumo-tagged precursor peptide gene of M5	Kan	This study
pYZ95	RSF	PT7: <i>nisB</i> and N-terminal Sumo-tagged precursor peptide gene of RL6	Kan	This study

pYZ96	RSF	PT7: <i>nisB</i> and N-terminal Sumo-tagged precursor peptide gene of RL8	Kan	This study
pYZ97	RSF	PT7: <i>nisB</i> and N-terminal Sumo-tagged precursor peptide gene of S29A	Kan	This study
pYZ99	RSF	PT7: <i>nisB</i> and N-terminal Sumo-tagged NisZ	Kan	This study
pYZ81	P15A	PT7: <i>nisC</i>	CmR	This study
pYX106	RSF	PT7: <i>nisB</i> and N-terminal His-tagged precursor peptide gene of RL6, and PT7: <i>nisC</i>	Kan	This study
pYX125	P15A	PT7: <i>nisB</i>	CmR	This study
pYX126	P15A	PT7: <i>nisC</i> and PT7: <i>nisB</i>	CmR	This study
pYX105	RSF	PT7: <i>nisB</i> and N-terminal Sumo-tagged precursor peptide gene of S29A, and PT7: <i>nisC</i>	Kan	This study
pYX122	RSF	PT7: <i>nisB</i> and N-terminal Sumo-tagged precursor peptide gene of RL13, and PT7: <i>nisC</i>	Kan	This study
pYX123	RSF	PT7: <i>nisB</i> and N-terminal Sumo-tagged precursor peptide gene of RL14, and PT7: <i>nisC</i>	Kan	This study

Plasmids for nisin overexpression in *L. lactis*

pRL415	pWV01	Pnis: <i>nisZ</i>	Emr	This study
pRL423	pWV01	Pnis: <i>nisZ</i> and <i>nisB</i>	Emr	This study

Table S5. Gene sequences of hybrid precursor peptides.

>RL1

ATGAGTACAAAAGATTTTAACTTGGATTTGGTATCTGTTTCGAAGAAAGATTCAGGTGC
ATCACCACGCATCACCGTGCCTAGCAAGAGCCTGTGCACCCCGGGTTGCATTACCGGT
CCGCTGCGTACCTGCTACCTGTGCTTCCCGACCCACGTTAACTGCTAA

>RL2

ATGAGTACAAAAGATTTTAACTTGGATTTGGTATCTGTTTCGAAGAAAGATTCAGGTGC
ATCACCACGCATCACCTGGAAGAGCGAGAGCCTGTGCACCCCGGGTTGCGTGACCGG
CGTTCTGCAGACCTGCTTCTGCAAACCATCACCTGCAACTGCAAGATTAGCAAATAA

>RL3

ATGAGTACAAAAGATTTTAACTTGGATTTGGTATCTGTTTCGAAGAAAGATTCAGGTGC
ATCACCACGCGTGACCAGCAAGAGCCTGTGCACCCCGGGTTGCATCACCGGCATTCTG
ATGTGCCTGACCCAGAACAGCTGCGTTAGCTGCAACAGCTGCATCAAATGCTAA

>RL4

ATGAGTACAAAAGATTTTAACTTGGATTTGGTATCTGTTTCGAAGAAAGATTCAGGTGC
ATCACCACGCATCACAGCAAAAGCCTGTGCACCCCGGGTTGCGTGACCGGCATTCTG
ATGACCTGCCCGGTTTCAGACCCGCGACCTGCGGTTGCCAAATCACCGGCAAATAA

>RL5

ATGAGTACAAAAGATTTTAACTTGGATTTGGTATCTGTTTCGAAGAAAGATTCAGGTGC
ATCACCACGCATCACAGCAAGAGCCTGTGCACCCCGGGTTGCATCACCGGCATTCTG
ATGTGCCTGACCCAGAACAGCTGCGTGAGCTGCAACAGCTGCATTTCGTTGCTAA

>RL6

ATGAGTACAAAAGATTTTAACTTGGATTTGGTATCTGTTTCGAAGAAAGATTCAGGTGC
ATCACCACGCATCACAGCGTGAGCCTGTGCACCCCGGGTTGCAAGACCGGTGCGCTG
ATGGGTTGCAACATGAAAACCGCGAGCTGCGGCTGCCACGTGCACGTTAGCAAGTAA

>RL7

ATGAGTACAAAAGATTTTAACTTGGATTTGGTATCTGTTTCGAAGAAAGATTCAGGTGC
ATCACCACGCATCACAGCGTGAGCCTGTGCACCCCGGGTTGCGTGACCGGCGTTCTG

ATGTGCCCGGGTAACACCATTAGCTGCAACGGCCACTGCAGCATCCACATTACCGGTTA

A

>RL8

ATGAGTACAAAAGATTTTAACTTGGATTTGGTATCTGTTTCGAAGAAAGATTTCAGGTGC
ATCACCACGCGTGACCAGCAAGAGCCTGTGCACCCCGGGTTGCAAAACCGGCATCCT
GCAGACCTGCGCGATTAAGAGCGCGACCTGCGGTTGCAGCATCCACATTGGCAAATAA

>RL9

ATGAGTACAAAAGATTTTAACTTGGATTTGGTATCTGTTTCGAAGAAAGATTTCAGGTGC
ATCACCACGCGTGACCAGCAAGAGCCTGTGCACCCCGGGTTGCATCACCAGGCGTGCTG
ATGTGCCTGACCCAGAACAGCTGCGTTAGCTGCAACAGCTGCATTAATGCTAA

>RL10

ATGAGTACAAAAGATTTTAACTTGGATTTGGTATCTGTTTCGAAGAAAGATTTCAGGTGC
ATCACCACGCATCACCGTGAAGATTACCAGCTACAGCCTGTGCACCCCGGGTTGCAAG
ACCGGTGCGCTGATGGGTTGCACCATGAAAACCGCGAGCTGCGGCTGCCACGTTTACA
TCAGCAAATAA

>RL11

ATGAGTACAAAAGATTTTAACTTGGATTTGGTATCTGTTTCGAAGAAAGATTTCAGGTGC
ATCACCACGCATCACCTGGAAGAGCGAGAGCCTGTGCACCCCGGGTTGCATTACCGGC
GTGCTGCAGACCTGCTTCTGCAAACCATCACCTGCAACTGCCACATTAGCAAATAA

>RL12

ATGAGTACAAAAGATTTTAACTTGGATTTGGTATCTGTTTCGAAGAAAGATTTCAGGTGC
ATCACCACGCATCACAGCTACAGCCTGTGCACCCCGGGTTGCATTACCGGCGTTCTGA
TGGGTTGCCACATCCAGAGCATTGGCTGCAACGTGCACGTTACGTTGAGCAAGTAA

>RL13

ATGAGTACAAAAGATTTTAACTTGGATTTGGTATCTGTTTCGAAGAAAGATTTCAGGTGC
ATCACCACGCATCACAGCAAGAGCCTGTGCACCCCGGGTTGCAAAACCGGCGCGCT
GATGACCTGCCCGATTAAGACCGCGACCTGCGGTTGCCACTTCGGCAACTAA

>RL14

ATGAGTACAAAAGATTTTAACTTGGATTTGGTATCTGTTTCGAAGAAAGATTTCAGGTGC
ATCACCACGCATCACAGCAAGAGCCTGTGCACCCCGGGTTGCGTGACCGGCGTTCTG
ATGGGTTGCGCGCTGAAAACCATCACCTGCAACTGCAGCGTGGGTATTGGCAAGAAAT

AA

>RL15

ATGAGTACAAAAGATTTTAACTTGGATTTGGTATCTGTTTCGAAGAAAGATTTCAGGTGC
ATCACCACGCATCACAGCAAGAGCCTGTGCACCCCGGGTTGCGTTACCGGTCTGCTG
ATGGGTTGCGCGGGTAGCAGCGCGACCTGCAACTGCAGCGTGCACGTTGGTTAA

>RL16

ATGAGTACAAAAGATTTTAACTTGGATTTGGTATCTGTTTCGAAGAAAGATTTCAGGTGC
ATCACCACGCATCACAGCAAGAGCCTGTGCACCCCGGGTTGCGTGACCGGCGTTCTG
ATGGGTTGCAACAACAAAACCGCGACCTGCAACTGCAGCGTGCACGTTGGCTAA

>RL17

ATGAGTACAAAAGATTTTAACTTGGATTTGGTATCTGTTTCGAAGAAAGATTTCAGGTGC
ATCACCACGCATCACCCAGTTCAAGAGCATTAGCCTGTGCACCCCGGGTTGCCCGACC
GGTATCCTGATGGGTTGCCATAAGTGCCCGAGCGGTAGCGACACCGTGTACACCAAAT

AA

>RL18

ATGAGTACAAAAGATTTTAACTTGGATTTGGTATCTGTTTCGAAGAAAGATTTCAGGTGC
ATCACCACGCATCACAGCCCGCAGATTACCAGCGTGAGCCTGTGCACCCCGGGTTGC
CAGACCGGCTTCTGGCGTGCTTTAGCCAAGCGTGCAACCCGACCGGTGGCTGCAAG
ATCAGCAAATAA

>NisZ

ATGAGTACAAAAGATTTTAACTTGGATTTGGTATCTGTTTCGAAGAAAGATTTCAGGTGC
ATCACCACGCAGTATTTTCGCTATGTACACCCGGTTGTAAAACAGGAGCTCTGATGGGTT
GTAACATGAAAACAGCAACTTGTAATTGTAGTATTACGTAAGCAAATAA

> Bagelicin

ATGAGTACAAAAGATTTTAACTTGGATTTGGTATCTGTTTCGAAGAAAGATTTCAGGTGC
ATCACCACGCGTGACCAGCATCAGCCTGTGCACCCCGGGTTGCAAGACCGGCATCCTG

ATGACCTGCGCGATTAAAACCGCGACCTGCGGTTGCCACTTCTAA

>M4

ATGAGTACAAAAGATTTTAACTTGGATTTGGTATCTGTTTCGAAGAAAGATTTCAGGTGC
ATCACCACGCATTACAAGTAAGTCGCTATGTACAACCGGTTGTCTGACAGGAGCGCTG
ATGGGTTGTAACATGAAAACAGCGACTTGTAATTGTGATATTCACGTAAGCAAATAA

>M5

ATGAGTACAAAAGATTTTAACTTGGATTTGGTATCTGTTTCGAAGAAAGATTTCAGGTGC
ATCACCACGCATTACAAGTCGGTCGCTATGTACACCCGGTTGTTGGACAGGACCTCTGA
TGGGTTGTAACATGAAAACAAAGACTTGTAATTGTGATTCACGTAAGCAAATAA

>S29A

ATGAGTACAAAAGATTTTAACTTGGATTTGGTATCTGTTTCGAAGAAAGATTTCAGGTGC
ATCACCACGCATCACCAGCATTAGCCTGTGCACCCCGGTTGCAAGACCGGTGCGCTG
ATGGGTTGCAACATGAAAACCGCGACCTGCCACTGCGCGATCCACGTGAGCAAAGTAA

Note: leader peptide sequence is gray marked, core peptide sequence is underlined.

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