

A Novel β -Hairpin Peptide Z-d14CFR Enhances Multidrug-Resistant Bacterial

Clearance in a Murine Model of Mastitis

Table S1 Information of bacterial strains used in this study

Bacteria	Genotype	Source
<i>Escherichia coli</i> ATCC ¹ 25922		Purchased from China Institute of Veterinary Drug Center
<i>Escherichia coli</i> CVCC ² 1450		Purchased from China Institute of Veterinary Drug Center
<i>Escherichia coli</i> CAU 201919	(<i>tet(A)</i> , <i>bla</i> CTX-M-65, <i>aac(3)</i> -IIId, <i>aph(3'')</i> -Ib, <i>aph(3')</i> -IIa, <i>aph(6)</i> -Id, <i>formA</i> , <i>mdf(A)</i> , <i>sul2</i>)	Isolated from unpasteurized milk
<i>Escherichia coli</i> CAU 201920	(<i>tet(A)</i> , <i>aph(3'')</i> -Ib, <i>aph(6)</i> -Id, <i>mdf(A)</i> , <i>bla</i> TEM-1, <i>sul2</i>)	Isolated from unpasteurized milk
<i>Salmonella typhimurium</i> ATCC14028		Purchased from China Institute of Veterinary Drug Center
<i>Klebsiella pneumoniae</i> CAU202084	(<i>tet(A)</i> , <i>oqxAB</i> , <i>bla</i> CTX-M-3, <i>fosA5</i>)	Isolated from unpasteurized milk
<i>Proteus vulgaris</i> CVCC1971		Purchased from China Institute of Veterinary Drug Center
<i>Staphylococcus aureus</i> ATCC29213		Purchased from China Institute of Veterinary Drug Center
<i>Staphylococcus haemolyticus</i> CAU202078	(<i>tet(M)</i> , <i>drf(A)</i> , <i>ermC</i> , <i>blaZ</i>)	Isolated from unpasteurized milk
<i>Bacillus cereus</i> CAU 202020	(<i>tet(M)</i> , <i>bla</i> TEM-1, <i>sul2</i>)	Isolated from unpasteurized milk
MRSA ³ ATCC33591		Purchased from China Institute of Veterinary Drug Center
<i>Streptococcus suis</i> CVCC3307		Purchased from China Institute of Veterinary Drug Center

¹ American Type Culture Collection; ² China Veterinary Culture Collection Center. ³ Methicillin-resistant *S. aureus*.

Table S2 Primers used for Real-Time PCR

Gene	Accession NO.	Prime sequence (5'-3')
β -actin	NM_007393.5	f- GCTCTTTTCCAGCCTTCCTT

IL1 β NM_008361.4
 TNF- α XM_006504297.5

r- GATGTCAACGTCACACTT
 f- TGCCACCTTTTGACAGTGATG
 r- AAGGTCCACGGGAAAGACAC
 f- ACGGCATGGATCTCAAAGAC
 r-AGATAGCAAATCGGCTGACG

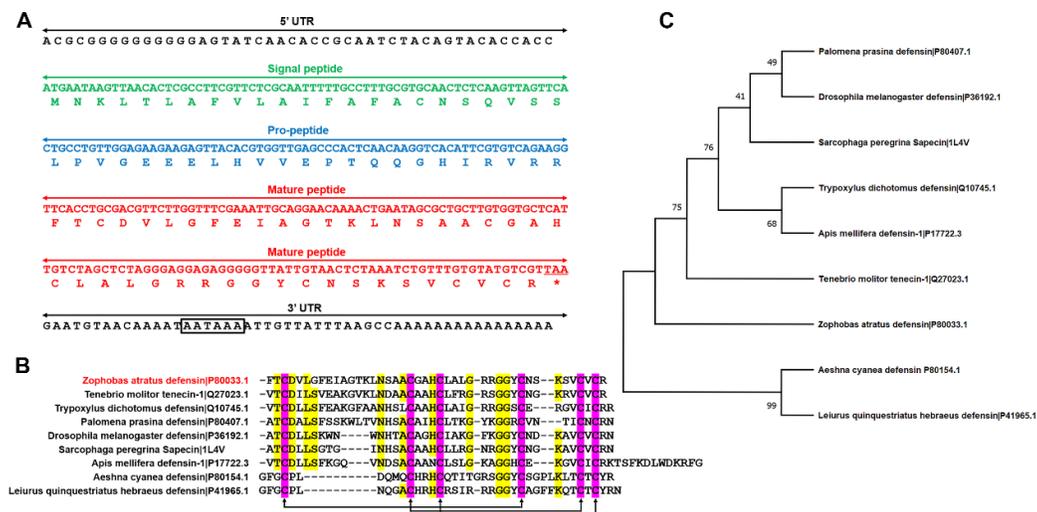


Figure S1 Amplification and analysis of *Z. atratus defensin*. (A) Analysis of *Z. atratus defensin* transcript, the 5' UTR and 3' UTR are marked with black, the putative polyA additional signal at 3' UTR (AATAAA) is boxed. The signal peptide, pro-peptide and mature peptide of *Z. atratus defensin* and their corresponding nucleotide sequence are respectively marked with green, blue and red. (B) Multiple sequence alignment of *Zophobas atratus* (*Z. atratus*) defensin. *Z. atratus defensin* (ZA-defensin) has six conserved cysteine residues like other insect defensins, and forms three disulfide bonds between Cys1-Cys4, Cys2-Cys5, Cys3-Cys6 (cysteines are shadowed in yellow and other identical residues are shadowed in grey). (C) A neighbor-joining distance tree

constructs from the alignment of defensin sequences presented in (B), numbers on

interior branches represent bootstrap values

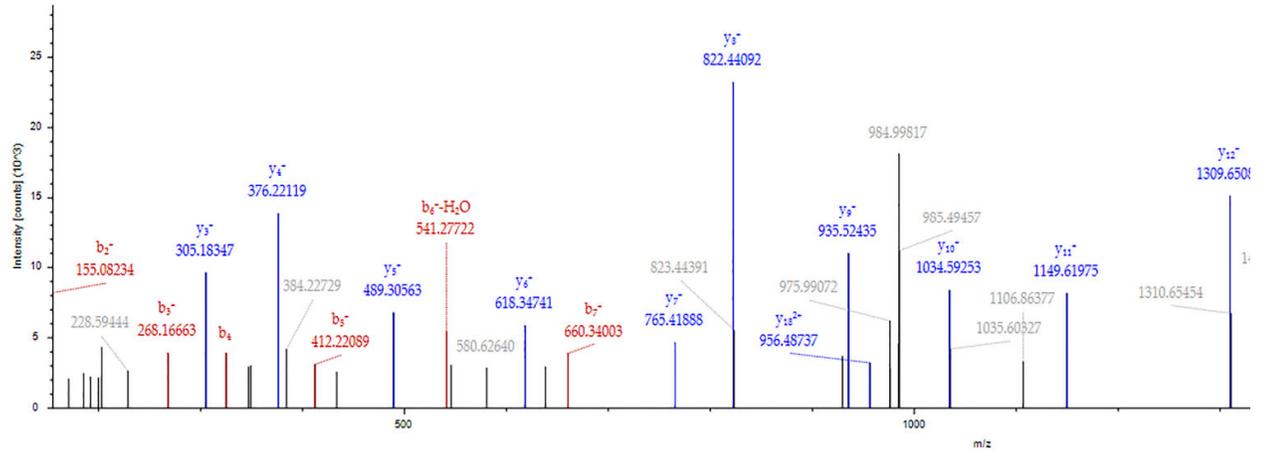
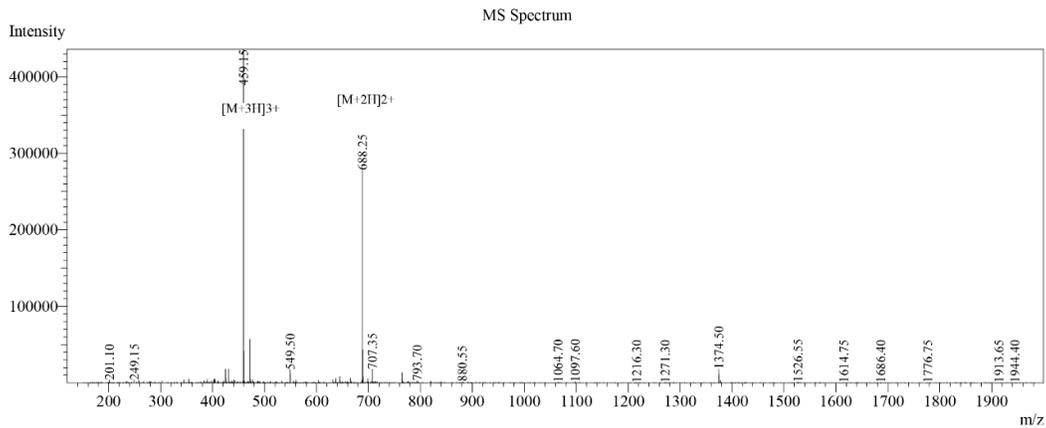
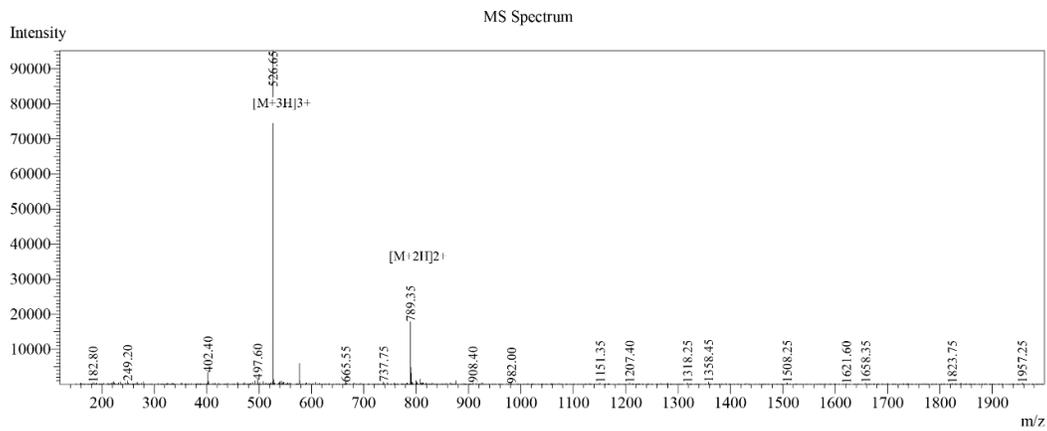


Figure S2 Mass spectrometry of recombinant *Z. atratus* defensin.

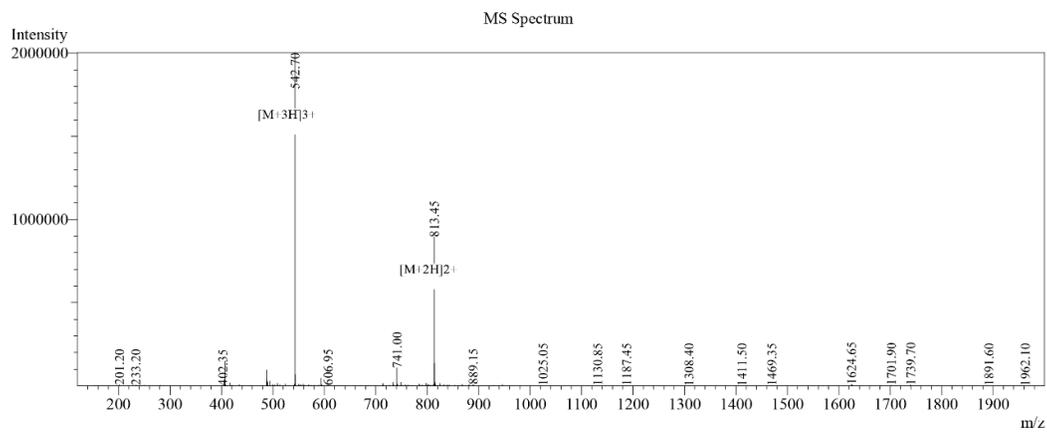
Z-d13



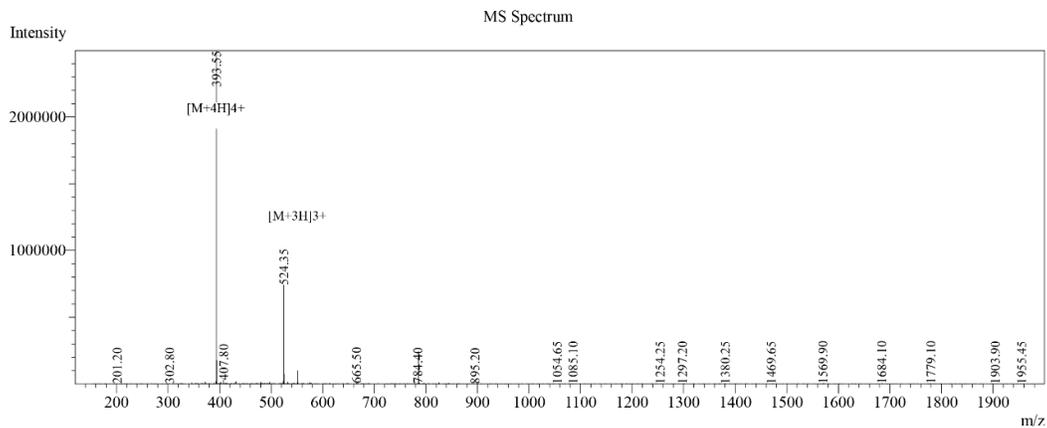
Z-d114C



Z-d114CF



Z-d114CR



Z-d114CFR

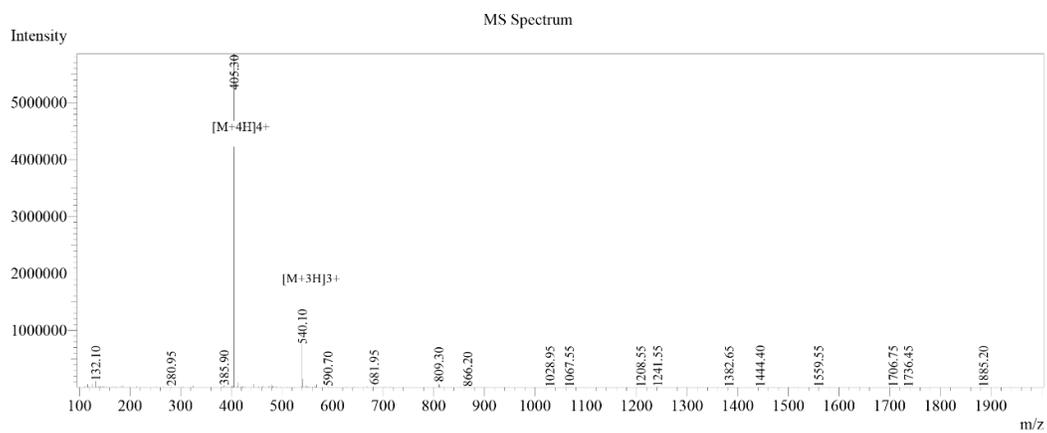


Figure S3 Mass spectrometry of the synthesized peptides.