

Internalization, distribution, and activity of peptide H2 against the intracellular multidrug-resistant bovine mastitis-causing bacterium *Staphylococcus aureus*

Xiao Wang^{1,2*}, Da Teng^{1,2*}, Xiumin Wang^{1,2}, Ya Hao^{1,2}, Huixian Chen^{1,2}, Ruoyu Mao^{1,2**} & Jianhua Wang^{1,2**}

¹ Key Laboratory of Feed Biotechnology, Ministry of Agriculture, Beijing 100081, People's Republic of China. ² Gene Engineering Laboratory, Feed Research Institute, Chinese Academy of Agricultural Sciences, Beijing 100081, People's Republic of China. * These authors contributed equally. ** Correspondence and requests for materials should be addressed to R.Y.M. (maoruoyu@caas.cn) and J.H.W. (email: 2681298635@qq.com)

SUPPORTING INFORMATION

Supplementary 1: Materials and Methods

Antimicrobial susceptibility testing of *S. aureus*. Antimicrobial susceptibility testing of *S. aureus* (subclinical isolate *S. aureus* CVCC3051 and E48) was performed by Kirby Bauer disc diffusion method according to the standards procedures recommended by Clinical Laboratory Standards Institute (CLSI 2012)^{1,2}. The antibiotics used in this study were same with our previous research³.

SCCmec and *spa* typing of *S. aureus*. The bacteria DNA was extracted and then used as a template in a 50-μl PCR. The SCCmec and x region of the *spa* gene was amplified by PCR with the same primers as Wang³. The x region of the *spa* genes was sequenced by Sangon Biotech (Shanghai, China) and the types were determined with the database accessible via <http://spa.ridom.de/spatypes.shtml>.

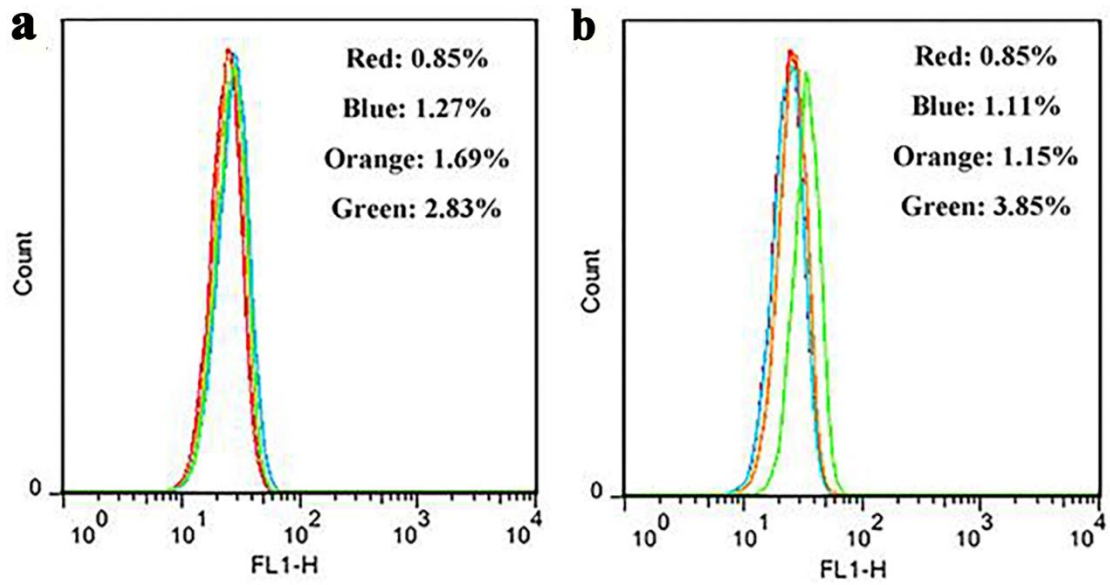
FITC-labeled peptides. The FITC-labeled peptides were carried out in ChinaPeptides Co., Ltd. (Shanghai, China) as previously described⁴.

References

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34 **Supplementary 2: Figure**



36 **Fig. S1**

37 **Figure 1** Effect of H2 and NZ2114 on the cell membrane of MAC-T cells. Cells were incubated with
38 different concentrations of H2 (**a**) and NZ2114 (**b**) for 24 h prior to staining with 5 µg/ml PI at 37 °C for
39 10 min. PI fluorescence in MAC-T cells was measured by flow cytometry. Red line: control; blue line:
40 2.5 µg/ml peptides; orange line: 25 µg/ml peptides; green line: 250 µg/ml peptides.

Supplementary 3: Tables

Table 1 Antimicrobial susceptibility patterns of *S. aureus* CVCC3051 and E48

Antibiotics	<i>S. aureus</i> CVCC3051	<i>S. aureus</i> E48
Vancomycin	S	S
Lincomycin	I	I
(AMX/20) Amoxicillin	S	S
Ciprofloxacin	S	I
Amikacin	S	S
Ampicillin	S	R
Oxacillin	S	S
Erythrocine	I	I
Tetracycline	S	S
Bacitracin	R	R
Norfloxacin	S	S
Sulfisoxazole	R	R
Neomycin	I	I
Azithromycin	S	S
Kanamycin	I	I
Streptomycin	R	R
Cefotaxime	S	I
Gentamicin	S	S
Chloramphenicol	S	S
Cefazolin	S	S
Penicillin	S	S

R: resistant; I: Intermediate; S: susceptible.

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Table 2 Sources, types and susceptibilities of *S. aureus* CVCC3051 and E48

<i>S. aureus</i>	Source	SCCmec type	Spa type	Antibiotic resistance profile	Biosafety level
<i>S. aureus</i> CVCC3051	Clinical isolate	-	t3297	Bacitracin, sulfisoxazole streptomycin	III
<i>S. aureus</i> E48	Clinical isolate	-	t3583	Ampicillin, bacitracin sulfisoxazole, streptomycin	N

65 - : negative. N: unknown

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67 **Table 3** Effect of cathepsin B on the MICs of H2, NZ2114 and vancomycin toward three different *S.*

68 *aureus* strains

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Drugs	MICs (µg/ml) AMP			MICs (µg/ml) AMP + cathepsin		
	ATCC43300	CVCC3051	E48	ATCC43300	CVCC3051	E48
H2	0.5	0.5	1	0.5	0.5	1
NZ2114	0.25	0.5	1	0.25	0.5	1
Vancomycin	1	1	1	1	1	1

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