Received: 16 May 2019 | Revised: 20 June 2019 | Accepted: 30 June 2019

DOI: 10.1002/mbo3.910

ORIGINAL ARTICLE

Novel peptides screened by phage display peptide library can mimic epitopes of the FnBPA-A protein and induce protective immunity against *Staphylococcus aureus* in mice

Jin-Nian Li | Hong Wang | Yu-Xi Han | Yu-Ting Zhao | Huan-Huan Zhou | Jun Xu | Lin Li

Anhui Province Key Laboratory of Veterinary Pathobiology and Disease Control, College of Animal Science and Technology, Anhui Agricultural University, Hefei, China

Correspondence
Lin Li, Anhui Province Key Laboratory of Veterinary Pathobiology and Disease Control, College of Animal Science and Technology, Anhui Agricultural University, Hefei 230036, China.
Email: lilinah@126.com

Funding information
National Natural Science Foundation of China, Grant/Award Number: 31672698, 31772802

Abstract

Fibronectin-binding protein A (FnBPA) is a key adhesin of *Staphylococcus aureus*, and the protein binding to fibrinogen and elastin is mediated by its N-terminal A domain. Thus, FnBPA-A has been considered a potential vaccine candidate, but the relevant epitopes are not fully understood. Here, purified rabbit anti-FnBPA-A antibodies were produced and used to screen for peptides corresponding to or mimicking the epitope of native FnBPA-A protein by using a phage random 12-mer peptide library. After four rounds of panning, 25 randomly selected phage clones were detected by phage-ELISA and competition-inhibition ELISA. Then, eight anti-rFnBPA-A antibody-binding phage clones were selected for sequencing, and six different 12-mer peptides were displayed by these phages. Although these displayed peptides shared no more than three consecutive amino acid residues identical to the sequence of FnBPA-A, they could be recognized by the FnBPA-A-specific antibodies in vitro and could induce specific antibodies against FnBPA-A in vivo, suggesting that these displayed peptides were mimotopes of FnBPA-A. Finally, the protective efficiencies of these mimotopes were investigated by mouse vaccination and challenge experiments. Compared with that of control group mice, the relative percent survival of mice immunized with phage clones displaying a mimotope was 13.33% (C2 or C15), 0% (C8), 6.67% (C10), 26.67% (C19 or 1:2 mixture of C23 and C19), 53.33% (C23), 33.33% (1:1 mixture of C23 and C19), and 66.67% (2:1 mixture of C23 and C19). Overall, five peptides mimicking FnBPA-A protein epitopes were obtained, and a partially protective immunity against *S. aureus* infection could be stimulated by these mimotope peptides in mice.

KEYWORDS
fibronectin-binding protein A, immunoprotection, mimotope, phage display technology, *Staphylococcus aureus*

Jin-Nian Li and Hong Wang contributed equally to this work.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.
© 2019 The Authors. *MicrobiologyOpen* published by John Wiley & Sons Ltd.

1 | INTRODUCTION

Staphylococcus aureus (*S. aureus*) is a major zoonosis pathogen that causes various infections in humans and mastitis in cows. At present, even though antibiotics are still used to treat *S. aureus*-induced cow mastitis, multiantibiotic resistance is a significant problem (Uhlemann, Otto, Lowy, & DeLeo, 2014). Therefore, development of a vaccine, such as an epitope-based vaccine, to control *S. aureus*-induced cow mastitis is urgently need (Broughan, Anderson, & Anderson, 2011; Proctor, 2012).

Attachment and colonization of *S. aureus* to the mammary epithelial cell surface via adhesins is the key step that initiates mastitis (Gong et al., 2010). Currently, several proteins have been proven to be adhesins of *S. aureus*, including fibronectin-binding protein A and B (FnBPA/B), cell-bound clumping factor A (ClfA), collagen-binding protein (Cnbp) and protein A (Foster, Geoghegan, Ganesh, & Hook, 2014). Among these adhesins, the surface protein FnBPA is ubiquitous in clinical isolates of *S. aureus*, which not only mediates the binding of *S. aureus* to elastin and fibrinogen but also is a relatively conserved protective antigen. Previous studies have ascertained that the FnBPA protein consists of mainly four domains (A, B, C, and D), and binding of the protein to fibrinogen and elastin is mediated by its N-terminal A domain, which includes three subdomains of N1, N2, and N3 (Brouillette, Talbot, & Malouin, 2003; Keane, Clarke, Foster, & Weiss, 2007; Piroth et al., 2008). Therefore, the FnBPA-A protein is a potential vaccine candidate, but relevant epitopes are not fully clear.

Phage display technology, also known as selection technology in vitro, is a biotechnology that combines peptides or proteins with the coat protein of a bacteriophage to display on the surface of the bacteriophage (Wu, Liu, Lu, & Wu, 2016). One of the most promising applications of phage display technology is to pan random peptide libraries (RPLs) against a specified target for the identification of linear epitopes or mimotopes that can effectively mimic the epitope structures present in antigen (Ahmad, Eweida, & Sheweita, 2016; Liu et al., 2015).

In this paper, the mimotopes of FnBPA-A proteins were identified through RPLs screening with the FnBPA-A-specific polyclonal antibodies. Then their immunogenicity and immunoprotection were investigated in vivo. Our findings would be conducive to the development of epitope-based vaccines against *S. aureus*-induced cow mastitis and comprehending the antigenic structure of the protein.

2 | MATERIALS AND METHODS

2.1 | Bacterial strains, plasmids, phage peptide libraries and experimental animals

Escherichia coli BL21 (DE3), *S. aureus* strain WWGSP-30 isolated from diseased cows with mastitis, and the pET-32a vector were all stored in our laboratory. The Ph.D.-12™ phage display peptide library kit was purchased from New England BioLabs, which contains the *E. coli* host ER2738 and _96gIII sequencing primers required for the assay. New Zealand white rabbits (weighing 2 kg) and ICR mice (weighing 18–22 g) were purchased from Experimental Animal Center of Anhui Medical University.

2.2 | Expression and purification of recombinant FnBPA-A

The gene encoding of the FnBPA-A protein was amplified from the genomic DNA of *S. aureus* strain WWGSP-30 by PCR using specific primers (F: 5′-CGCGGATCCGTGAAAAACAATCTTAGGTACGGC-3′,R:5′-CCGCTCGAGTTAAGCTGTGTGGTAATCAATGTCAAG-3′, underlined for *BamH* I and *Xho* I restriction sites). Then, the PCR products were cloned into the *BamH* I and *Xho* I sites of the pET-32a(+) vector to construct the recombinant plasmid pET-32a-FnBPA-A. The recombinant plasmid was verified by enzyme digestion and sequencing and then transformed into *E. coli* strain BL21 (DE3) competence cells.

The recombinant plasmid pET-32a-FnBPA-A and the control plasmid pET-32a were induced with 0.3 mmol/L isopropyl-β-D-thiogalactopyranoside (IPTG, Sigma) for 5.5 hr at 30°C. The soluble recombinant FnBPA-A protein (rFnBPA-A) was collected and purified with nickel-nitrilotriacetic acid (Ni-NTA) resin affinity chromatography (Qiagen) according to the manufacturer's instructions. The purity, concentration, and immunoreactivity of the purified protein were analyzed by 13% SDS-PAGE, BCA Protein Assay Kit (Kang Wei, China) and western blot, respectively.

2.3 | Production and purification of polyclonal antibodies against rFnBPA-A

New Zealand white rabbits were immunized via multiple subcutaneous injections with 0.5 mg of purified rFnBPA-A protein emulsified with an equal volume of Freund's complete adjuvant (Sigma), followed by boosts with the same dose at 2-week intervals. On the 28th day after primary immunization, the cardiac blood from immunized rabbits was collected, and the immune serum was isolated from coagulated blood.

Anti-FnBPA-A antibodies in the immune serum were purified using a HiTrap Protein G HP Column (Pharmacia, Sweden) according to the manufacturer's instructions. The purity and concentration of the purified antibodies were determined by 12% SDS-PAGE and BCA Protein Assay Kit, respectively. The titer of the purified antibodies was detected by indirect ELISA. Briefly, the purified rFnBPA-A protein (20 μg/well) was coated onto ELISA plates overnight at 4°C. The plates were washed with PBST (PBS plus 0.05% Tween-20) and blocked with 5% nonfat milk for 2 hr at 37°C. Then, the plates were incubated with serially diluted immune serum for 2 hr at 37°C. After washing, the plates were incubated with a 1:5,000 dilution of HRP-conjugated goat anti-rabbit IgG (Novagen), and 3,3′,5,5′-tetramethylbenzidine (TMB) was used for color development. The reaction was terminated with 2 mol/L H_2SO_4, and the

OD_{450} of each well was measured using a microplate reader (Model 450; Bio-Rad Laboratories). Endpoint titers were expressed as the highest dilution that yielded an $OD_{450} \geq 2.1$ times the mean value of the control serum (normal rabbit serum).

2.4 | Screening a random phage-displayed 12-peptide library with anti-rFnBPA-A antibodies

To obtain phages binding to anti-rFnBPA-A antibodies, a random Ph.D.-12^{TM} phage display peptide library (New England Biolabs) was screened with purified anti-FnBPA-A antibodies according to the manufacturer's instructions. For each round of biopanning, phages (1.5×10^{12} PFU/mL diluted with pure normal rabbit IgG) were applied to a 96-well plate precoated with anti-rFnBPA-A antibodies (10 μg/well). Twenty-five individual phage clones were randomly picked from the fourth round of biopanning, and preliminarily identified by phage-ELISA. Briefly, purified anti-rFnBPA-A antibodies or normal rabbit serum (negative control) were added to 96-well plates (10 μg/well) overnight at 4°C. Unbound antibodies were removed, and the wells were blocked with 5% nonfat milk for 2 hr at 37°C. Selected phage clones were added to plates (1×10^9 PFU/well) and incubated for 2 hr at 37°C. After washing with PBST, a 1:5,000 diluted anti-phage M13 monoclonal antibody (Pharmacia) was added to the plates and incubated for 1 hr at 37°C, and the remaining steps were the same as in section 2.4. If the ratio of test well OD_{450}/control well $OD_{450} \geq 2.1$, the phage clones were preliminarily regarded as positive clones that were recognized by anti-FnBPA-A antibodies.

Positive phage clones identified in the phage-ELISA were further explored for their specific binding to purified anti-FnBPA-A antibodies by a competition-inhibition ELISA. Briefly, the different concentrations of purified rFnBPA-A competitor (12.5, 25, 50, 100 and 200 μg/ml) were diluted by an equal volume of phage solution (2×10^9 PFU/well). The mixture or the pure phage solution (non-inhibition control) was added to antibody-coated plates for 1 hr at 37°C, respectively. As mentioned above, mouse anti- HRP-labeled M13 monoclonal antibody was used to detect the titer of bound bacteriophage. The OD_{450} was measured, and the inhibition ratio was calculated according to the following formula: inhibition ratio = (OD_{450} value unsuppressed − OD_{450} value after suppression)/OD_{450} value unsuppressed × 100%. An inhibition percentage equal to or greater than 50% was considered anti-rFnBPA-A antibody-binding phage according to the literature (Li, Han, Li, & Lei, 2009). Each assay was carried out in triplicate.

2.5 | DNA sequencing and homology analysis

Antibody-binding phage clones identified by the competition-inhibition ELISA were amplified. Then, the single-stranded phage DNAs were extracted with a Single-stranded DNA Extraction Kit according to the manufacturer's instructions, and sequenced using the _96 gIII primer. The 12-mer peptides displayed on the antibody-binding phage clones were deduced from their nucleotide sequences and aligned with the FnBPA-A sequences, which were accessed on NCBI (acession No: WP_049307669.1, CAO77276.1, AFJ20680), using the MEGALIGN program in DNASTAR to determine the relationships among the peptides.

2.6 | Detection of the reactivity of the identified mimotopes with anti-rFnBPA-A antibodies

To verify whether the identified mimotopes could be recognized by anti-rFnBPA-A antibodies, the identified mimotope peptides and an irrelevant control peptide (MPKLNRCAIAIL) were synthesized (Shanghai Amoy Cape Technology Co., Ltd) in vitro and used as the coated antigen (30 μg/ml) for peptide-ELISA, in which a 1:500 dilution of purified anti-rFnBPA-A antibodies was used as preliminary antibodies, and commercial rabbit anti-His tag antibodies (Novagen) and nonimmunized rabbit serum were included as controls, respectively. The test procedure was the same as that for the indirect ELISA (section 2.4). The OD_{450} of the negative control (normal rabbit serum) was supposed to be 2.1 times higher than that of the anti-His tag antibodies, indicating the absence of unspecific reaction. On this premise, if the ratio of the OD_{450} of the FnBPA-A antibodies to that of the negative control ≥ 2.1, the synthetic mimotope peptide was thought to be specifically recognized by anti-FnBPA-A antibodies. Each assay was performed in triplicate.

2.7 | Mouse immunization and challenge

To explore the immunogenicity and immunoprotection of the identified mimotopes, six purified positive phage clones displaying mimotope peptide were used to immunize ICR mice (SPF, weighing 18–22 g). The mice were randomly divided into 12 groups (n = 20 in each group). Groups 1–6 were injected intraperitoneally with 1×10^{12} PFU of different positive phage clones per mouse; groups 7–9 were given 1×10^{12} PFU of the mixture of C23 and C19 at the number ratios of 1:1, 1:2 and 2:1; group 10 was immunized with 30 μg of the rFnBPA-A protein emulsified with 50 μl of Freund's adjuvant per mouse; and groups 11–12 were inoculated with the wild type M13 phage at a dose of 1×10^{12} PFU or 0.2 ml of PBS, respectively, for controls. Two booster immunizations were carried out on days 14 and 28.

On the 35th day after primary immunization, sera were collected from 5 randomly selected mice in each group, and then the titers of antibodies against FnBPA-A were detected by indirect ELISA (vide supra). The remaining 15 mice per group were challenged intraperitoneally with 0.2 ml of *S. aureus* strain WWGSP-30 that was resuspended in PBS to 2.2×10^7 CFU/ml (equal to 10 LD_{50} determined in a pre-experiment). Mortality was recorded at daily for 7 days after the challenge, and dying mice were sampled for bacterial recovery from different tissues. Relative percent survival (RPS) was calculated according to the following formula: $1 - \frac{\%\text{ mortality of immunized mice}}{\%\text{ mortality of control mice}} \times 100$ (Amend, 1981).

FIGURE 1 (a) SDS-PAGE analysis of rFnBPA-A before and after purification. lane M, protein molecular weight standard; lane1, supernatant of the cell lysate of pET-32a-FnBPA-A/BL21 after induction; lane2, uninduced whole bacterial cell lysate of the pET-32a-FnBPA-A/BL21; lane 3, induced whole bacterial cell lysate of the pET-32a/BL21; lane 4, uninduced whole bacterial cell lysate of the pET-32a/BL21; lane5, purified rFnBPA-A. (b) Western blotting analysis of purified rFnBPA-A. lane M, prestained protein molecular weight standard; lane1, purified rFnBPA-A; lane 2, Trx-His-tag

2.8 | Statistical analysis

The data were presented in the form of means and standard deviations. The differences in antibody titers in different groups were analyzed by one-way analysis of variance (ANOVA) followed by the Duncan method. The differences in relative percent survival (RPS) of immunized mice in different groups were determined with the log-rank test. In all cases, the differences were considered significant at a p value < .05 and extremely significant at a p value < .01.

3 | RESULTS

3.1 | Expression and characterization of the recombinant FnBPA-A protein

As shown in Figure 1a, the rFnBPA-A protein (approximately 81 kDa) was expressed in the supernatant of recombinant *E. coli* BL21 (DE3) after IPTG induction and purified by a His-band purification kit. Western blotting analysis revealed that the purified rFnBPA-A protein could react with rabbit anti-*S. aureus* strain WWGSP-30 antibodies (prepared by our laboratory), whereas the Trx-His-tag control could not (Figure 1b). These results showed that rFnBPA-A was expressed correctly and that the purified protein might be used as an immunogen for immunization.

3.2 | Purity, concentration and titer of purified anti-rFnBPA-A antibodies

On the 28th day after immunization, immune sera were separated and purified by using a HiTrap Protein G HP Column. The purified antibodies (IgG) showed 95% purity and two protein bands by SDS-PAGE gel (Figure 2), and the sizes of the two protein bands were approximately 55 kDa and 25 kDa, which is consistent with the theoretical molecular mass of the heavy and light chains of rabbit IgG reduced by β-mercaptoethanol. The concentration of purified antibodies was approximately 2.5 mg/ml, as detected by the BCA Protein Assay Kit, and the titer of the anti-FnBPA-A antibodies was more than 1:8,388,600, as detected

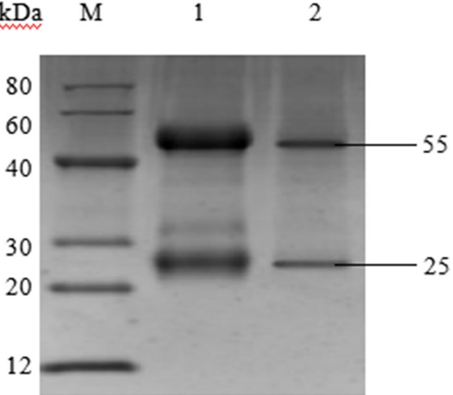

FIGURE 2 SDS-PAGE analysis for the purified rabbit anti-rFnBPA-A polyclonal antibodies. lane M, protein molecular weight standard; lane 1, rabbit immune serum; lane 2, purified IgG of rabbit anti-rFnBPA-A antibodies

by indirect ELISA. These results indicated that the antibodies could be used to screen phage peptide libraries.

3.3 | Identification of anti-rFnBPA-A antibody-binding phage clones and sequence alignment

Twenty-five phage clones were randomly picked and preliminarily identified by phage-ELISA. Then, 16 positive clones identified by phage-ELISA were further confirmed by competition-inhibition ELISA. As shown in Figure 3, rFnBPA-A could competitively inhibit phage binding to rFnBPA-A antibodies in a concentration-dependent manner. When the concentration of rFnBPA-A competitor was 200 μg/ml, its inhibition rate on eight phage clones (C2, C8, C10, C11, C15, C19, C21, and C23) was greater than 50%, suggesting that these eight clones were positively recognized by anti-rFnBPA-A antibodies according to the literature (Liu et al., 2009). The DNA sequences and deduced amino acid sequences of eight positive clones are shown in Table 1. There were six different peptides displayed by the eight positive phage clones. The clones C8,

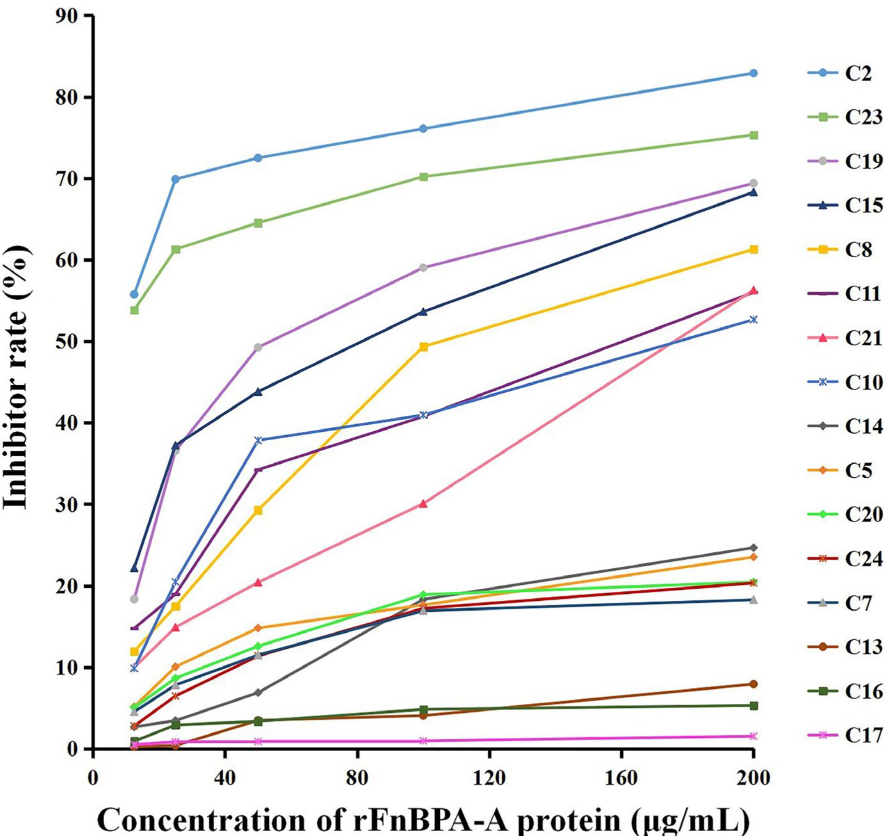

FIGURE 3 Competitive inhibition of primary positive phage clones binding to coated anti-rFnBPA-A antibodies in the presence of the purified rFnBPA-A competitor. The average inhibition rates (means ± *SE*s, *n* = 3) are shown under different concentrations of rFnBPA-A. The binding of clone 2, 8, 10, 11, 15, 19, 21, and 23 to coated antibodies was inhibited by rFnBPA-A in a concentration-dependent manner

C11, and C21 shared the same displayed peptide, and the D-GFPG motif was shared by the clones C8, C10, C11, and C21. Alignment revealed that six different displayed peptides shared less than three continuous amino acid residues identical to the available sequences of FnBPA-A registered in GenBank, indicating that these peptides were likely the mimotopes of the FnBPA-A protein.

3.4 | Reactivity of the identified mimotopes with anti-rFnBPA-A antibodies

As shown in Figure 4, all six mimotope peptides synthesized in vitro could react with anti-FnBPA-A antibodies, whereas they did not react with anti-His tag monoclonal antibodies or negative serum. A nonspecific reaction was excluded because the irrelevant control peptide did not react with anti-FnBPA-A antibodies or anti-His tag monoclonal antibodies. These results indicated that these mimotopes could be specifically recognized by anti-FnBPA-A antibodies.

3.5 | Development of anti-FnBPA-A antibodies and protective immunity elicited by displayed mimotope phage clones

As shown in Figure 5, the titers of serum antibodies against rFnBPA-A in each immunization group at 35 days postvaccination were 1:2,560 (C8), 1:5,120 (C10), 1:10,240 (C2 or C15), 1:81,920 (C23), 1:20,480 (C19 or 1:2 mixed of C19 and C23), 1:40,960 (1:1 mixed of C19 and C23), 1:163,840 (2:1 mixed of C19 and C23), and 1:327,680 (rFnBPA-A mixed with adjuvant), while no FnBPA-A-specific antibodies were detected in the M13 phage and PBS control groups, indicating that all mimotope peptides possessed immunogenicity at different degrees.

After challenge, the pathological changes of dead mice were seroperitoneum, splenic necrosis, and hemorrhage in the eye, nose, claw, and lung. No pathogens other than *S. aureus* were isolated from dead mice. The RPS of mice immunized with different displayed mimotope phage clones or rFnBPA-A protein was 0% (C8), 6.67% (C10), 13.33% (C2, C15), 26.67% (C19, 1:2 mixture of C23 and C19), 53.33% (C23), 33.33% (1:1 mixture of C23 and C19), 66.67% (2:1 mixture of C23 and C19), and 73.33% (rFnBPA-A), compared with that of the control group mice (Figure 6). The results of statistical analysis (log-rank test) showed that the RPS of immunized mice was significantly higher in both the C19 and C23 groups than in the remaining four single phage clones groups ($p < .05$), but there was no significant difference between the C19 and C23 groups ($p > .05$). Interestingly, the RPS of mice was significantly higher in the group immunized with the mixture of C23 and C19 at the number ratio of 2:1 than in the single clone C19 or C23 groups ($p < .05$), but there was no significant difference between the rFnBPA-A protein group and the 2:1 ratio mixed biphage clones group ($p > .05$). These results indicated that, except for the mimotope peptide displayed on C8, the other five mimotope peptides could induce a partially protective immunity against *S. aureus* challenge.

TABLE 1 The amino acid and nucleotide sequences of the 12-mer peptides displayed on the positive phage clones

Positive phage clones	Nucleotide sequence	Amino acid sequence
C2	GGTTTGCATACTTCGGCTACTAATCTGTATTTGCAT	SLHTGATNLYLH
C8,C11,C21	GGGTATTTTGATGTGGTGTTGGGGGGTTTTGGTCCG	GYF**D**VVLG**GFGP**
C10	TTTATTCGTCCTAATGATTGGGGGTTTGGTCCGTGG	FIRPN**D**W**GFGP**W
C15	CATGTTTTGAATTCTACTGTTTGGAATACGCGTATT	HVLNSTVWNTRI
C19	CATACGGAGCAGGGGACTTTGTTTTTGAAGATGCCG	HSAQASITIKMA
C23	AGTTATTTTGATGCGCTTGAGAGGATGTTGCCGGGG	SYFDALERMLPG

Note: The consensus motif of D-GFPG displayed on the clones 8, 10, 11, and 21 is shown in bold and underlined.

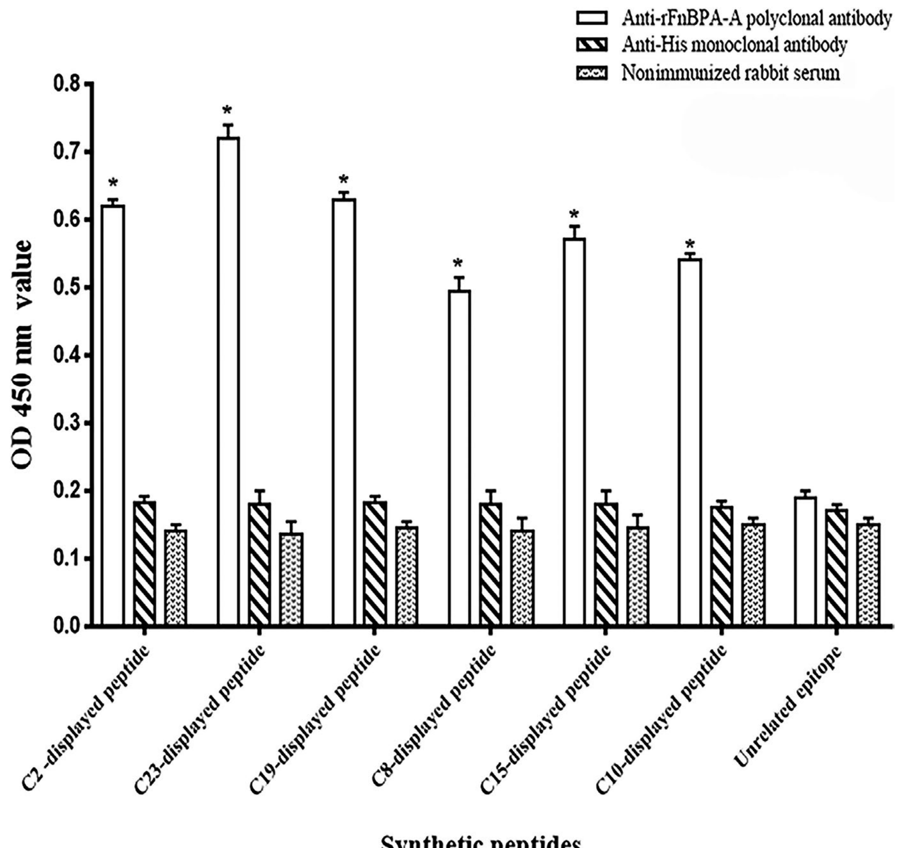

FIGURE 4 Peptide-ELISA analysis for the reactivity of identified mimotope peptides with anti-rFnBPA-A antibodies. Six identified mimotope peptides and one unrelated peptide were synthesized in vitro used to coat ELISA plates. Rabbit anti-rFnBPA-A antibodies were used as preliminary antibodies, whereas commercial rabbit anti-His tag antibodies and nonimmunized rabbit serum were included as controls. Each column represents the mean OD_{450} with a standard deviation bar (n = 3). *p < .05 indicates significant differences between the rFnBPA-A antibodies group and the two control groups

4 | DISCUSSION

As is known, epitopes are a set of residues on the surface of an antigen and can be readily recognized by either specific B/T-cell receptors or particular antibody molecules. The potential advantages of the epitope-based vaccine include mainly safety, intense immune responses, and the breadth of rationally engineered epitopes (Zhang, Zhang, Li, Liu, & Li, 2014). Therefore, epitope identification of protective antigens from pathogens has important scientific significance and practical value. The important role of FnBPA adhesin in *S. aureus* pathogenesis has made it a potentially interesting vaccine target. The N-terminal A domain of FnBPA is consistent with the A region of other staphylococcal bacteria "microbial surface components recognizing adhesive matrix molecules" (MSCramms), which may bind to fibrinogen and elastin (Keane, Loughman, et al., 2007; Wann, Gurusiddappa, & Höök, M., 2000; Zuo et al., 2014), whereas its C-terminal B-DuC-D domains were responsible for binding to fibronectin (Ulrich et al., 2003). Considering that, the epitope of FnBPA-D was clear (Casolini et al., 1998) and there have been few studies on the epitopes of FnBPA-A, we decided to identify the B-cell epitopes of FnBPA-A protein.

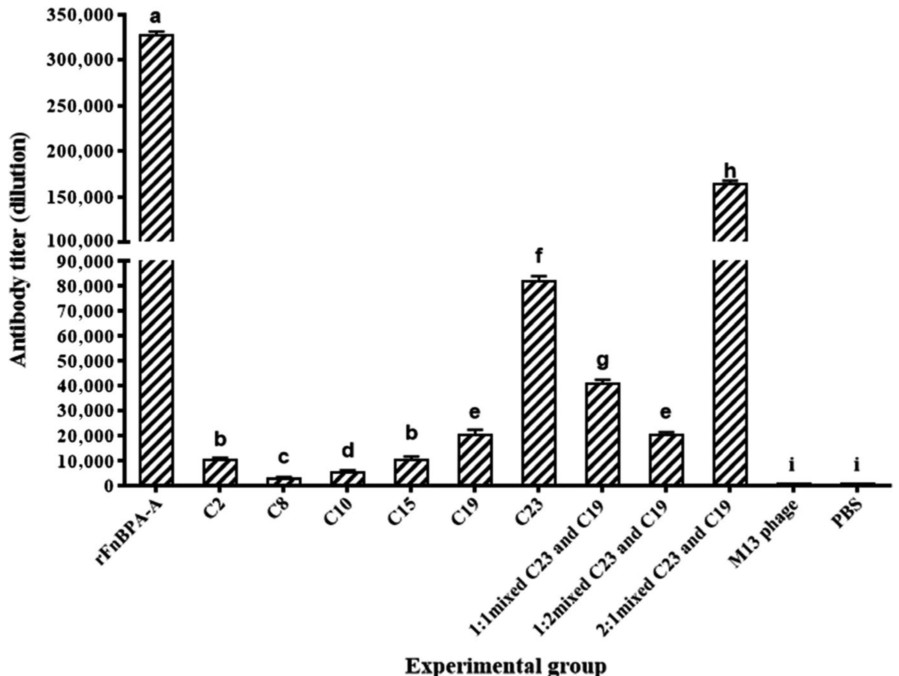

FIGURE 5 Indirect ELISA detection of serum anti-rFnBPA-A antibody titers in mice vaccinated with rFnBPA-A protein and different positive phage clones displaying mimotope. Negative control mice were injected with PBS or M13 phage. Each datum column represents the mean of antibody titer with a standard deviation bar (n = 5). The lowercase letters indicate a significant difference ($p < .05$)

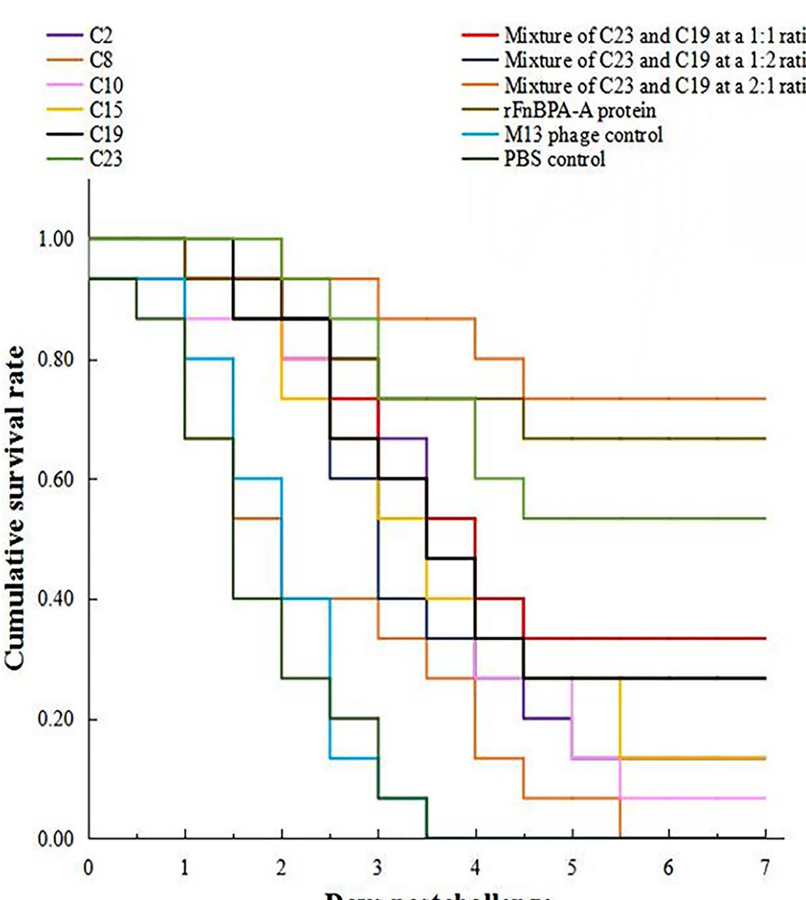

FIGURE 6 Cumulative survival rate of immunized mice when challenged with 10 LD_{50} of *S. aureus* strain WWGSP-30 on the 35th day postvaccination

Several methods have been used to map antigenic epitope, including chemical synthesis (Geysen, Rodda, & Mason, 1986), pepscan (Estepa & Coll, 1996), X-ray crystallography (Rux & Burnett, 2000), nuclear magnetic resonance spectroscopy (Mayer & Meyer, 2001), bioinformatics predictions (Greenbaum et al., 2007), and phage display technology. The phage display technology is simpler, faster, and more convenient than other methods for mapping epitopes (Leili et al., 2016). Zuo et al., (2014) reported that the region covering 110 to 263 amino acid residues was the immunodominant region of FnBPA protein. Ma et al. (2018) defined a B-cell linear epitope ($N2N3_{159\text{-}171}$) of the FnBPA-A protein by selecting a random phage-displayed 12-mer peptide library using a McAb against the N2N3 subdomain of FnBPA-A. In the current study, rabbit anti-FnBPA-A antibodies were used to a screen 12-mer peptide library. Sixteen of 25 phage clones picked randomly from the fourth round were preliminarily positive in phage-ELISA analysis. However, there were many interfering factors in the phage-ELISA, which can produce nonspecific signals. Additionally, competitive inhibition ELISA analyses further confirmed these suspect clones, and 8 of the suspect clones could be positively recognized by anti-rFnBPA-A antibodies. It was notable that six different 12-mer peptides displayed on the eight positive clones shared no more than three continuous amino acid residues identical to the sequence of FnBPA-A protein, but these peptides could be recognized by FnBPA-A-specific antibodies in vitro and could induce antibodies against FnBPA-A in immunized mice, which suggested that these peptides were mimotopes that mimicked conformational epitopes of the FnBPA-A protein.

The epitopes screened by the phage random 12-peptide library are usually mimotopes for the following reasons. First, B-cell epitopes can be divided into linear epitopes and conformational epitopes based on their spatial structure. A linear epitope consists of consecutive residues, while a conformational epitope consists of nonconsecutive fragments that are close in spatial proximity when the corresponding antigen is folded. More than 90% of B-cell epitopes have been shown to be conformational epitopes. Therefore, the screening probability of mimotopes from random peptide libraries is much higher than that of linear epitopes. Second, the peptide segments with low content may be lost during the amplification of the original peptide library, resulting in a low possibility that the obtained displayed peptide is consistent with the amino acid sequences of the natural antigen in the primary structure.

The immunization and challenge tests indicated that all six phage clones displaying mimotopes without additional adjuvant could induce FnBPA-A-specific antibodies, and except for the C8-displayed mimotope, the remaining five mimotopes could provide partially protective immunity against challenge infection. Previous studies have shown that filamentous phages themselves possess adjuvant effects, thus enhancing the immunogenicity and immunoprotection of mimotopes (Grabowska et al., 2000; Guo et al., 2010; Wang et al., 2005). Our results were also in line with this view. Interestingly, significant protective immunity was observed following immunization with the mixture of C23 and C19 at the ratio of 2:1 compared to that with the single clone. We speculated that the clones mixed in an appropriate proportion might cover more target sites and mimic epitopes to the greatest extent from different perspectives, thus inducing increased immunoprotection.

In human medicine, *S. aureus* mutliantigen vaccines are currently in clinical trials. Although these vaccines produced strong humoral immunity and have proven effective in preclinical models, they did not prevent or reduce infection in clinical trials (Fowler & Proctor, 2014; Proctor, 2012). This is somewhat unsurprising, as *S. aureus* can generate over 50 virulence factors to acclimatize itself to multiple host niches and enable diverse infections (Lacey, Geoghegan, & Mcloughlin, 2016). Meanwhile, the humoral immune responses are thought to be important in preventing *S. aureus*, but antibody responses seem to be insufficient to clear bacterial colonization and impair inflammatory damage. In addition, there is growing evidence that T cells have an important role in the protective immunity against *S. aureus*. For example, Joshi et al. (2012) suggested that in mice, IL-17A producing Th17 cells played an essential role in IsdB vaccine-mediated defense against invasive *S. aureus* infection. Narita, Asano, and Nakane (2017) reported that ClfA or FnBPA could induce IL-17A-mediated cellular immunity, which was associated with a protective effect against *S. aureus* infection. Brown et al. (2015) found that *S. aureus*-specific Th1 cells can protect host from *S. aureus* infection. Zhang et al. (2018) demonstrated that during $FnBPA_{110\text{–}263}$ vaccination against *S. aureus* sepsis and skin infection in mice, IL-17A from Th17 cells played a critical role. Therefore, future studies are needed to identify B- and T-cell epitopes or mimotopes of multiple antigens from *S. aureus* and optimize the combination presentation of these epitopes or mimotopes to develop an efficient vaccine against *S. aureus*.

In conclusion, six mimotopes of the FnBPA-A protein were identified through screening phage-displayed random peptide libraries with rabbit anti-FnBPA-A antibodies in the present work. Except for the C8-displayed mimotope, the remaining five mimotopes could induce a partially protective immunity against *S. aureus* challenge. Two mimotope peptides displayed by the C19 and C23, as vaccine candidates, should be studied further.

ACKNOWLEDGMENTS

This work was supported by the grants from the China Natural Science Foundation, Project No.31672698, 31772802.

CONFLICT OF INTERESTS

The authors have no conflicts of interest.

AUTHOR CONTRIBUTIONS

Jin-nian Li performed the main experiments and analyzed the data. Hong Wang performed some experiments and wrote the manuscript. Huan-huan Zhou performed some experiments and analyzed the data. Yu-xi Han and Yu-ting Zhao participated in the

animal treatment and sample collection. Lin Li conceived and designed the study.

ETHICS STATEMENT

All animal experiments were performed strictly according to the Guide for the Care and Use of Laboratory Animals of the national laboratory animal welfare ethics guidelines, and protocols concerning animals were approved by the Ethical Committee of the Faculty of Veterinary Science of Anhui Agricultural University (permit number: 20170312). We reduced the number of animals used to the maximum extent and reduced animal suffering as much as possible.

DATA AVAILABILITY STATEMENT

All data are provided in full in the results section of this paper and available from the corresponding author on reasonable request.

ORCID

Jin-Nian Li https://orcid.org/0000-0003-1634-6359
Lin Li https://orcid.org/0000-0003-4421-7984

REFERENCES

Aghebati-Maleki, L., Bakhshinejad, B., Baradaran, B., Motallebnezhad, M., Aghebati-Maleki, A., Nickho, H., ... Majidi, J. (2016). Phage display as a promising approach for vaccine development. *Journal of Biomedical Science*, *23*, 66–83. https://doi.org/10.1186/s12929-016-0285-9

Ahmad, T. A., Eweida, A. E., & Sheweita, S. A. (2016). B-cell epitope mapping for the design of vaccines and effective diagnostics. *Trials in Vaccinology*, *5*, 71–83. https://doi.org/10.1016/j.trivac.2016.04.003

Amend, D. (1981). Potency testing of fish vaccines international symposium on fish biologics: Serodiagnostics and vaccines. *Developments in Biological Standardization*, *49*, 447–454.

Broughan, J., Anderson, R., & Anderson, A. S. (2011). Strategies for and advances in the development of *Staphylococcus aureus* prophylactic vaccines. *Expert Review of Vaccines*, *10*, 695–708.

Brouillette, E., Talbot, B. G., & Malouin, F. (2003). The fibronectin-binding proteins of *Staphylococcus aureus* may promote mammary gland colonization in a lactating mouse model of mastitis. *Infection and Immunity*, *71*, 2292–2295. https://doi.org/10.1128/IAI.71.4.2292-2295.2003

Brown, A. F., Murphy, A. G., Lalor, S. J., Leech, J. M., O'Keeffe, K. M., Mac Aogáin, M., ... McLoughlin, R. M. (2015). Memory Th1 cells are protective in invasive *Staphylococcus aureus* infection. *PLoS Path*, *11*, e1005226. https://doi.org/10.1371/journal.ppat.1005226

Casolini, F., Visai, L., Joh, D., Conaldi, P. G., Toniolo, A., Hook, M., & Speziale, P. (1998). Antibody response to fibronectin-binding adhesin FnbpA in patients with *Staphylococcus aureus* infections. *Infection and Immunity*, *66*, 5433–5442.

Estepa, A., & Coll, J. M. (1996). Pepscan mapping and fusion-related properties of the major phosphatidylserine-binding domain of the glycoprotein of viral hemorrhagic septicemia virus, a salmonid rhabdovirus. *Virology*, *216*, 60–70. https://doi.org/10.1006/viro.1996.0034

Foster, T. J., Geoghegan, J. A., Ganesh, V. K., & Hook, M. (2014). Adhesion, invasion and evasion: The many functions of the surface proteins of *Staphylococcus aureus*. *Nature Reviews Microbiology*, *12*, 49–62. https://doi.org/10.1038/nrmicro3161

Fowler, V. G., & Proctor, R. A. (2014). Where does a *Staphylococcus aureus* vaccine stand? *Clinical Microbiology and Infection*, *20*, 66–75. https://doi.org/10.1111/1469-0691.12570

Geysen, H. M., Rodda, S. J., & Mason, T. J. (1986). A priori delineation of a peptide which mimics a discontinuous antigenic determinant. *Molecular Immunology*, *23*, 709–715. https://doi.org/10.1016/0161-5890(86)90081-7

Gong, R., Hu, C., Xu, H., Guo, A., Chen, H., Zhang, G., & Shi, L. (2010). Evaluation of clumping factor A binding region A in a subunit vaccine against *Staphylococcus aureus*-induced mastitis in mice. *Clinical and Vaccine Immunology*, *17*, 1746–1752. https://doi.org/10.1128/CVI.00162-10

Grabowska, A. M., Jennings, R., Laing, P., Darsley, M., Jameson, C. L., Swift, L., & Irving, W. L. (2000). Immunisation with phage displaying peptides representing single epitopes of the glycoprotein G can give rise to partial protective immunity to HSV-2. *Virology*, *269*, 47–53. https://doi.org/10.1006/viro.2000.0185

Greenbaum, J. A., Andersen, P. H., Blythe, M., Bui, H.-H., Cachau, R. E., Crowe, J., ... Peters, B. (2007). Towards a consensus on datasets and evaluation metrics for developing B-cell epitope prediction tools. *Journal of Molecular Recognition*, *20*, 75–82. https://doi.org/10.1002/jmr.815

Guo, A., Cai, X., Jia, W., Liu, B., Zhang, S., Wang, P., ... Luo, X. (2010). Mapping of *Taenia solium* TSOL18 antigenic epitopes by phage display library. *Parasitology Research*, *106*, 1151–1157. https://doi.org/10.1007/s00436-010-1786-1

Joshi, A., Pancari, G., Cope, L., Bowman, E. P., Cua, D., Proctor, R. A., & Mcneely, T. (2012). Immunization with *Staphylococcus aureus* iron regulated surface determinant B (IsdB) confers protection via Th17/IL17 pathway in a murine sepsis model. *Human Vaccines and Immunotherapeutics*, *8*, 336–346.

Keane, F. M., Clarke, A. W., Foster, T. J., & Weiss, A. S. (2007). The N-terminal A domain of *Staphylococcus aureus* fibronectin-binding protein A binds to tropoelastin. *Biochemistry*, *46*, 7226–7232.

Keane, F. M., Loughman, A., Valtulina, V., Brennan, M., Speziale, P., & Foster, T. J. (2007). Fibrinogen and elastin bind to the same region within the A domain of fibronectin binding protein A, an MSCRAMM of *Staphylococcus aureus*. *Molecular Microbiology*, *63*, 711–723. https://doi.org/10.1111/j.1365-2958.2006.05552.x

Lacey, K. A., Geoghegan, J. A., & Mcloughlin, R. M. (2016). The role of *Staphylococcus aureus* virulence factors in skin infection and their potential as vaccine antigens. *Pathogens*, *5*, 22–39. https://doi.org/10.3390/pathogens5010022

Li, Y., Han, W. Y., Li, Z. J., & Lei, L. C. (2009). *Klebsiella pneumoniae* MrkD adhesinmediated immunity to respiratory infection and mapping the antigenic epitopeby phage display library. *Microbial Pathogenesis*, *46*, 144–149. https://doi.org/10.1016/j.micpath.2008.11.006

Liu, G. W., Livesay, B. R., Kacherovsky, N. A., Cieslewicz, M., Lutz, E., Waalkes, A., ... Pun, S. H. (2015). Efficient identification of murine M2 macrophage peptide targeting ligands by phage display and next-generation sequencing. *Bioconjugate Chemistry*, *26*, 1811–1817. https://doi.org/10.1021/acs.bioconjchem.5b00344

Ma, J., Wei, Y., Zhang, L., Wang, X., Yao, D. I., Liu, D., ... Cui, Y. (2018). Identification of a novel linear B-cell epitope as a vaccine candidate in the N2N3 subdomain of *Staphylococcus aureus* fibronectin-binding protein A. *Journal of Medical Microbiology*, *67*, 423–431. https://doi.org/10.1099/jmm.0.000633

Mayer, M., & Meyer, B. (2001). Group epitope mapping by saturation transfer difference NMR to identify segments of a ligand indirect contact with a protein receptor. *Journal of the American Chemical Society*, *123*(25), 6108–6117. https://doi.org/10.1021/ja0100120

Narita, K., Asano, K., & Nakane, A. (2017). IL-17A plays an important role in protection induced by vaccination with fibronectin-binding domain of fibronectin-binding protein A against *Staphylococcus aureus*

infection. *Medical Microbiology and Immunology*, *206*, 225–234. https://doi.org/10.1007/s00430-017-0499-9

Piroth, L., Que, Y. A., Widmer, E., Panchaud, A., Piu, S., Entenza, J. M., & Moreillon, P. (2008). The fibrinogen-and fibronectin-binding domains of *Staphylococcus aureus* fibronectin-binding protein A synergistically promote endothelial invasion and experimental endocarditis. *Infection and Immunity*, *76*, 3824–3831. https://doi.org/10.1128/IAI.00405-08

Proctor, R. A. (2012). Challenges for a universal *Staphylococcus aureus* vaccine. *Clinical Infection Diseases*, *54*(8), 1179–1186. https://doi.org/10.1093/cid/cis033

Proctor, R. A. (2012). Is there a future for a *Staphylococcus aureus* vaccine? *Vaccine*, *30*, 2921–2927. https://doi.org/10.1016/j.vaccine.2011.11.006

Rux, J. J., & Burnett, R. M. (2000). Type-specific epitope locations revealed by X-ray crystallographic study of adenovirus type 5 hexon. *Molecular Therapy*, *1*, 18–30. https://doi.org/10.1006/mthe.1999.0001

Uhlemann, A. C., Otto, M., Lowy, F. D., & DeLeo, F. R. (2014). Evolution of community and healthcare-associated methicillin-resistant Staphylococcus aureus. *Infection Genetics and Evolution*, *21*, 563–574. PMID: 23648426. https://doi.org/10.1016/j.meegid.2013.04.030

Ulrich, S. L., Werner, J. M., Pickford, A. R., Gurusiddappa, S., Kim, J. H., Pilka, E. S., ... Potts, J. R. (2003). Pathogenic bacteria attach to human fibronectin through a tandem beta-zipper. *Nature*, *423*, 177–181.

Wang, M., Yi, X. Y., Li, X. P., Zhou, D. M., Larry, M. R., & Zeng, X. F. (2005). Phage displaying peptides mimic schistosoma antigenic epitopes selected by rat natural antibodies and protective immunity induced by their immunization in mice. *World Journal of Gastroenterology*, *11*, 2960–2966. https://doi.org/10.3748/wjg.v11.i19.2960

Wann, E., Gurusiddappa, S., & Höök, M. (2000). The fibronectin-binding MSRAMM FnbpA of *Staphylococcus aureus* is a bifunctional protein that also binds to fibrinogen. *Journal of Biological Chemistry*, *275*, 13863–13871.

Wu, C. H., Liu, I. J., Lu, R. M., & Wu, H. C. (2016). Advancement and applications of peptide phage display technology in biomedical science. *Journal of Biomedical Science*, *23*, 1–14. https://doi.org/10.1186/s12929-016-0223-x

Zhang, R., Lia, S., Zhang, X. K., Wang, Y., Yang, L. Y., Zeng, H., Zuo, Q. F. (2018). Mechanisms of fibronectin-binding protein A (FnBPA110-263) vaccine efficacy in Staphylococcus aureus sepsis versus skin infection. *Clinical Immunology*, *194*, 1–8.

Zhang, Y. Q., Zhang, T. T., Li, J. N., Liu, X. L., & Li, L. (2014). Design and evaluation of a tandemly arranged outer membrane protein U (OmpU) multi-epitope as a potential vaccine antigen against *Vibrio mimicus* in grass carps (*Ctenopharyngodon idella*). *Veterinary Immunology and Immunopathology*, *160*, 61–69. https://doi.org/10.1016/j.vetimm.2014.03.016

Zuo, Q. F., Cai, C. Z., Ding, H. Y., Wu, Y. I., Yang, L.-Y., Feng, Q., ... Zou, Q.-M. (2014). Identification of the immunodominant regions of *Staphylococcus aureus* fibronectin-binding protein A. *PLoS ONE*, *9*, e95338. https://doi.org/10.1371/journal.pone.0095338

How to cite this article: Li J-N, Wang H, Han Y-X, et al. Novel peptides screened by phage display peptide library can mimic epitopes of the FnBPA-A protein and induce protective immunity against *Staphylococcus aureus* in mice. *MicrobiologyOpen*. 2019;8:e910. https://doi.org/10.1002/mbo3.910