

1 **Applied Microbiology and Biotechnology**
2 **Supplementary Material**

3 **Therapeutic potential of a designed CS α peptide ID13 in *Staphylococcus aureus*-induced endometritis of mice**

4 **Bing Li^{1,2*} · Na Yang^{1,2*} · Yuxue Shan^{1,2,3*} · Xiumin Wang^{1,2} · Ya Hao^{1,2} · Ruoyu Mao^{1,2**} · Da Teng^{1,2**} · Huan**
5 **Fan³ · Jianhua Wang^{1,2**}**

6 ¹ Gene Engineering Lab, Feed Research Institute, Chinese Academy of Agricultural Science, Beijing 100081, P. R. China

7 ² Key Laboratory of Feed Biotechnology, Ministry of Agriculture and Rural Affairs, Beijing 100081, P. R. China

8 ³ Tianjin Animal Science and Veterinary Research Institute, Tianjin 300381, P. R. China

9 * These three authors Bing Li, Na Yang and Yuxue Shan contributed equally into this paper.

10 ** Correspondence should be addressed to:

11 (A) Outstanding Prof., Ph.D., PI. and Director Jianhua Wang:

12 Team of Alternatives to Antibiotics, Gene Engineering Laboratory, Feed Research Institute, Chinese Academy of
13 Agricultural Sciences, Beijing 100081, People's Republic of China

14 E-mail address: wangjianhua@caas.cn; 2681298635@qq.com

15 Phone: 0086-10-82106081

16 (B) Professor Ph.D., Da Teng, and her email address: tengda@caas.cn

17 (C) A/C professor Ph.D., Ruoyu Mao, and his email address: maoruoyu@caas.cn

18

19 **Supplementary Figures**



Blank control

Negative control

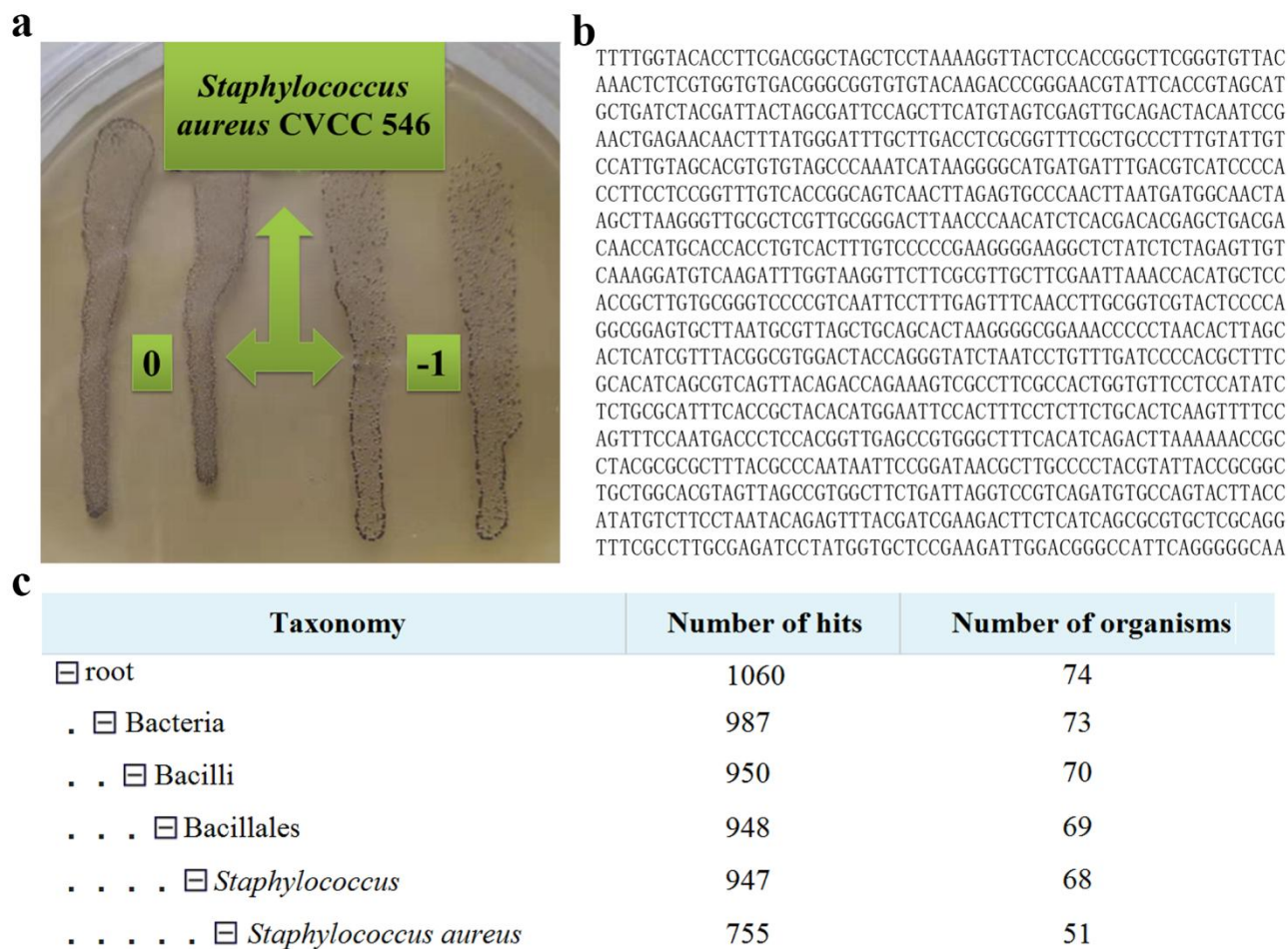
20

21 **Fig. S1** Photos of uteri morphology changes in mice between the blank and negative control.

22

23

24



25

26 **Fig. S2** Pathogens isolation and identification. (a) Homogenate of uterus (0) and 10 times dilutions (-1) were dropped on
 27 BP agar supplemented with potassium tellurite egg-yolk reagent for *S. aureus* CVCC 546. (b) 16S rRNA gene sequence
 28 of *S. aureus* CVCC 546. (c) The taxonomy report of *S. aureus* CVCC 546 via 16S rRNA gene sequence BLAST on
 29 NCBI (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>).

30

31 **Supplementary Tables**32 **Table S1.** Selected DEGs of ID13 treated-*S. aureus* CVCC546 related to membrane potential.

Gene name	Gene product	log2.fold change	qvalue	Protein type
<i>mnhA</i>	Na(+)/H(+) antiporter subunit A1	-1.6	7.3E-194	
<i>mnhB</i>	Na(+)/H(+) antiporter subunit B1	-1.5	2.2E-13	
<i>mnhC</i>	Na(+)/H(+) antiporter subunit C1	-1.6	2.3E-10	
<i>mnhD</i>	Na(+)/H(+) antiporter subunit D1	-1.5	1.3E-100	
<i>mnhE</i>	Na(+)/H(+) antiporter subunit E1	-1.1	5.3E-16	
<i>mnhF</i>	Na(+)/H(+) antiporter subunit F1	-1.8	9.2E-04	
<i>fabD</i>	Malonyl CoA-acyl carrier protein transacylase	-1.0	6.2E-40	
<i>ebh</i>	Extracellular matrix-binding protein ebh	2.2	1.3E-03	
<i>mtlA</i>	PTS system mannitol-specific EIICB component	1.8	8.0E-04	ion binding and transport
<i>sdrC</i>	Serine-aspartate repeat-containing protein C	3.6	1.2E-03	
<i>sdrD</i>	Serine-aspartate repeat-containing protein D	6.2	3.7E-21	
<i>sdrE</i>	Serine-aspartate repeat-containing protein E	2.5	5.8E-11	
<i>copA</i>	Copper-exporting P-type ATPase A	1.4	2.2E-19	
<i>hutG</i>	Formimidoylglutamase	2.1	5.7E-34	
<i>zinT</i>	Zinc-binding lipoprotein	1.7	6.0E-05	
<i>lytM</i>	Glycyl-glycine endopeptidase	-3.2	6.2E-72	
<i>ureF</i>	Urease accessory protein UreF	-1.3	2.9E-03	
<i>ipdC</i>	Indole-3-pyruvate decarboxylase	-1.4	0.0E+00	
<i>nreB</i>	Oxygen sensor histidine kinase NreB	1.7	3.8E-04	
<i>ftnA</i>	Bacterial non-heme ferritin	1.3	7.5E-11	
<i>narG</i>	Nitrate reductase alpha chain	6.2	1.2E-87	
<i>narH</i>	Nitrate reductase beta chain	6.0	2.5E-21	
<i>narT</i>	Probable nitrate transporter NarT	7.8	1.2E-94	
<i>nasD</i>	Nitrite reductase [NAD(P)H]	5.2	5.8E-46	
<i>pflA</i>	Pyruvate formate-lyase-activating enzyme	4.2	5.5E-209	
<i>pflB</i>	Formate acetyltransferase	4.1	0.0E+00	
<i>trxA</i>	Thioredoxin	-1.3	0.0E+00	energy metabolism
<i>trxB</i>	Thioredoxin reductase	-1.5	2.4E-12	
<i>glpD</i>	Aerobic glycerol-3-phosphate dehydrogenase	-3.6	0.0E+00	
<i>AdhI</i>	Alcohol dehydrogenase	5.6	2.1E-196	
<i>ldhA</i>	L-lactate dehydrogenase	3.3	8.4E-125	
<i>ldhB</i>	L-lactate dehydrogenase	2.0	3.1E-11	
<i>natA</i>	ABC transporter ATP-binding protein	3.9	3.2E-121	
<i>fda</i>	Fructose-bisphosphate aldolase class 1	-1.6	0.0E+00	
<i>pgk</i>	Phosphoglycerate kinase	-2.3	0.0E+00	
<i>eno</i>	Enolase	-1.4	0.0E+00	

33

34

35 **Table S2.** Selected DEGs of ID13 treated-*S. aureus* CVCC546 related to infections.

Gene name	Gene product	log2.fold change	qvalue	Protein type
<i>clfB</i>	Clumping factor B	2.1	1.5E-12	
<i>fnbA</i>	Fibronectin-binding protein A	-1.9	3.9E-03	
<i>eno</i>	Enolase	-1.4	0.0E+00	
<i>spa</i>	Immunoglobulin G-binding protein A	5.8	0.0E+00	
<i>agrA</i>	Accessory gene regulator protein A	2.2	5.1E-208	
<i>agrB</i>	Accessory gene regulator protein B	2.7	0.0E+00	
<i>agrC</i>	Accessory gene regulator protein C	1.8	1.1E-119	
<i>sdrC</i>	Serine-aspartate repeat-containing protein C	3.6	1.2E-03	Colonization factors and toxins
<i>sdrD</i>	Serine-aspartate repeat-containing protein D	6.2	3.7E-21	
<i>sdrE</i>	Serine-aspartate repeat-containing protein E	2.5	5.8E-11	
<i>ebh</i>	Extracellular matrix-binding protein ebh	2.2	1.3E-03	
<i>sarA</i>	Transcriptional regulator SarA	1.2	5.2E-32	
<i>sarS</i>	HTH-type transcriptional regulator SarS	3.2	2.2E-73	
<i>sarV</i>	HTH-type transcriptional regulator SarV	2.9	1.1E-36	
<i>sar1418</i>	TelA-like protein SAR1418	-1.4	6.2E-105	
<i>sar0193</i>	PTS system EIIBC component SAR0193	2.9	2.4E-47	
<i>secA</i>	Protein translocase subunit SecA	1.3	4.5E-07	
<i>hly</i>	Alpha-hemolysin	4.7	0.0E+00	
<i>cidA</i>	Holin-like protein CidA	-4.2	6.2E-10	
<i>cidB</i>	Holin-like protein CidB	2.7	6.1E-167	
<i>lrgB</i>	Antiholin-like protein LrgB	2.5	1.5E-06	
<i>lytR</i>	Transcriptional regulator LytR	-1.3	4.0E-193	
<i>lytM</i>	Glycyl-glycine endopeptidase LytM	-3.2	6.2E-72	
<i>capA</i>	Capsular polysaccharide synthesis protein cap5A	-2.8	5.4E-63	Autolysis and biofilm-related proteins
<i>capB</i>	Putative tyrosine-protein kinase CapB	-3.5	1.1E-20	
<i>capC</i>	Putative tyrosine-protein phosphatase CapC	-3.5	4.1E-38	
<i>capD</i>	UDP-glucose 4-epimerase	-2.6	6.7E-28	
<i>capG</i>	UDP-N-acetylglucosamine 2-epimerase	-3.5	3.2E-50	
<i>capL</i>	Probable glycosyltransferase	-3.6	2.5E-47	
<i>capM</i>	Uncharacterized sugar transferase	-2.3	5.2E-14	
<i>capN</i>	UDP-glucose 4-epimerase	-2.9	5.3E-28	

36

37

38 **Table S3.** Changes of different white blood cells in mice between negative and blank control (n=5).

Group	WBC^a (10⁹ cell/L)	NEU^b (10⁹ cell/L)	LYM^c (10⁹ cell/L)	NEUT%	LYM%
BC^d	5.14±1.22	2.24±0.34	2.03±0.40	47.80±5.13	39.73±1.75
NC^e	14.56±3.00**	9.65±2.00***	3.25±0.83*	66.24±1.51***	22.24±2.33****

39 ^aWBC: white blood cells; ^bNEU: neutrophils; ^cLYM: lymphocytes; ^d BC: blank control; ^eNC: negative control, *p<0.05,

40 **p<0.01, ***p<0.001, ****p<0.0001.

41