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Immunogenicity and protective capacity of EF-Tu and FtsZ of *Streptococcus suis* serotype 2 against lethal infection

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ABSTRACT

Vaccine development efforts against *Streptococcus suis* serotype 2 (*S. suis* 2) are often constrained by strain/serotype antigen variability. Bioinformatics analyses revealed two highly conserved *S. suis* 2 factors, EF-Tu and FtsZ. Murine immunization with recombinant proteins emulsified in white oil adjuvant or eukaryotic DNA vaccine vectors provided significant protection against lethal *S. suis* 2 challenge. Immune responses elicited by recombinant protein immunization revealed the robust generation of humoral immune responses, with a mixed induction of Th1-type and Th2-type responses. Furthermore, the antiserum from mice immunized with recombinant proteins significantly inhibited the growth of *S. suis* 2 in healthy pig whole blood, suggesting the triggering of a strong opsonizing response. Histological examination found that immunizing mice with purified recombinant proteins reduced neutrophil and macrophage accumulation in brain and lung tissues after challenge with virulent *S. suis*. Taken together, these findings reveal that EF-Tu and FtsZ may be promising targets for subunit and DNA vaccine candidates against *S. suis* 2 infection.

1. Introduction

Streptococcus suis (*S. suis*) is an emerging zoonotic pathogen responsible for substantial economic losses in the worldwide swine industry [1,2]. *S. suis* infection can present as various manifestations in pigs, notably including arthritis, pneumonia, septicemia, meningitis and even acute death [1,2]. Humans may become incidentally infected with *S. suis* through contact with diseased pigs or contaminated pork-derived products, potentially resulting in permanent hearing loss, meningitis [3], and septic shock [1,4]. Among the 33 *S. suis* serotypes identified on the basis of capsular polysaccharides (types 1 to 31, 33, and 1/2), *S. suis* serotype 2 (*S. suis* 2) is considered to be the most virulent serotype responsible for severe porcine and human disease in endemic countries [5,6]. In 1998 and 2005, two large outbreaks of human *S. suis* 2 infections in China have attracted great public concern [6].

Current strategies to control *S. suis* infection largely depend upon antibiotic treatment, drug-resistant strains of *S. suis* have been widely reported [7,8]. Commercially available inactivated whole cell bacterin vaccines fail to elicit protection against heterologous strains [9]. Subunit vaccines comprised of externally-exposed muramidase-released protein [10], suilysin, and extracellular protein factor [11,12] elicit protection in pigs against both homologous and heterologous *S. suis* 2 strains; however, these factors are not conserved in all virulent strains [13].

Bacterial extracellular proteins are attractive targets for *S. suis* vaccine development as host protection against infection is primarily mediated by Th1-type immune responses and opsonophagocytosis [14,15]. Within the past decade, several immunogenic extracellular proteins of *S. suis* 2 have been identified through immunoproteomic approaches [15–17]. Among these, Enolase [18,19], HP0197 [18], HP0272 [20], HP0245 [21] and SsPepO [22] demonstrated significant protection in mice and/or pigs against *S. suis* 2 challenge. However, vaccine development efforts dependent upon outer membrane factors are often constrained by strain/serotype specificity [20,23]. It is imperative to characterize highly conserved immunogenic factors that are cap-

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Table 1
Plasmids and primers used in this study.

Plasmids or Primers	Sequence	Source or Restriction site
Plasmids		
pET-30a (+)	/	Novagen
pET-30a-*tuf*	/	This study
pET-30a-*ftsZ*	/	This study
pcDNA3.1	/	Invitrogen
pcDNA3.1-*tuf*	/	This study
pcDNA3.1-*ftsZ*	/	This study
Primers for incorporation of target genes into pET-30a (+)		
tuf-F1	5′-CGCCGAATTCATGGCAAAAGAAAAATACGATC-3′	*Eco*R I
tuf-R1	5′-CCCCGTCGACTTAAGCTTCGATTTCTGTAACCA-3′	*Sal* I
ftsZ-F1	5′-CGCCGAATTCATGGCATTTTCATTTGAAGCA-3′	*Eco*R I
ftsZ-R1	5′-CCCCGTCGACTTAGCGATTACGGAAGAATGG-3′	*Sal* I
Primers for incorporation of target genes into pcDNA3.1		
tuf-F2	5′-CCGGAATTCATGGCAAAAGAAAAATACGATC-3′	*Eco*R I
tuf-R2	5′-CCGCTCGAGTTAAGCTTCGATTTCTGTAACCA-3′	*Xho* I
ftsZ-F2	5′-CCGGAATTCATGGCATTTTCATTTGAAGCA-3′	*Eco*R I
ftsZ-R2	5′-CCGCTCGAGTTAGCGATTACGGAAGAATGG-3′	*Xho* I

able of eliciting protection against a expansive repertoire of *S. suis* strains.

As determined through bioinformatics analysis, we evaluated the protective efficacy and immune responses elicited by two extensively conserved *S. suis* 2 proteins, including EF-Tu (BLA49_07145, Elongation factor Tu) and FtsZ (BLA49_06910, cell division protein). Through immunization with purified recombinant proteins as well as DNA vaccine strategies, our findings demonstrated significant protection against lethal infection by *S. suis* 2 in a mouse model. Immunization with purified recombinant EF-Tu and FtsZ elicited strong IgG induction, encompassing both Th1-type and Th2-type responses. Antisera generated against the recombinant proteins reduced viable *S. suis* in healthy pigs blood, indicative of the triggering of opsonizing responses. These findings demonstrate that the highly conserved EF-Tu and FtsZ may be prime targets for vaccine candidates against *S. suis* 2.

2. Materials and methods

2.1. Bacterial strains and culture conditions

S. suis 2 strain SC19 was isolated from a diseased pig during the large-scale outbreak in the Sichuan province of China in 2005 (Accession NO. MNPY00000000 in GenBank) [24]. SC19 was grown in Tryptic Soy Broth (TSB, Difco laboratories, Detroit, MI, USA) supplemented with 10% newborn bovine serum (Sijiqing Biological Engineering Materials Co., Ltd. Hangzhou, China) at 37 °C. Tryptic Soy Agar (TSA) containing 10% newborn bovine serum was used as solid culture medium. *Escherichia coli* (*E. coli*) strains DH5α and BL21 (DE3) (TransGen Biotech, Beijing, China) were used for gene cloning and protein expression, respectively. The *E. coli* strains were grown in Luria-Bertani (LB) medium at 37 °C. Kanamycin (25 μg/mL, Sigma) was added to media for the selection of pET-30a transformants. Ampicillin was used as the selectable marker for the pcDNA3.1 transformants.

2.2. DNA construction

The genes encoding each of the proteins indicated in this study were amplified from the chromosomal DNA of SC19. For making recombinant EF-Tu and FtsZ, the sequences were amplified using the primers in Table 1. All the PCR products were digested with *Eco*R I/*Sal* I (TaKaRa) and cloned into pET-30a (+) vector (Novagen) to yield plasmid pET-30a-*tuf* and pET-30a-*ftsZ*. For construction of the eukaryotic expression vectors, the coding sequences *tuf* and *ftsZ* were amplified using the respective primer pairs (Table 1). Both the coding sequences were cloned into the pcDNA3.1 at *Eco*R I and *Xho* I sites obtaining the pcDNA3.1-*tuf* and pcDNA3.1-*ftsZ* vectors respectively.

2.3. Purification of recombinant proteins, SDS-PAGE

The plasmids pET-30a-*tuf* and pET-30a-*ftsZ* were transformed into *E. coli* BL21 (DE3) for prokaryotic expression. Overnight cultures of *E. coli* BL21 (DE3) harboring pET-30a-*tuf* and pET-30a-*ftsZ* were inoculated into LB medium supplemented with 25 μg/mL of kanamycin which was incubated at 37 °C with shaking. When the optical density at 600 nm (OD_{600}) of the culture was approximately 0.6, 1 mM IPTG (Sigma, USA) was added to induce production of the recombinant proteins and incubation was continued for 4 h. The recombinant proteins were purified from the supernatant by metal affinity chromatography using Ni-NTA (GE Healthcare, Germany), the purified protein was condensed by membrane ultrafiltration (Millipore, USA) and then stored at −20 °C. Cell lysates or purified proteins were separated in a 12% acrylamide gel.

2.4. Immunization of mice and lethal challenge

The animal experiments were approved by the Laboratory Animal Monitoring Committee of Huazhong Agricultural University and performed according to their guidelines. Mice were immunized with either whole recombinant proteins or with pcDNA3.1 DNA vaccine vectors.

For recombinant immunization, 50 μg of each individual purified protein suspended in 100 μL PBS was emulsified with an equal volume of white oil adjuvant and then administered to 10 female BALB/c mice (6-week-old) by intraperitoneal injection. Five additional mice per group were immunized for harvesting the spleen. Control groups consisted of 10 mice immunized with either 100 μL PBS or PBS emulsified with an equal volume of white oil adjuvant. A vaccine using inactivated whole cells (commercial inactivated vaccine) was also used as the positive control ('vaccine'). Subsequent booster injections were administered after 14 days with the same antigen. All mice in each group were challenged with 3×10^9 CFU of SC19 by intraperitoneal injection 21 days after boost immunization.

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