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The Identification of Six Novel Proteins with Fibronectin or Collagen Type I Binding Activity from *Streptococcus suis* Serotype 2

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***Streptococcus suis*, a major swine pathogen, is an emerging zoonotic agent that causes meningitis and septic shock. Bacterial cell wall and secreted proteins are often involved in interactions with extracellular matrix proteins (ECMs), which play important roles in the initial steps of pathogenesis. In this study, 2D SDS-PAGE, western blotting-based binding affinity measurements, and microtiter plate binding assays were used to identify cell wall and secreted proteins from *S. suis* that interact with fibronectin and collagen type I. We identified six proteins from *S. suis*, including three proteins (translation elongation factor G, oligopeptide-binding protein OppA precursor, and phosphoglycerate mutase) that show both fibronectin and collagen type I binding activity. To the best of our knowledge, these three newly identified proteins had no previously reported fibronectin or collagen type I binding activity. Overall, the aim in this study was to identify proteins with ECM binding activity from *S. suis* and it represents the first report of six new proteins from *S. suis* that interact with fibronectin or collagen type I.**

Keywords: *Streptococcus suis*, cell wall proteins, secreted proteins, extracellular matrix protein

Introduction

Streptococcus suis is the causative agent of numerous infections in pigs (Chanter *et al.*, 1993; Staats *et al.*, 1997). It causes a range of serious pathologies in swine, including pneumonia, endocarditis, meningitis, and arthritis. Among the 33 known serotypes of *S. suis*, serotype 2 is known to be the most virulent and frequently isolated (Higgins and Gottschalk, 2000). In addition to causing disease in pigs, it can also cause serious enzootic infections in humans (Yu *et al.*, 2006). Notably, two large-scale *Streptococcus* outbreaks were caused by *S. suis* serotype 2 in 1998 and 2005 in China. These outbreaks led to severe economic losses in the swine industry and posed significant public health concerns worldwide (Yao *et al.*, 1999; Tang *et al.*, 2006).

Several bacterial components, such as extracellular and surface proteins, have been proposed to be virulence factors; however, the precise roles of these components in the pathogenesis or virulence of *S. suis* have not yet been established (Fittipaldi *et al.*, 2012). Pathogens have evolved diverse strategies to successfully colonize a broad range of host tissues. A common strategy is the initiation of infection by adhering to specific host macromolecules (Finlay, 1990), such as extracellular matrix (ECM) proteins secreted by host cells, under either stringent or hostile conditions. Indeed, it has been established that *S. suis* binds to several immobilized serum and ECM proteins, such as both plasma and cellular fibronectins and collagen types I, III, and V (Esgleas *et al.*, 2005). Recently, many *S. suis* proteins have been proposed to contribute to the colonization of organs because of their ability to bind host ECM proteins, such as FBPS, α-enolase, GAPDH, and Autolysin (Quessy *et al.*, 1997; de Greeff *et al.*, 2002; Jobin *et al.*, 2004; Esgleas *et al.*, 2008; Ju *et al.*, 2012). This study aimed to identify novel cell wall and secreted proteins from *S. suis* that interact with fibronectin and collagen type I. The identification of such proteins could serve as a foundation for further research into the precise roles of these components in the pathogenesis or virulence of *S. suis*.

Materials and Methods

Bacterial strains and culture conditions

The *S. suis* serotype 2 strain (*S. suis* HA9801) was isolated in 1998 (Yang *et al.*, 2008) and grown in Todd-Hewitt broth (THB) medium (Difco Laboratories, USA) or plated on THB agar supplemented with 5% (v/v) sheep blood. *Escherichia coli* DH5α and *E. coli* BL21 (DE3) pLysS were grown in Luria Bertani (LB) broth liquid medium or plated on LB agar. We used 50 μg kanamycin/ml (Kan; Sigma-Aldrich, USA) in growth media when required.

Preparation of cell wall and secreted proteins

Cell wall proteins were prepared as described previously with a few modifications (Ling *et al.*, 2004; Wu *et al.*, 2008b). *S. suis* HA9801 was cultured to an OD_{600nm} of approximately 0.8. Bacterial cultures (200 ml) were harvested by centrifugation for 10 min at 10,000 × *g* and 4°C. The resulting pellets were dissolved in 10 ml mutanolysin solution (125 U/ml mutanolysin in 25% sucrose, 3 mM $MgCl_2$, and 30 mM Tris-Cl, pH 7.5) and then incubated for 90 min at 37°C. Soluble proteins released from bacteria were collected

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from the supernatant after centrifugation (10 min at 10,000 × *g* at 4°C). The supernatants were precipitated using trichloroacetic acid and acetone. Then, the supernatants were mixed with prechilled 100% trichloroacetic acid to a final concentration of 10% and incubated in ice-cold water for 30 min. After centrifugation at 10,000 × *g* for 10 min at 4°C, the pellet was resuspended in 10 ml prechilled acetone and washed twice. The final pellet was air-dried.

Secreted proteins were prepared as described previously (Wu *et al.*, 2008a). Briefly, culture supernatant of strain HA9801 was harvested by centrifugation (10,000 × *g* for 15 min at 4°C). Residual bacteria in the supernatants were removed through 0.22 μm membrane filters. Trichloroacetic acid was added to the filtrate to a final concentration of 10%, and then the filtrate was incubated in ice-cold water for 30 min. After centrifugation for 15 min at 10,000 × *g* and 4°C, the pellet was washed with prechilled acetone twice. The pellet was air-dried.

Isoelectric focusing

Isoelectric focusing (IEF) was performed using an Ettan IPGphor-3 IEF system (GE Healthcare, UK). Before rehydration, the precipitated proteins were treated using a 2-DE Clean-Up Kit (GE Healthcare) to remove contaminants that can interfere with electrophoresis. We rehydrated 13 cm immobilized pH gradient (IPG) strips (Immobiline DryStripk, pH 4–7; GE Healthcare) overnight at room temperature (RT) using rehydration solution [7 M urea, 2 M thiourea, 2% CHAPS, 0.2% DTT, 0.5% IPG buffer (pH 4–7), and 0.002% bromophenol blue]. Each strip was loaded with 300 μg cell wall and secreted protein, and IEF was carried out at 20°C for 13.5 h (maximum voltage, 4,000 V; maximum current, 50 μA per IPG strip; total, 26,000 Vh).

2D SDS-PAGE

Prior to 2D SDS-PAGE, each IPG strip was washed in 3 mL equilibration buffer 1 (375 mM Tris-HCl; pH 8.8, 6 M urea, 2% SDS, and 2% DTT) for 15 min, followed by incubation in 3 mL equilibration buffer 2 [375 mM Tris-HCl; pH 8.8, 6 M urea, 2% SDS, and 2.5% iodoracetamide) for 15 min. Each IPG strip plus an SDS-PAGE molecular weight standard (Beyotime Institute, China) was loaded onto a homogeneous 12% polyacrylamide gel and sealed with 1% agarose. Electrophoresis was performed at 15°C using an initial voltage of 110 V for 30 min, followed by 220 V until the tracking dye reached the bottom of the gel. All gels were stained with colloidal Coomassie brilliant blue G-250 (GE Healthcare) and three replicates were run for each sample.

ECM protein-binding assay

A western blot assay was used to identify ECM adhesive proteins from *S. suis* by detecting bacterial cell wall and secreted proteins that interacted with fibronectin and collagen type I (Patti *et al.*, 1992; Zhang *et al.*, 2013). Protein samples from each SDS-PAGE gel were transferred onto polyvinylidene fluoride (PVDF) membranes (GE Healthcare) for 2 h at 0.65 mA/cm^2 using a semi-dry blotting apparatus (TE77, GE Healthcare). Additional protein-binding sites were blocked by incubating samples at room temperature for 1 h in 3% (w/v) non-fat dry milk in TBS (20 mM Tris and 150 mM NaCl, pH 7.4). Membranes were washed and incubated with either Human Collagen type I (Santa Cruz Biotechnology, USA) or Human Fibronectin (R&D, USA) in 3% (w/v) non-fat dry milk in TBS (pH 8.0) overnight at 30°C. Membranes were washed again and incubated with either rabbit anti-collagen I (Sangon Biotech, China) or rabbit anti-fibronectin (Boster, USA) antibody in TBS for 2 h. After washing with TBS containing 0.1% Tween 20 (TBST), goat anti-rabbit IgG-peroxidase-labeled antibody (Beyotime Institute) was added. SuperSignal West Pico Chemiluminescent Substrate (Pierce, USA) was used according to the manufacturer's instructions.

MALDI-TOF MS and database searching

Protein spots were excised from stained 2-D gels and sent to Shanghai GeneCore BioTechnologies Co. Ltd. (Gene Core Ltd., China) for in-gel trypsin digestion and matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS; TOF Ultraflex II mass spectrometer, Bruker Daltonics, USA). Peptide mass fingerprinting (PMF) data was analyzed using the MASCOT server (http://www.matrixscience.com). MASCOT searches were used identify

Table 1. Primer sequences used for cloning extracellular matrix protein adhesion genes candidates

Spot no.	Sequences (5′–3′)[a]	Restriction enzyme	Length of PCR products (bp)	Expression vector
1	CGGATCCATGGCACGCGAATTTTCAT	*Bam*HI	2082	pET-28a
	CCTCGAGTTATGCATTGCCACCGTTT	*Xho*I		
2	GGAATTCGGTTCAAAACAATCAGGTG	*Eco*RI	1719	pET-28a
	CCTCGAGTTATTCTGCCACTACACCC	*Xho*I		
7	GGAATTCGTAAAATTGGTTTTTGCTC	*Eco*RI	666	pET-28a
	CCTCGAGCACAATGTTCAAGTTTTCA	*Xho*I		
9	GGAATTCGCAAAATTGACTGTTAAAG	*Eco*RI	1197	pET-28a
	CCTCGAGCTATTTTTCAGTCAAAGCT	*Xho*I		
10	CGCGAATTCATGCAACAATCCGTGAT	*Eco*RI	924	pET-28a
	CCCTCGAGGCTAGTCTACAAACACATC	*Xho*I		
11	CGGATCCATCAAATTTGCAGCAGATG	*Bam*HI	1611	pET-28a
	CCTCGAGTTAGTAGCCCATACCCATT	*Xho*I		

[a] The underlined sequences indicate restriction sites.

peptides that were considered to show a significant signal; these peptides were used to generate the combined peptide scores. The extent of sequence coverage, number of matched peptides, and the score probability obtained from the PMF data were all used to validate protein identifications. Low-scoring proteins were either verified manually or rejected.

Bioinformatics analysis

Sequences of the identified proteins were queried using the BLASTX server (http://www.ncbi.nlm.nih.gov/BLASTX/) to identify homologous sequences. The PSORT server (http://www.psort.org/), LocateP program (http://www.cmbi.ru.nl/locatep-db), and Gpos-mPLoc program (http://www.csbio.sjtu.edu.cn/bioinf/Gpos-multi/) were used to predict the subcellular localizations of the proteins.

Expression of recombinant proteins

We identified six genes that encoded proteins that potentially promoted extracellular matrix protein adhesion and amplified them by PCR from the genomic DNA of *S. suis* HA9801; PCR primers are listed in Table 1. PCR products were cloned into the pET-28a or pET-30b expression vectors; the resulting plasmids were introduced into *E. coli* BL21 (DE3) pLysS for IPTG-inducible expression of recombinant proteins. Under native conditions, the His-tagged fusion proteins were purified by HisTrap chromatography according to the manufacturer's protocols (GE Healthcare). Protein-containing fractions were identified using SDS-PAGE.

Microtiter plate binding assays

The binding of proteins to Human Collagen type I (Santa Cruz Biotechnology) or Human Fibronectin (R&D) were also measured by ELISA (Esgleas *et al.*, 2008). Microtitre 96-well plates were coated with 100 µl serially diluted purified proteins (the protein concentration varied depending on the experiment) in 0.1 M carbonate coating buffer [0.15% (w/v) Na_2CO_3, 0.1% (w/v) $MgCl_2{\cdot}6H_2O$, and 0.3% (w/v) $NaHCO_3$ (pH 9.6)] and incubated overnight at 4°C. Casein-coated wells served as controls for non-specific adhesion. Plates were washed three times with PBS (pH 7.4) containing 0.05% (v/v) Tween 20 (PBST) and 200 µl 3% (w/v) non-fat dried milk in PBST were added to each well to prevent non-specific binding. After 1 h at 37°C, the wells were washed three times with PBST. Next, 100 µl 10 µg collagen type I/ml or 1 µg fibronectin/ml were added and plates were incubated for 2 h at 37°C. After three washes with PBST, 1:200 diluted collagen type I or 1:800 diluted fibronectin-specific antibodies (Boster) were added to the corresponding wells and plates were incubated for 1 h at 37°C. Wells were washed three times with PBST and then goat anti-rabbit IgG-peroxidase-labeled antibody (Beyotime Institute) was added. Plates were then incubated for 1 h at 37°C with secondary antibody and 3,3′,5,5′-tetramethylbenzidine (Beyotime Institute) was used as the enzyme substrate according to the manufacturer's instructions. The reactions were stopped by adding 50 µl H_2SO_4 (0.5 M) and were read at 450 nm using a microplate reader.

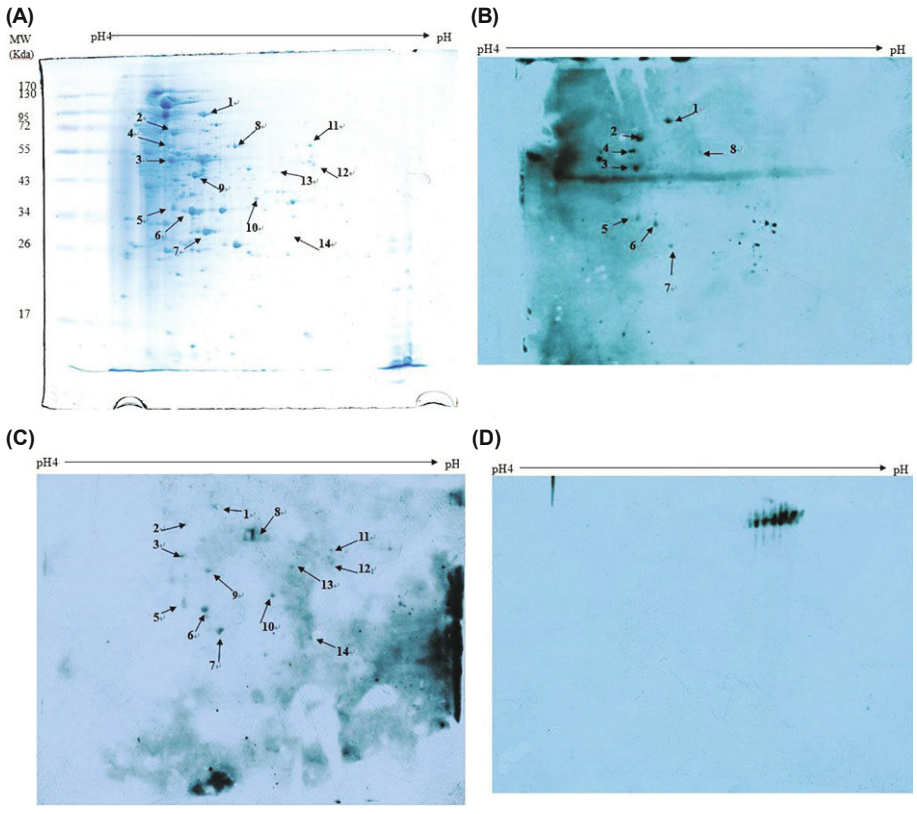

Fig. 1. 2-DE gel and western blotting to measure the affinity of *S. suis* cell wall and secreted protein interactions with collagen type I and fibronectin. (A) HA 9801 cell wall and secreted proteins (pH 4–7, 13 cm) were stained with colloidal Coomassie brilliant blue G-250. (B) A 2-DE blot of HA9801 cell wall and secreted proteins that show interactions with collagen type I. (C) A 2-DE blot of HA9801 cell wall and secreted proteins that show interactions with fibronectin. (D) A 2-DE blot of HA9801 cell wall and secreted proteins incubated without collagen type I; the 2-DE blot of HA9801 cell wall and secreted proteins incubated without fibronectin is not shown.

Table 2. **Protein spot interactions with collagen type I identified by MALDI–TOF/TOF MS**

Spot number	Protein identified[a]	BLASTX similarity matched protein/species/ identity score	Theoretical MW (Da)/pI[b]	Experimental MW (Da)/pIw	Mascot score[c]	No. of peptides matched[d]	Coverage (%)[e]
1	gi 223933803	translation elongation factor G	76677/4.85	82000/5.0	280	16	24
2	gi|253752531	oligopeptide-binding protein OppA precursor	65631/4.91	68000/4.8	94	13	29
3	gi|146321359	enolase gene product	47066/4.66	47000/4.5	131	15	46
4	gi|146319837	inosine 5′-monophosphate dehydrogenase	52743/5.61	56000/4.6	296	20	44
5	gi|253751156	surface-anchored protein	75540/4.63	38000/4.5	247	26	39
6	gi|330832175	fructose-bisphosphate aldolase	31120/4.90	34000/4.9	208	19	55
7	gi|146319292	phosphoglycerate mutase	21516/5.08	30000/5.0	91	9	48
8	gi|146318198	pyruvate kinase	54710/5.12	55000/5.4	148	18	43

[a] gi number in NCBI.
[b] The theoretical pI was calculated using AnTheProt (http://antheprot-pbil.ibcp.fr/).
[c] The Mascot score obtained for the peptide mass fingerprint (PMF). The significance threshold was 70.
[d] The number of peptides that match the predicted protein sequence.
[e] The percentage of predicted protein sequences covered by the matched peptides.

Statistical analysis

ELISA tests were performed at least three times for each binding assay. Statistical analyses were carried out using the GraphPad Prism 5 software package (Graphpad, USA).

Results

Identification of *S. suis* proteins that adhered to extracellular matrix proteins

Bacterial cell wall and secreted proteins from *S. suis* were separated by 2-D SDS-PAGE over a pH range of 4–7 (IPG linear gradient; Fig. 1A) and transferred to PVDF membranes. After incubation with collagen type I and fibronectin, 14 potential *S. suis* proteins that adhered to the ECM were observed (Figs. 1B and 1C). No specific protein spots were observed when the blot was incubated without collagen type I (Fig. 1D). The 14 spots were then characterized by MALDI-TOF-MS and data were compared to those in the NCBI sequence database. The probability scores, molecular weights (MWs), isoelectric points (pIs), number of peptide matches, and the percentages of total translated ORF sequence covered by the peptides were used to confirm spot identification. Seven proteins (spots 1, 2, 3, 5, 6, 7, and 8) showed potential binding affinities for both fibronectin and collagen type I. The results are summarized in Tables 2 and 3.

Microtiter plate binding assays for detecting recombinant protein interactions

To verify the fibronectin- and collagen type I-binding activities of the potential ECM-adhering proteins, six recombinant proteins (spots 1, 2, 7, 9, 10, and 11) were expressed. SDS-PAGE showed that the recombinant 1(1'), 2(2'), 9(9'), 7(7'), 10(10'), and 11(11') proteins were approximately 80, 72, 45, 30, 40, and 60 kDa, respectively (Fig. 2). Purified recombinant proteins were obtained after performing Ni-NTA affinity chromatography. Analyzed by RONN to predict disordered protein structures, the region between residues 285 and 308 of EF-G showed a high probability of disorder, which accounts for the appearance of the two protein bands that we observed during purification. We used ELISA to show

Table 3. **Protein spot interactions with fibronectin identified by MALDI–TOF/TOF MS**

Spot number	Protein identified[a]	BLASTX similarity matched protein/species/ identity score	Theoretical MW (Da)/pI[b]	Experimental MW (Da)/pIw	Mascot score[c]	No. of peptides matched[d]	Coverage (%)[e]
1	gi 223933803	translation elongation factor G	76677/4.85	82000/5.0	280	16	24
2	gi|253752531	oligopeptide-binding protein OppA precursor	65631/4.91	68000/4.8	94	13	29
3	gi|146321359	enolase gene product	47066/4.66	47000/4.5	131	15	46
5	gi|253751156	surface-anchored protein	75540/4.63	38000/4.5	247	26	39
6	gi|330832175	fructose-bisphosphate aldolase	31120/4.90	34000/4.9	208	19	55
7	gi|146319292	phosphoglycerate mutase	21516/5.08	30000/5.0	91	9	48
8	gi|146318198	pyruvate kinase	54710/5.12	55000/5.4	148	18	43
9	gi|146317815	phosphoglycerate kinase	42048/4.85	44000/4.9	132	12	47
10	gi|253752506	pyruvate dehydrogenase E1 component, alpha subunit	35240/5.25	37000/5.5	124	19	50
11	gi|253751059	chaperonin GroEL	57037/4.70	57000/5.8	148	19	35
12	gi|253752618	adenylosuccinate synthetase	51985/5.65	50000/5.9	218	22	47
13	gi|146317908	glutamate dehydrogenase	48751/5.43	49000/5.5	166	20	42
14	gi|146319457	3-ketoacyl-ACP reductase	25573/5.53	27000/5.6	163	15	63

[a] gi number in NCBI.
[b] The theoretical pI was calculated using AnTheProt (http://antheprot-pbil.ibcp.fr/).
[c] The Mascot score obtained for the peptide mass fingerprint (PMF). The significance threshold was 70.
[d] The number of peptides that match the predicted protein sequence.
[e] The percentage of predicted protein sequences covered by the matched peptide.

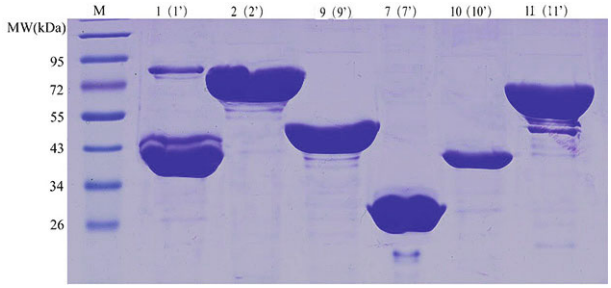

Fig. 2. SDS-PAGE analysis of recombinant 1(1'), 2(2'), 9(9'), 7(7'), 10(10'), and 11(11') proteins purified from the inducible protein producing *E. coli* strains; molecular size markers are shown in kDa (Lane M).

that the immobilized bacterial proteins could specifically interact with fibronectin or collagen type I in a saturable, dose-dependent manner. These results were confirmed by western blotting to show binding affinity. Under similar assay conditions, immobilized bacterial proteins failed to interact with casein, which was used as a negative control (Fig. 3).

Discussion

This is the first report of the use 2D SDS-PAGE and western blot affinity assays as a tool to identify fibronectin- or collagen type I-binding activity proteins from *S. suis*. In this study, six ECM adhering proteins from *S. suis* were identified, including three proteins (spots 1, 2, and 7) that showed both fibronectin- and collagen type I-binding activity. To the best of our knowledge, these proteins had no previously reported fibronectin- or collagen type I-binding activity, and their identification serves as a foundation for further research to investigate the precise roles of these components in the pathogenesis or virulence of *S. suis*.

Translation elongation factors are responsible for two of the main processes involved in protein synthesis on the ribosome. Spot 1 corresponded to *S. suis* translation elongation factor G (EF-G), which was previously identified as a secreted immunogenic protein in the *Bacillus cereus* group and *S. suis* serotype 9 (Delvecchio *et al.*, 2006; Wu *et al.*, 2011), although this is the first report of EF-G binding to either collagen or fibronectin. Translation elongation factor Tu (EF-Tu), which is homologous to EF-G, has been reported to bind fibronectin and act as a mediator of microbial colonization and tissue tropism in *Mycoplasma pneumoniae* and *Acinetobacter baumannii* (Balasubramanian *et al.*, 2009; Dallo *et al.*, 2012). When analyzed by RONN to predict disordered protein structures, the region between residues 285 and 308 of EF-G showed a high probability of disorder. Disordered or unfolded proteins are prone to degradation by the proteolytic enzyme HtrA in *E. coli* (Kim *et al.*, 1999), which accounts for the appearance of the two protein bands that we observed during purification.

In gram-positive bacteria, the Opp system, notably OppA (oligopeptide-binding protein), is involved in different aspects of cell physiology, including intercellular communication and binding to host proteins (Nepomuceno *et al.*, 2007). The binding of fibronectin by OppA may be important both for spirochete-host interactions in the subgingival environment and for the uptake of peptide nutrients (Fenno *et al.*, 2000). Spot 2 contains an OppA precursor and this is the first report of OppA in *S. suis*. Spot 7 contains phosphoglycerate mutase, which was first identified in a comparative proteome analysis of secreted proteins from *S. suis* serotype 9 isolates obtained from diseased and healthy pigs (Wu *et al.*, 2008a).

Spot 9 contains phosphoglycerate kinase (PGK), an immunogenic protein from *S. suis* biofilms (Wang *et al.*, 2012). In group B *Streptococci* (GBS), PGK binds to plasminogen and actin and can alter the epithelial cell cytoskeleton (Boone *et al.*, 2011; Boone and Tyrrell, 2012). Spot 10 contains pyruvate dehydrogenase E1 component, alpha subunit (PDH), which is an important part of the cytoskeleton of *M. pneumoniae* and has been linked to cell adhesion (Francolini and Donelli, 2010), in which it is thought to be involved in binding to fibronectin (Savini *et al.*, 2010). PDH is an immunogenic protein with increased expression levels in *S. suis* biofilms (Wang *et al.*, 2012). Spot 11 contains the chaperonin

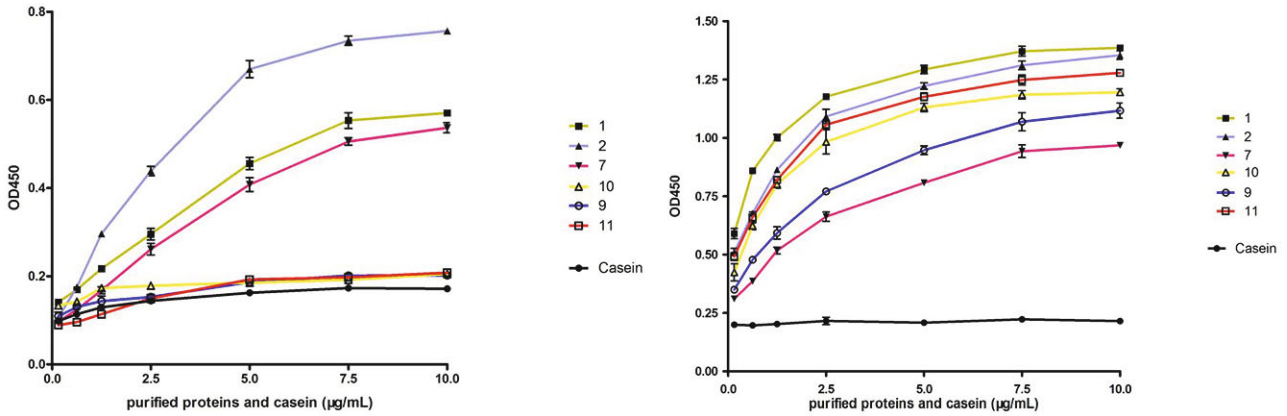

Fig. 3. A microtiter plate binding assay for recombinant proteins. (A) A representative ELISA analysis of the binding of collagen type I to immobilized purified recombinant proteins and casein (negative control). (B) A representative ELISA analysis of the binding of fibronectin to immobilized purified recombinant proteins and casein (negative control).

GroEL. A previous study found that the lifespan of mice immunized with GroEL from *S. pneumoniae* was not significantly affected, and all of the mice died by seven days postinfection (Khan *et al.*, 2009). In *S. mutans*, GroEL affects the expression of key virulence traits, including biofilm formation and acid tolerance, and supports the concept that it evolved in this organism to accommodate unique roles that allow it to adapt to its niche (Lemos *et al.*, 2007). However, FBPS and autolysin (described in the introduction) were not detected by our tests, possibly as a consequence of the low abundance of FBPS and autolysin in the protein preparations. GAPDH has also been found to have albumin binding activity (Quessy *et al.*, 1997; Jobin *et al.*, 2004).

The eight proteins that were identified by 2D SDS-PAGE and western blotting assays to measure binding affinity may have fibronectin- and collagen type I-binding activities, but we could not verify this by the microtiter plate binding assays. Spot 3 contains enolase, which is localized at the cell surface and has been shown to have fibronectin-binding affinity, which may contribute to the virulence of *S. suis* (Esgleas *et al.*, 2008). To date, there have been no reports of its collagen-binding abilities. Spot 5 contains a surface-anchored protein of a currently unknown function. Spot 6 contains fructose-bisphosphate aldolase (FBA) of *S. suis*. This cell surface-associated protein elicits protective immune responses in mice and affords significant protection against respiratory challenge with virulent *S. pneumoniae* (Ling *et al.*, 2004). It was also identified as an immunogenic surface protein of *S. suis* serotype 9 (Wu *et al.*, 2008b), and it shows decreased expression levels in *S. suis* biofilms (Wang *et al.*, 2012). Spot 8 contains pyruvate kinase, which is an immunogenic protein found in the cell walls of *S. pyogenes* and *S. suis* serotype 9 (Cole *et al.*, 2005; Wu *et al.*, 2008b). Spot 4, which was identified as inosine 5′-monophosphate dehydrogenase (IMPDH), was shown to have collagen-binding activity, and is considered to be a potential virulence factor of *S. suis* (Zhang *et al.*, 2009).

Spots 12–14 were shown to have fibronectin-binding abilities. Spot 12 contains adenylosuccinate synthetase, which has not been previously reported in *S. suis*. Spot 13 contains glutamate dehydrogenase (GDH), a cell surface protein that serves as an antigen of diagnostic importance for the detection of *S. suis* infection (Okwumabua *et al.*, 2001). GDH ETs and sequence types may serve as useful markers for predicting the pathogenic behavior of *S. suis* (Kutz and Okwumabua, 2008). Spot 14 contains 3-ketoacyl-ACP reductase, which has been found in virulent strains in a comparative proteomics assay of *S. suis* serotype 2 (Zhang and Lu, 2007).

In conclusion, the identification of these proteins serves as a strong foundation for the future elucidation of the roles of these proteins in the pathogenesis or virulence of *S. suis*.

Acknowledgements

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