academicJournals

Vol. 7(20), pp. 2316-2323, 14 May, 2013
DOI: 10.5897/AJMR12.2092
ISSN 1996-0808 ©2013 Academic Journals
http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

A study of expression performed for the construction of Staphylococcus aureus adhesins FnBPB/ClfA and immunogenicity of the expressed product

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Accepted 26 April, 2013

In this this study, a cloning plasmid named pMD19-FnBPB-ClfA was constructed, by PCR specific amplifications of genes from region D, fibronectin-binding protein B (FnBPB) and region A in clumping factor A (ClfA), all from Staphylococcus aureus. Splicing by overlap extension using PCR tandem gene FnBPB-ClfA was performed and then gene segments of pMD19-FnBPB-ClfA were inserted into a prokaryotic expression vector named pET-32a (+). They were ultimately transferred into the host strain BL21 (DE3), resulting in the expression plasmid pET-FnBPB-ClfA. SDS-PAGE demonstrated an extrinsic protein belt consistent with the desired protein at 51 kDa, when the expression constructed was induced with 1 mmol/L isopropyl β-D-1-Thiogalactopyranoside (IPTG). Western-blot identification demonstrated consensus between the expressed protein and the endogenous protein. After purification and emulsification with Freund's adjuvant, the expressed protein was used to immunize mice. After three subsequent immunizations in the same mice, we gained a highly effective antiserum. Through ELISA, tube agglutination, phagocytosis of opsonized experiment, and antibodies against S. aureus adhesion ability test, it was demonstrated that the fusion gene was successfully expressed in prokaryotic cells, the expressed protein was adherence active, the prepared immune antiserum was capable of preventing S. aureus from adhering to bovine fibrinogens and the antiserum had functions of phagocytosis and opsonization.

Key words: Staphylococcus aureus, FnBPB, ClfA, splicing by overlap extension polymerase chain reaction (PCR), prokaryotic expression.

INTRODUCTION

Staphylococcus aureus is one of the most common and important pathogenic bacteria that cause mastitis infection, responsible for significant economic loss in cow breeding and dairy industry (Olde Riekerink et al., 2006). It has been demonstrated in multiple studies, that surface-associated adhesins, caspsular polysaccharide (CP), toxins and enzymes are all important factors influencing the pathogenicity of S. aureus (Brouillette et al., 2003). Among them, adhesins, also named microbial surface components recognizing adhesive matrix molecules (MSCRAMMs), consist of specific proteins expressed on the surface of S. aureus, and are able to specifically recognize and attach to components of the extracellular matrix (ECM) of an organism (Patti et al., 1994). In the early phrase of S. aureus infection, they are the most important pathogenic factors (Barkema et al., 2006). Therefore, blocking bacteria from adhering to and colonizing the mucosal surface in this phrase might

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Table 1. Primers sequences, restriction enzymes and respective digest sites and PCR amplification conditions.

Primer	Primer sequence (5'-3')
FnBPB up	CGC GAA TTC GGC CAA AAT AGC GGT AAT CAG TC
FnBPB down	aga acc gcc tcc tcc ATG ACC ACT TAC TTG TGG
ClfA up	gga gga ggc ggt tct GTA GCT GCA GAT GCA CC
ClfA down	CCG CTC GAG TTA CTC ATC AGG TTG TTC AGG

The underlines represent the restrict enzyme sites and the lower letters represent the introduction of four glycine and serine sequence.

be one of the most effective strategies for preventing infection (Zhou et al., 2006).

It is known that S. aureus can express a series of cell wall-associated proteins that are capable of facilitating their clinging to host cells, extracellular matrixes and soluble plasma proteins (Nour El-Din, et al., 2006). These cell wall-associated proteins include clumping factors A and B (ClfA and ClfB), fibronectin-binding proteins A and B (FnBPA and FnBPB), collagen-binding protein (CnBP) and protein A (Nour El-Din et al., 2006). A number of studies have confirmed that ClfA and FnBPs are the most crucial virulence factors for the S. aureus-caused mastitis infection in cows (Hettinqa et al., 2008). The antigenic epitope in FnBPA and FnBPB, which has almost the same ligand combination area in its primary gene structures lies in region D (Joh et al., 1994), while that of ClfA lies in region A (Hettinqa et al., 2008).

Studies have shown that S. aureus expresses different adhesins responsible for mastitis infection in cows from different sources, sometimes FnBPs only or at other times ClfA only. Antibodies against FnBPs and ClfA can only inhibit adherence to mammary tissues of the corresponding adhesins (Jiang and Hao 2009; Shi et al., 2010). In this context, the aim of this study is to clone genes from region D of FnBPB and region A of ClfA, by constructing the expression plasmid through splicing by overlap extension using PCR tandem gene FnBPB-ClfA and immunogenically analyzing the expressed product. This study provided theoretical basis and experimental evidence for developing genetically-engineered subunit vaccine against cow mastitis infection caused by S. aureus.

METHODS AND MATERIALS

Experimental materials

S. aureus, Escherichia coli DH-5α, BL21 (DE3), pET-32a (+)and antisera against FnBPB and ClfA were obtained from the Microbiology Laboratory, Veterinary Science Faculty, Agricultural University of the Inner Mongol. The cloning vector pMD19-T Simple, pfu mix DNA polymerase, dNTP Mix, and restriction enzyme were purchased from TaKaRa Company in Dalian, Liaoning Province. Bacterial genome extraction kits were purchased from Tiangen Biotech (BEIJING, China) CO.LTD. Gel extraction and plasmid kits were purchased from Axygen Company (San Jose, USA). Primers were synthesized by Shanghai Bioengineering Company (Shanghai, China). Goat anti mice IgG marked with alkaline phosphatase was purchased from Promega Beijing Biotech Co. Ltd (Beijing, China).

DNA extraction of S. aureus genome

After collection of bacteria, DNA extraction of S. aureus genome was done with cetyltrimethylammonium bromide (CTAB)-DNA precipitation method (Corinaldesi C, Danovaro R .2005).

Connection of region D in FnBPB and region A in ClfA

By using Linker and PCR overlapping extension, region D in FnBPB and region A in ClfA were connected in the order of FnBPB-Linker-ClfA (Table 1), where lowercase letters represent Linker sequences and underlined sequence signifies restriction enzyme cutting sites.

PCR

PCR amplification of region A in ClfA was carried out, using the extracted S. aureus genomic DNA as a template and ClfA up and ClfA down as primers. The reaction condition (Jiang and Hao 2009) is as follows: Cycling conditions were one cycle of 94°C for 2 min, followed by 30 cycles of 94°C for 1 min, annealing by 70°C for 30 s and extension by 72°C for 30 s, followed by a final extension of 72°C for 8 min. The PCR product was identified by 1% agarose gel electrophoresis (AGE), and the product was extracted using gel extraction kits from Axygen Company (San Jose, USA).

PCR amplification of region D in FnBPB was performed using the extracted S. aureus genomic DNA as a template and FnBPB up and FnBPB down as primers. The reaction conditions are as follows (Shi et al., 2010) : Cycling conditions were one cycle of 94°C for 2 min, followed by 35 cycles of 94°C for 1 min, annealing by 68°C for 30 s and extension by 72°C for 30 s, followed by a final extension of 72°C for 8 min. The PCR product was identified by 1% AGE, and the product was extracted using gel extraction kits from Axygen Company (San Jose, USA).

Splicing by overlap extension PCR (SOE-PCR)

PCR amplification of the FnBPB-ClfA tandem gene was done using the gel extracted products of ClfA and FnBPB as templates and FnBPB up and ClfA down as primers (Anthony et al., 1997). Conditions were: Cycling conditions were one cycle of 94°C for 2 min, followed by 30 cycles of 94°C for 1 min, annealing by 65°C for 45 s, and extension by 72°C for 2 min plus 30 s, followed by a final extension of 72°C for 8 min. The PCR product was identified by 1% AGE, and the product was extracted using gel extraction kits from Axygen Company (San Jose, USA).

Table 2. Immunization groups of laboratory animals.

Group	Antigen	Adjuvant	Animal numbers
A	pClfA	Freund's complete adjuvant / Freund's incomplete adjuvant	10
B	pFnBPB	Freund's complete adjuvant / Freund's incomplete adjuvant	10
C	pClfA+pFnBPB	Freund's complete adjuvant / Freund's incomplete adjuvant	10
D	pClfA-FnBPB	Freund's complete adjuvant / Freund's incomplete adjuvant	10
E	PBS		10

Construction of the recombinant expression vector

The recombinant plasmid was confirmed by sequencing and digested by endonucleases EcoR I and Xho I; and then the target fragments were respectively recovered and ligated by T4 DNA ligase. The ligated products were then transferred into BL21 (DE3) competent cells, and the transformants were identified by double digestion of EcoR I and Xho I. The ones proved to be correct were the prokaryotic expression vector, pET32a-FnBPB-ClfA. After being verified by further double-enzyme cleavage of EcoR I and Xho I, it was delivered to Shanghai Biosynthesis Company (Shanghai, China) for sequencing and comparison of the sequencing results.

Induced expression of the fusion gene

The positive strain BL21 (DE3) was inoculated in 4 ml LB containing 100 µg/ml ampicillian (Amp) medium, the bacterium of pET-32a (+) empty vector not inducted by IPTG as a negative control. After overnight culturing at 37°C and 200 rpm shaking, 100 µl was removed to inoculate a fresh 10 ml LB containing 100 µg/ml Amp culture medium. Culturing at 37°C and 200 rpm shaking were done again until the culture reached an OD600 of 0.6~0.8. IPTG was then added to the final concentration of 1mmol/L. After a further growth, under the same conditions listed above, samples were removed at 3, 4, 5 and 6 h after being inducted by IPTG, and the induction time was confirmed by the brightness of the band through the electrophoretic results.

Culture time points with positive expression induced protein, in contrast to control, where the bacterium of pET-32a (+) empty vector was inducted by IPTG, and the molecular weight of expression protein was approximately 51 kDa bands as the proposed protein bands. The supernatants containing soluble proteins and retained for further purification were analyzed by SDS-PAGE.

Western blot analyses

Purified recombinant protein was electrophoresed, and the best electrophoresis was chosen for transmembrane. The expressed products were electro-transferred to a nitrocellulose filter (NC) membrane using the BIO-RAD system. The membranes were sealed blocked with 5% (W/V) skim milk powder, washed with PBST for 20 min and incubated with the antiserum against FnBPB (SHI DY,et al.2010) and antiserum against ClfA (Jiang and Hao 2009) overnight at 4°C, respectively.

Membranes were washed with PBST (six times for 5 min) and goat anti rabbit IgG labeled with alkaline phosphatase was utilized for secondary antibody. Finally, results were visualized by electrochemiluminescence liquid (ECL) method and pictures were taken.

Animal immunization

Grouping

Female BALB/c mice, aged 6 to 8 weeks, were purchased from Experimental Animal Center of Inner Mongolia University, and evenly divided into five groups of ten mice each (Table 2).

Immunization procedure

For the first immunization, the fusion protein was blended and emulsified at 1:1 in Freund's complete adjuvant. A protein concentration of 1 µg/µl was used to immunize the mice by intramuscular injection in their hind limbs. For the booster immunizations given at a 10 days interval, the fusion protein was blended and emulsified at 1:1 in Freund' s incomplete adjuvant; and also, a protein concentration of 1 µg/µl was used to immunize the mice by intramuscular injection in their hind limbs (Table 3).

Tests on expressed products

ELISA

100 µl protein with a final concentration of 5 µg/ml was used to package the 96-well microtiter plates, and the plates were incubated at 4°C overnight; the next day PBST (PBS with high-temperature and pressure sterilized with 0.05% Tween) was used to wash the plates three times (each well by adding 200 µl), and every wash lasted for 5 min. Then they were then dried. After the last wash, the plates were made to dry with absorbent paper. Every hole of the 96-well microtiter plates was blocked with 200 µl, 5% non-fat dry milk at 37°Cfor 3 h; after washing, the untested sera were added. Sera were added to every hole after titra ratio dilution, they were mixed well and incubated at 37°C for 1.5 h. After washing the secondary antibody was added. To every hole, 100 µl 1:3000 dilution of sheep anti-mouse antibody labeled horseradish peroxidase (HRP) was added and incubated at 37°C for 2 h. Again, after washing, to every hole, 100 µl TMB solution (Tiangen Biotech Co, Beijing, China) was added, a plate reader was placed for 10 min and bright light was avoided. Then 50 µl H_2SO_4 (2M sulfuric acid) was added to every hole to terminate the reaction. The absorbance values were 450 nm

Serum agglutination test (SAT)

S. aureus was regulated at the exponential growth phase until it reached OD600 of 1. The prepared serum was diluted into eight small tubes numbered from 1 to 8 (Table 4). After the dilution, each of the tubes was supplemented with 0.5 ml of the exponential growth phase of S. aureus, and after blending, they were deposited

Table 3. Strategies of mice immunization and blood collection.

Group	Protein vaccine	Injection and/or blood collection				
		Day 0	Day 10	Day 14	Day 21	Day 28
A	pClfA	▵/○	○	▵	▵/○	○
B	pFnBPB	▵/○	○	▵	▵/○	○
C	pClfA+pFnBPB	▵/○	○	▵	▵/○	○
D	pClfA-FnBPB	▵/○	○	▵	▵/○	○
E	PBS	▵/○	○	▵	▵/○	○

Triangles (▵) show immunizations and circles (○) show serum collections.

Table 4. Tube agglutination test of the antibody.

Codes	1	2	3	4	5	6	7	8
The final serum density	1 : 25	1 : 50	1 : 100	1 : 200	1:400	1:800	1:1600	Sa
0.5% Carbolic acid	2.3	0.5	0.5	0.5	0.5	0.5	0.5	0.5
physiological saline (ml)								
self-restraint serum (ml)	0.2	0.5	0.5	0.5	0.5	0.5	0.5	discard 0.5
		discard 0.5						
Sa. (OD600=1)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5

into 37°C constant incubators for 10 h cultivation. The cultures where then placed at room temperature for 14 h for observation (Table 4).

Antibody cytophagic test

S. aureus, at exponential growth phase, was monitored until an OD600 of 1 was achieved, and then a 1:10,000 dilution was made by PBS. Blood was collected from eyeballs of mouse under aseptic conditions. 20 µl diluted S. aureus solution was combined with 200 µl of newly-collected anticoagulant blood from eye of mouse containing sodium citrate, with 200 rpm shaking and incubated at 37°C for 3 h. The solution was evenly coated onto sterile plain agar culture plates with sterile sticks. At the same time, 20 µl diluted S. aureus solution was coated onto another group of plain agar culture plates for a blank control. After inverted cultivation at 37°C for 18 to 24 h, bacterial colonies were counted and a sterilizing rate was calculated according to the formula: sterilizing rate = [1-Sa.the number of blood-incubated bacterial colonies/the number of bacterial colonies in control]×100%. The experiment was to be repeated three times.

T cell proliferation assay

Preparation of T-cells from mouse spleen: Three immunized mice were killed by cervical dislocation and steeped in 75% alcohol for 5 min. Spleens were removed from the bodies under aseptic conditions and put into Petri dishes containing 2 ml PRMI 1640 solution. They were grinded with sterilized syringes and made into cell suspension, which were then filtered into 10 ml centrifuge tubes with 200-eyed sterile copper nets. After centrifugation at 2000 rpm for 10 min, the upper layer of the culture solution was removed and the remaining solution was combined with 2 ml Red Cell Lysis Buffer (RCLB) for 2 min. Following the lysis, PRMI 1640 solution (v:v) was added to stop the reaction. After a centrifugation at 2000 rpm for 10 min, the upper layer of the culture solution was again removed and RPMI 1640 suspension containing 2 ml 10% fetal calf serum (FCS) was added. Cells were then counted under a microscope. Cell density was adjusted to 106/ml for cultivation on a 96-well culture plate.

Testing the living cell numbers with the 3-(4,5)-dimethylthiahiazo (-z-y1)-3,5-di- phenytetrazoliumromide (MTT) method: The following was added to each well of a 96-well culture plate: 0.lml of the cell suspension; ConA (final concentration being 10 µg/ml); fusion

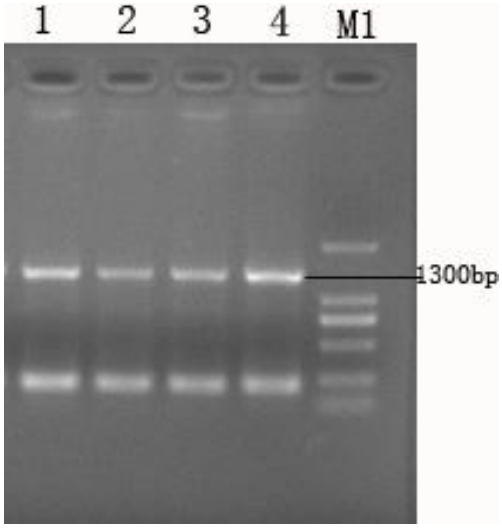

Figure 1. Amplification of the FnBPB-ClfA tandem gene by PCR. M1: DL2000 DNA Marker pET-32a-FnBPB-ClfA (*ECoR I/ Xho* I); 1, 2, 3,4: PCR products of FnBPB-ClfA.

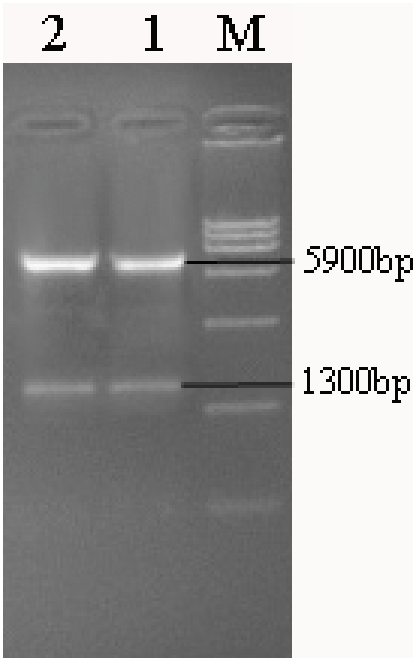

Figure 2. Restriction enzyme digestion of the pET-32a-FnBPB-ClfA plasmid. M: DL2000 DNA Marker; 1, 2: pET-32a-FnBPB-ClfA(*ECoR I/ Xho* I).

protein that had been filtered and sterilized; and single adhesin protein and protein mixture (final concentration being 10 μg/ml) to stimulate T cells, with T cells cultivated under no stimulation for control. The plates were cultured for 48 h at 37°C, in which 150 μl dimethyl sulfoxide (DMSO) was most preferably preheated to 37°C, followed by a continuous shaking for 5 min. Finally, the OD570 light absorption value was measured with a Bio-Rad microplate reader (Bio-Rad, USA). The stimulation index was calculated according to the formula: stimulation index (SI) = OD value of tested holes/OD value of control. Each treatment was done in three wells for repetition (n = 3). Data collected were analyzed statistically.

RESULTS

PCR amplification and sequencing

Through the two PCR amplifications, a specific product of about 1300 bp was retrieved. This was consistent with what had been expected from the expressed product (Figure 1).

Construction and identification of the recombinant expression plasmid

By double-enzyme cleavage, with EcoRI and Xho I, of the recombinant expression plasmid pET-32a-FnBPB-ClfA, we got pET-32a (+) linear segments of approximately 5900 bp and insert segments of approximately 1300 bp. PCR tests also showed the successful construction of the prokaryotic expression plasmid pET-32a-FnBPB-ClfA (Figure 2).

SDS-PAGE analysis

Via IPTG inducement, pET-32a-FnBPB-ClfA was successfully expressed in E. coli BL21 (DE3). The results suggest that the aimed protein was expressed at high level in soluble form. It was confirmed that the highest induction was achieved at 5 h, and that the molecular weight of the expressed protein was 51 kDa (Figure 3).

Western blot analyses

Western blot analyses demonstrated that the expressed protein could react idiosyncratically to antisera directed against S. aureus FnBPB and ClfA (Figure 4). The recombined gene was successfully expressed and the gene product had fine reactionogenicity.

Results of the ELISA test

In this experiment, we detected the specific antibody IgG in serum with the method of indirect ELISA (enzyme-linked immunosorbent assay). Table 5 shows that the antibody was detected in all sera of the experimental rabbits immunized with the protein. In contrast to group E (the control group), antibody levels of all experimental groups demonstrated an increasing trend. Among them,

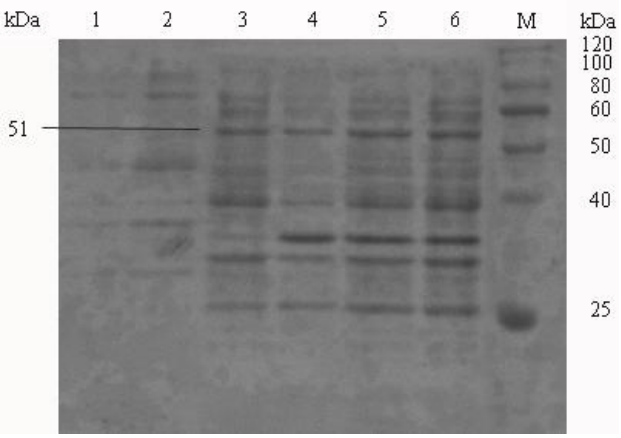

Figure 3. SDS-PAGE of the lysate of uninduced and induced host bacteria E. coli BL21(DE3) and time gradient of pET-32a-FnBPB-ClfA induced with IPTG unclear which fraction was analyzed. M: Protein marker (low); 1: pet-32a/BL21 (DE3) not induced with IPTG; 2: pet-32a/BL21 (DE3) induced with IPTG; 3 to 6: Expression products of 3-6h induced with IPTG (3: 3 h, 4: 4 h, 5: 5 h, 6: 6 h).

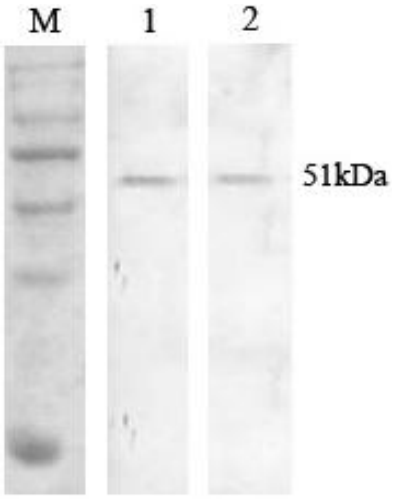

Figure 4. Western blot analyses of target proteins. M: Protein MW Marker (low); 1: Using antiserum against FnBPB protein; 2: Using antiserum against ClfA protein.

group A(the pClfA group) was higher than group B (pFnBPB) ($p<0.01$), which could be likely attributed to the fact that the pClfA protein has a large number of epitopes. Group C (the mixed immunization group) was extremely and significantly higher than groups A and B ($p<0.01$). This might be related to the intake efficiency of the antigen-presenting cells (APC). Group D (the expressed infusion group) also showed significant higher antibody levels as compared to the PBS groups ($p<0.01$).

Results of agglutination test

In contrast to the control, agglutination valence of serum from immunized mice increased remarkably. The increase of the antibody (anti S. aureus) level indicated that the expressed recombinant protein contains one of the components of S. aureus. The highest agglutination valence was found in the expression fusion immunity group, suggesting it contained more antibodies than the other groups (Table 6). Agglutination of S. aureus in the control serum was considered to be caused by protein A of S. aureus.

Results of cytophagic test

Counting of bacterial colonies was performed on the cultured product after the 18h cultivation at 37°C. Colonies above 300 were excluded (Table 7). As shown in Table 7, the number of bacterial colonies in the experimental groups was smaller than that in the control, which may be explained by the fact that the whole blood of the experimental mice was able to kill S. aureus. Colony numbers of the fusion protein immunized group and protein mixture immunized group and co-expression group were distinct significantly lower than the rest groups of the separate and combined Immunohis-tochemistry. This demonstrated that the combined fusion of two adhesins may be the most successful effective immunization against S. aureus.

Results of T lymphocyte proliferation test

T-cell suspension, prepared from spleens of the three immunized mice, was stimulated with the expressed protein and concanavalin A (ConA), so as to test the stimulation index of specificity and non-specificity of the T cells (Table 8).

Antigenic specific lymphocyte proliferation is one of the common methods for externally assessing immunity of animal cells (Jin et al., 1998). After ConA stimulation, the T lymphocyte stimulation indexes in mice of the groups-the single adhesin group, fusion group, mixture group and control group were all around 1.0, with no evident difference between the groups. However, things were much different in the case of antigen stimulation-stimulation with the expressed protein. Differences were found, not only between the experimental groups and the control group, but also among the experimental groups. Extremely significant differences were found in the T cell proliferation capabilities of the pFnBPB-ClfA fusion group and the pFnBPB+pClfA mixture group as compared to the control ($P<0.01$). Significant differences were also seen between the single adhesin groups and the control group ($P<0.05$), as well as the mixture group and the control group ($P<0.05$).

Table 5. Analysis of expressed product by ELISA.

Group	Protein vaccine	Blood collection			
		Day 0	Day 10	Day 21	Day 28
A	pClfA	0.147±0.021	0.256±0.016	0.468±0.018	0.579±0.012
B	pFnBPB	0.163±0.018	0.234±0.012	0.361±0.015	0.490±0.019
C	pClfA+ pFnBPB	0.151±0.014	0.297±0.012	0.518±0.011	0.653±0.09
D	pClfA-FnBPB	0.157±0.017	0.341±0.014	0.673±0.020	0.825±0.011
E	PBS	0.158±0.010	0.149±0.020	0.142±0.011	0.150±0.014

Table 6. Tube agglutination test.

Group	Antiserum dilution							
	1：25	1：50	1：100	1：200	1：400	1：800	1：1600	1：3200
pClfA	++++	++++	++++	++++	++++	++	-	-
pFnBPB	++++	++++	++++	+++	-	-	-	-
pClfA+pFnBPB	++++	++++	++++	++++	++++	+++	++	-
pClfA-FnBPB	++++	++++	++++	++++	++++	++++	+++	++
PBS	++++	++++	++	-	-	-	-	-

(+) represents the viable "lattice" structure; “++++” means that all the red cells form a uniform reddish color across the well; “+++” means that most of the red cells form a uniform reddish color across the well and a few red cells sink to the bottom of a well and form a button; “++” means that about half of the red cells form a lattice that coats the well with small area and other cells form a button; “+” means only a few of cells agglutinate.

Table 7. Average colony counts.

Protein vaccine	Dilution			
	10^{-3}	10^{-4}	10^{-5}	10^{-6}
pClfA	>300	69	13	0
pFnBPB	>300	92	35	2
pClfA + pFnBPB	>300	50	26	1
pClfA-FnBPB	>300	45	12	0
PBS	>300	>300	247	21

Table 8. T lymphocytes proliferation assays.

Group	ConA	Antigen
Contrast	0.140	0.149
pFnBPB	0.065	0.207
pC1fA	0.115	0.312
pFnBPB + pClfA	0.122	0.381
pFnBPB-ClfA	0.146	0.422

ConA = Concanavalin A.

DISCUSSION

FnBPs can promote bacterium invasion in host cells by mediating the fibronectin (Fn) that unites *S. aureus* with the cell surface and ultimately facilitates bacterial attachment. Most *S. aureus* derived from cow bind spe-

cifically with Fn in the extracellular matrix. ClfA is considered to be one of the primary adhesion factors for S. aureus residence in host cells, for it can bind specifically with fibrinogen (Fg) on the extracellular matrix.

Here, we measured the dynamic variation of antibody levels in sera of immunized mice by indirect ELISA. According to our results, after three consecutive immunizations, antibody was produced in all test groups and the antibody titer of all groups increased over the course of immunizations. Among the groups, the fusion and mixture groups were significantly different from the control, which suggests that fusion and mixture immunizations can enhance antibody level in the organism. However, immunization with fusion protein is better for application in the future in that it is more convenient for expression and purification. According to a report by Shkreta et al. (2004), after immunization of the mammary gland with a DNA vaccine composed of FnbP and ClfA, an FnbP-ClfA functional antibody was produced in seven months pregnant cows. This indicates that the protein vaccine anti FnbP and ClfA can bring about an immunity reaction in cells and humor in cows and, as a result, provide protection for their mammary glands. It also renders good immunity excitation conditions to immunized cows (Castagliuolo et al., 2006). Our results on mice are consistent with these conclusions.

According to the test of T cell proliferation with the MTT method, the mice produced antigens, regardless of whether they were immunized with a single adhesin protein, fusion protein or a mixed protein. The immunized mice also had their lymphocytes converted into immunelogical memory cells, which would process a rapid and effective immune response against future stimulation of the same antigens. Thus, it is clear that in this study, all single adhesin proteins, fusion proteins and mixed proteins could induce immunity in cells in the organism, which agrees with the report of Metcalf et al. (1986).

After IPTG induction, SDS-PAGE analyses and Western blot analyses, the recombinant prokaryotic expression plasmid pET-32a-FnBPB-ClfA constructed in this study has been demonstrated to express a protein of great immunogenicity, immunity response and anti-adhesion. The FnBPB-ClfA expressed fusion protein is promising as an antigen for the creation of a prophylactic and therapeutic mastitis vaccine for cows.

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