CHAPTER 2

Literature review

2.1 *Streptococcus suis*

2.1.1 General characteristics

Streptococcus suis (*S. suis*) is classified into the family *Streptococcaceae*, genus *Streptococcus*. It is a nonmotile, facultative anaerobic gram-positive coccus (1 micrometer in diameter) and is arranged in pairs or in short chains (Figure 1a). The colonies of *S. suis* are 1-2 mm in diameter, grey and produce alpha(α)- or beta(β)-haemolysis on blood agar plates (Figure 1b) (Staats *et al*, 1997). Originally, *S. suis* strains were classified as Lancefield groups R, S, RS and T (de Moor, 1963), but later strains of *S. suis* were shown to share antigenic features with group D streptococci (Elliott, 1966) with differences in genetics. and *S. suis* can be (Reuter, 1992; Kouki, 2012). The cell wall of *S. suis* consists of a cytoplasmic membrane and a thick peptidoglycan layer surrounded by a polysaccharide capsule (Figure 2), which is used to classify this bacterium into 33 serotypes including serotypes 1-31, 33 and 1/2 and 2 serotypes (serotypes 32 and 34) has been classified as *Streptococcus orisratti* (Hill *et al*., 2005; Paul Wichgers Schreur, 2012,).

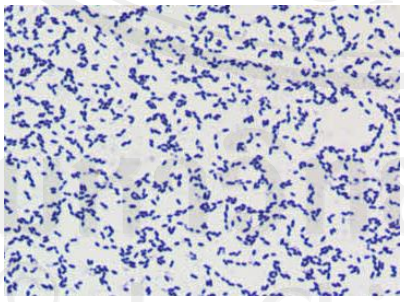
(a)  (b) 

Figure 1 Biological features of *S. suis* (a) Pairs and short chains of *S. suis* (b) Zones of alpha- or gamma- haemolysis of *S. suis* on blood agar.

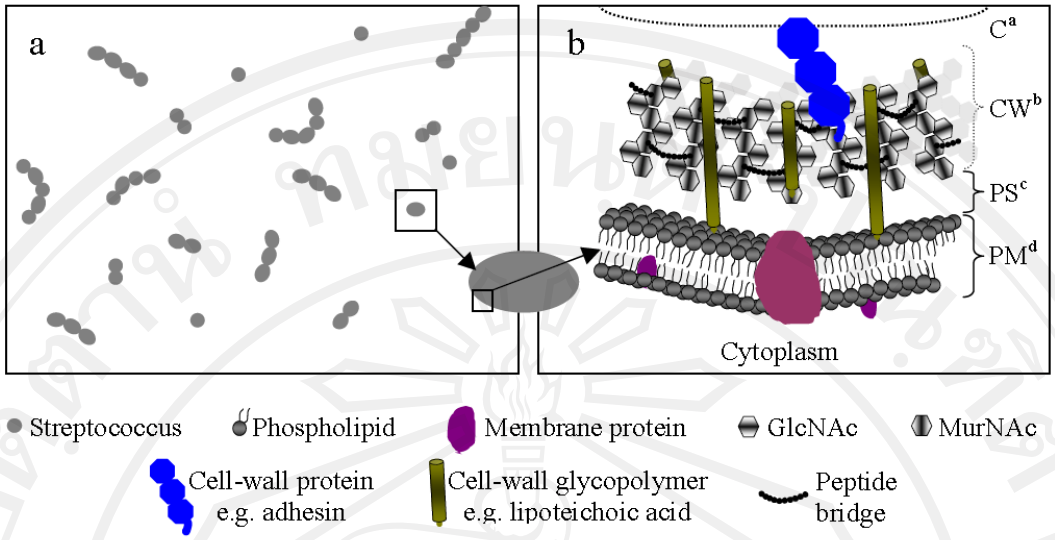


Figure 2 Structure of cell wall in *S. suis* (a) Chains of streptococci and (b) a simplified schematic cell wall of *S. suis* [a] C, capsule; [b] CW, cell wall; [c] PS, periplasmic space; [d] PM, plasma membrane (from Annika Kouki, 2012).

2.1.2 Epidemiology

S. suis distributes worldwide from North America, South America, Europe, Asia, Australia to New Zealand. The first case of *S. suis* infection in a human was reported in Denmark (Perch *et al.*, 1968) and more than 858 human cases have been reported worldwide since then (Kay *et al.*, 1995; Michaud *et al*, 1996; Arends and Zanen, 1988; Vilaichone *et al.*, 2002; Suankratay *et al.*, 2004; Ye *et al.*, 2006; Tang *et al.*, 2006; Mai *et al.*, 2008; Takamatsu *et al.*, 2008; Wertheim *et al.*, 2009; Haleis *et al.*, 2009; Princivall *et al.*, 2009; Fongcom *et al.*, 2009; Kerdsin *et al.*, 2009; Kerdsin *et al.*, 2011) as shown in Table 1. The clinical manifestations of patients infected with *S. suis* include acute meningitis and septicemia. A high incidence of *S. suis* infection occurs in Asia, particularly China, Vietnam and Thailand (Wertheim *et al.*, 2009). In Thailand, *S. suis* infection was firstly reported in 2 patients at Ramathibodi Hospital in 1987 (Phuapradit *et al.*, 1987) followed by 12 patients with meningitis from Chulalongkorn University Hospital (Suankratay *et al.*, 2004). Between 2 0 0 6 -2 0 0 8 , Kerdsin and colleagues reported 165 cases (serotype 2) and 12 cases (serotype 14) of *S. suis* infection from Thailand by isolation of the bacterium from blood and cerebrospinal

fluid of 1,154 samples suspected of Streptococcal infection. *S. suis* isolates were typed by multilocus sequence typing (MLST: base on various alleles found in certain housekeeping gene loci) and found that *S. suis* sequence type (ST) 1 and ST104 of serotype 2 were the 2 most prevalent of sequence type (n=103, 62.4% and n=42, 25.5%, respectively) (Kerdsin *et al.*, 2011) and ST105 and ST127 of serotype 14 are less founded (n=11, 91.7% and n=1, 8.3%, respectively), particularly in northern Thailand (Kerdsin *et al.*, 2009) as shown in Figure 3.

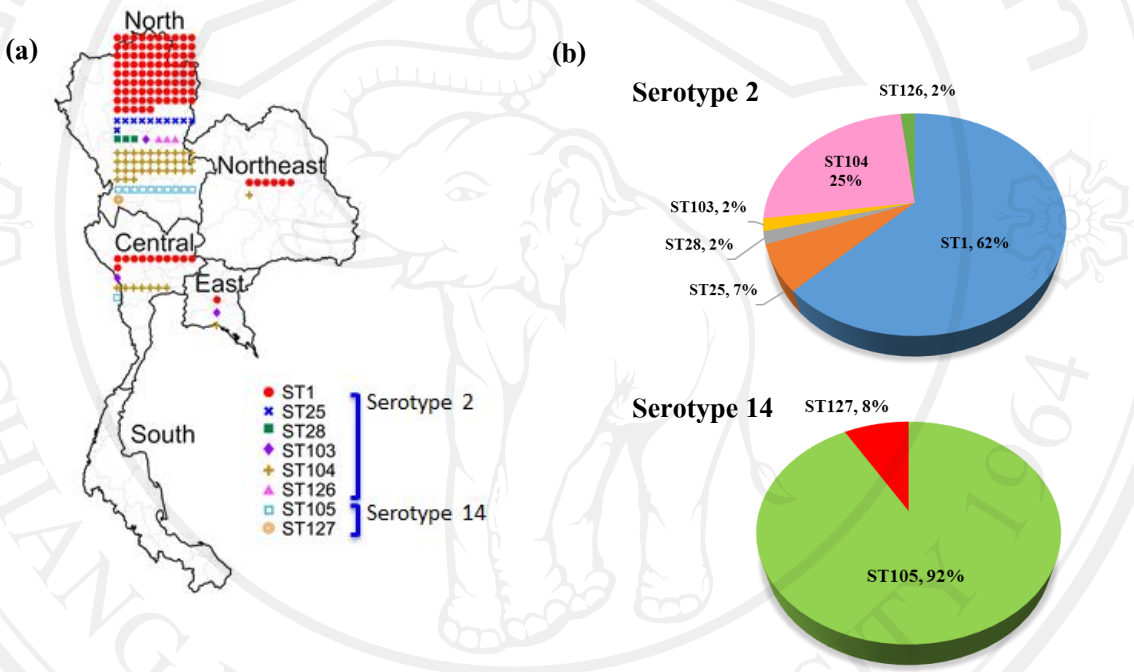


Figure 3 The prevalence of *S. suis* serotypes 2 and 14 in Thailand. (a) Distribution of sequence type of *S. suis* serotypes 2 and 14 isolated in the year 2006-2008. (b) Prevalence of the number sequence type of *S. suis* serotypes 2 and 14 (adapted from Kerdsin *et al.*, 2009; Kerdsin *et al.*, 2011).

ST1, ST25, ST28, ST103, ST104, ST126, ST105, ST127

Table 1 Examples of human cases infected with *S. suis* worldwide (1996 until 2011).

Country	Serotype	Cases	Deaths	Diseases and symptoms	References
Canada (Quebec)	2	1	0	Meningitis	Michaud *et al*., 1996
Netherlands	-	30	2	Meningitis	Arends and Zanen, 1988
Canada	14	1	0	Meningitis	Haleis *et al*., 2009
Italy	2	2	0	Meningitis	Princivall *et al*., 2009
Hongkong	-	21	1	Meningitis	Kay *et al*., 1995
Thailand	-	32	2	Meningitis, Sepsis	Vilaichone *et al*., 2002
Thailand	-	12	1	Meningitis	Suankratay *et al*., 2004
China	2 (ST7)	88	0	Meningitis, STSS[1]	Ye *et al*., 2006
China	-	204	38	STSS[1]	Tang *et al*., 2006
Vietnam	-	151	4	Meningitis	Mai *et al*., 2008
Thailand	2,14	20	6	Meningitis, Septicemia, Endocarditis, Neck stiffness, Pulmonary edema, deafness	Takamatsu *et al*., 2008
Thailand	-	43	12	Meningitis, Septicemia	Fongcom *et al*., 2009
Thailand	14	12	0	Meningitis, Sepsis, Septic arthritis	Kerdsin *et al*., 2009
Thailand	2	165	0	Meningitis, Sepsis	Kerdsin *et al*., 2011

[1]STSS: Streptococcal toxic shock syndrome

2.1.3 Virulence factors

Most of *S. suis* virulence factors reported nowadays are based on studies in *S. suis* serotype 2. The important virulence factors included the capsular polysaccharide (CPS), the virulence-related proteins (such as the muramidase-released protein (MRP), the extracellular protein factor (EPF), suilysin (haemolysin), enolase, the adhesins and other virulence factors (Gottschalk and Segura, 2000) as shown in Table 2 (Fittipaldi *et al*., 2012).

2.1.3.1 Capsular polysaccharide (CPS)

The capsular polysaccharide of *S. suis* serotype 2 consists of five different sugars, including galactose, glucose, N-acetylglucosamine, rhamnose, and N-acetyl neuraminic acid (sialic acid). The genes involved in capsule production of *S. suis* are capsular polysaccharide synthesis genes in the cps locus, which contains the serotype-specific genes at the last part of it (Smith *et al*., 1999). The capsular polysaccharide is show to interfere with phagocytosis and shield cell wall components that activate dendritic cells and induce cytokine productions (Fittipaldi *et al*., 2012). The unencapsulated mutants of *S. suis* serotype 2 were more susceptible to phagocytosis by macrophages compared to the parent strain.

2.1.3.2 Suilysin (Hemolysin)

Suilysin or hemolysin (54-kDa) produced by *S. suis* is a thiol-activated toxin that targets cholesterol in the membrane of eukaryotic cells (Jacobs *et al*., 1994; Gottschalk *et al*., 1995; Palmer, 2001). A gene encoding suilysin (*sly*) was found in various but not all invasive *S. suis* serotype 2 strains and different capsular serotypes (King *et al*., 2001). It has been shown that suilysin is able to destroy epithelial cells (Gottschalk and Segura, 2000), monocytes and neutrophils by forming transmembrane pores. It has also been suggested that suilysin plays a role in invasion and pathogenesis of *S. suis*. The toxin may be involved in the dissemination of the bacteria in the bloodstream as well as the disruption of the blood brain barrier (BBB) by affecting the

endothelial cells (Kouki, 2012; Haesebrouck *et al.*, 2004, Gottschalk *et al*., 2007; Lecours *et al.*, 2011). However, strains not producing suilysin are also able to reach the bloodstream, disseminate and cause diseases (Gottschalk *et al*., 2011). Although it is not essential for virulence of *S. suis*, suilysin has been proposed to play a role in host-pathogen interactions by modulating different host cell functions (Segura and Gottschalk, 2002; Chabot-Roy *et al*., 2006).

2.1.3.3 Muramidase-released protein (MRP, *mrp*)

MRP is a 136-kDa cell wall anchored protein which releases into the culture supernatant after muramidase treatment and during the stationary phase of bacterial growth (Vecht *et al*., 1991; Fittipaldi *et al*., 2012). MRP is not an essential virulence factor of *S. suis* as the *mrp* mutant was as virulent as the wild-type strain (Smith *et al*., 1996). MRP was found in various strains of serotypes 1, 2, 1/2, 14 and 15. Large (MRP*) and small (MRP^s) size variants have been described, and were detectable in *S. suis* strains of nearly all 16 serotypes investigated (Wisselink *et al*., 2000). Furthermore, investigation using *S. suis* serotype 2 and 9 found that MRP was a highly immunogenic protein which reacted with piglet sera (Baums and Valentin-Weigand, 2009). Convalescent sera from piglets infected with either MRP+ serotype 2 or MRP* serotype 9 strains generally contain high specific antibody titers (Zhang and Lu, 2007).

2.1.3.4 Extracellular protein factor (EF, *epf*)

EF is a 110-kDa secreted protein encoded by the gene *epf* and was previously identified as a protein associated with virulence in serotype 1 and 2 strains. Because, in previous study, most of the strains isolated from organs of diseased pigs mainly belonged to the MRP^+ EF^+ phenotype (77%), while isolates from tonsils of healthy pigs mainly had the MRP^- EF^- phenotype (86%). So, it had been suggested to be virulence factors that play a role in the pathogenesis of *S. suis* serotype 2 infections in pigs (Vecht *et al*., 1991). However, it had been shown that the *epf* mutants of serotypes 1 and 2 were as virulent as the wild-type in experimental infections indicating that EF is associated but not essential for virulence and the function of EF is still unknown. (Smith *et al*., 1996). EF producing strains have been identified only in serotypes 1, 2, 1/2, 14 and 15 (Wisselink *et al*., 2000). The EF and the high molecular variants of EF are immunogenic proteins. Convalescent phase sera from animals infected either with MRP+ EF+ or MRP+ EF* serotype 2 strains generally contain high antibody titers against EF (Baums and Valentin-Weigand, 2009).

2.1.3.5 The adhesins

In 2012, Fittipaldi and colleagues suggested that *S. suis* have many other proteins containing glyceraldehyde-3-phosphate dehydrogenase (GAPDH) involved in adhesion to host cells (Brassard *et al*, 2004). The 6-phosphogluconate-dehydrogenase (6-PGD), fibronectin binding proteins (Fbps), enolases, amylopullulanase A (AmyA) (Tan *et al*., 2008; Si *et al*., 2009; Ferrando *et al*., 2010) and LPXTG motif-containing adhesins that play a role in interactions with extracellular matrix (ECM) proteins such as fibronectin, plasminogen, fibrinogen, and collagen, (Fittipaldi *et al*., 2012).

2.1.3.6 Other virulence factors

Many other virulence factors of *S. suis* have specific role of the pathogenesis of the infection. Properties and functions of some virulence associated factors are shown in Table 2 and Figure 4.

Table 2 Examples of putative virulence-associated factors of *S. suis*

Properties	Virulence factor	Functions	References
Colonization	Fibronectin binding protein (Fbps)	-Binds human fibronectin and fibrinogen *in vitro*	De Greeff *et al*., 2002
	Enolase	-Binds human plasminogen and fibronectin -Induces the production of antibodies in infected pigs *in vivo*	Pancholi *et al*., 2001 Zhang *et al*., 2009; Esgleas *et al*., 2009
	Dipeptidylpeptidase DppIV	-Interacts with human fibronectin and fibrinogen	Ge *et al*., 2009
	Sortase (SrtA)	-Deletion of SrtA impair *S. suis* binding to ECM proteins	Vanier *et al*., 2008
	LPXTG motif-containing adhesins (including pili)	-Important for interactions of *S. suis* with ECM proteins	Vanier *et al*., 2008
	GAPDH	-Involves in the first step of bacterial adhesion to host cells	Brassard *et al*., 2004; Wang *et al*., 2007

Table 2 (Continued)

Properties	Virulence factor	Functions	References
	IgA protease-producing bacteria	-Blocks the access of intact antibodies	Weiser *et al*., 2003
	AmyA	-Adhesin (porcine epithelium and mucus)	Ferrando *et al*., 2010
	6-PGD	-Adhesin (HEp-2 and HeLa cells)	Tan *et al*., 2008
Avoidance of the host innate immune response	Peptidoglycan *N*-deacetylation	-Reduces killing by neutrophils, probably by providing the bacterium with enhanced resistance against the action of lysozyme	Fittipaldi *et al*., 2008a
	D-alanylation of the LTA	-Enhances resistance to host antimicrobial peptides and resistance to neutrophil killing	Fittipaldi *et al*., 2008b
	Serine protease (SspA)	-Degrades IL-8	Vanier *et al*., 2009
	A cell wall-anchored DNase	-Participates in NETs breakdown	Fontaine *et al*., 2004; Wartha *et al*., 2007
	Superoxide dismutase (SOD)	-Resists to the intracellular environment	Valentin-Weigand P., 2004; Langford *et al*., 1991

ECM: extracellular matrix; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; AmyA: Amylopullulanase A; 6-PGD: 6-phosphogluconate-dehydrogenase; LTA: lipoteichoic acid; NETs: neutrophil extracellular traps

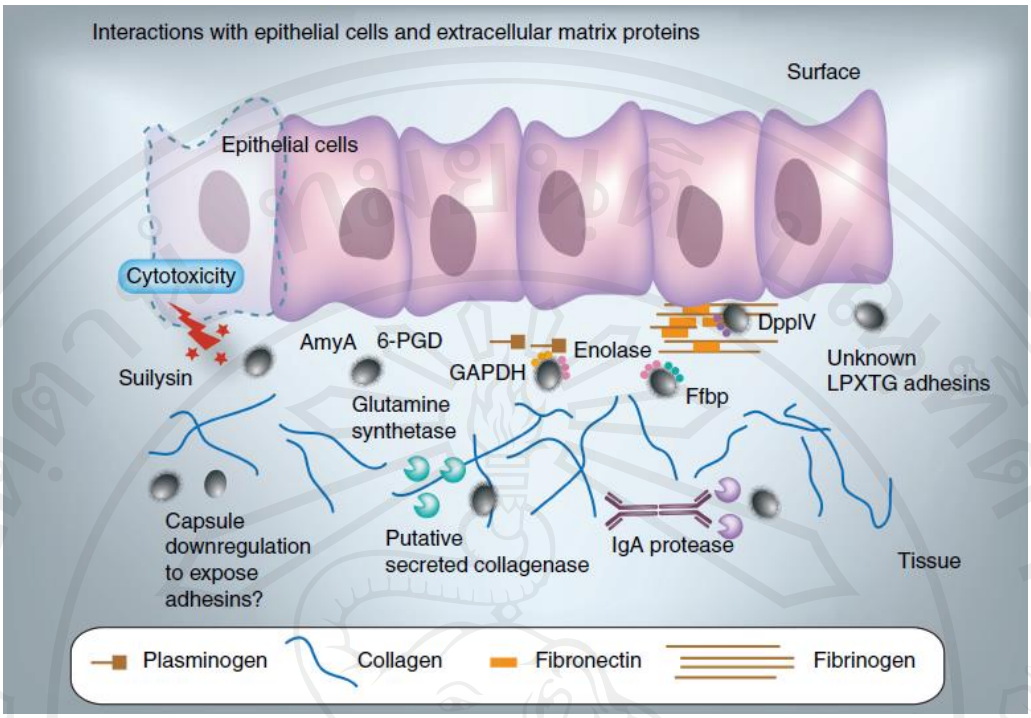


Figure 4 Interactions of *Streptococcus suis* with epithelial cells and extracellular matrix proteins (Fittipaldi *et al.*, 2012). *S. suis* proteins that are involved in cell adhesion are shown.

2.1.4 Pathogenesis of *S. suis* in human

The mechanism of *S. suis* infection is poorly understood as it has been unknown how *S. suis* initially adheres to host cell, how it travels in the bloodstream (Haesebrouck *et al*., 2004), how it is able to translocate through the blood brain barrier (BBB) or the blood cerebrospinal fluid barrier (CSF) in order to cause infections in the central nervous system (CNS). Gottschalk and Segura proposed the hypothesis involves the steps for pathogenesis of meningitis during *S. suis* serotype 2 infection that *S. suis* adhered on epithelial cell layers of upper respiratory tract (colonization) and invade to blood circulation. Hemolysin-positive (Hem+) strains may disrupt cells and invade to reach the blood stream, resulting in septicemia. While the mechanisms used by adhered hemolysin-negative (Hem-) strains are still uncertain (Gottschalk and Segura, 2000) but may use tight junction or alterations to reach the blood stream (Fittipaldi *et al*., 2012). Other routes of entry might inculde skin abrasions and oral contamination followed by intestinal tract translocation (Gottschalk and Segura, 2010). After entering the blood stream, *S. suis* could be, free or directly uptaken by monocytes or macrophages (Mo-associated bacteria) before invasion to blood-brain barrier (BBB). There are 2 proposed mechanisms; firstly, *S. suis* could across to the BBB by binding/adhering or being within monocytes (Trojan horse) and then across directly to the CNS. Secondaly, free *S. suis* could induce BBB permeability, via direct cell damage by suilysin-positive strains and induce local cytokine production that could also increase cell adhesion molecules (CAMs) expression. Consequently, this may lead to induction of leukocytes migration and help free bacteria to enter the CNS, resulting in meningitis (Gottschalk and Segura, 2010) as shown in Figure 5.

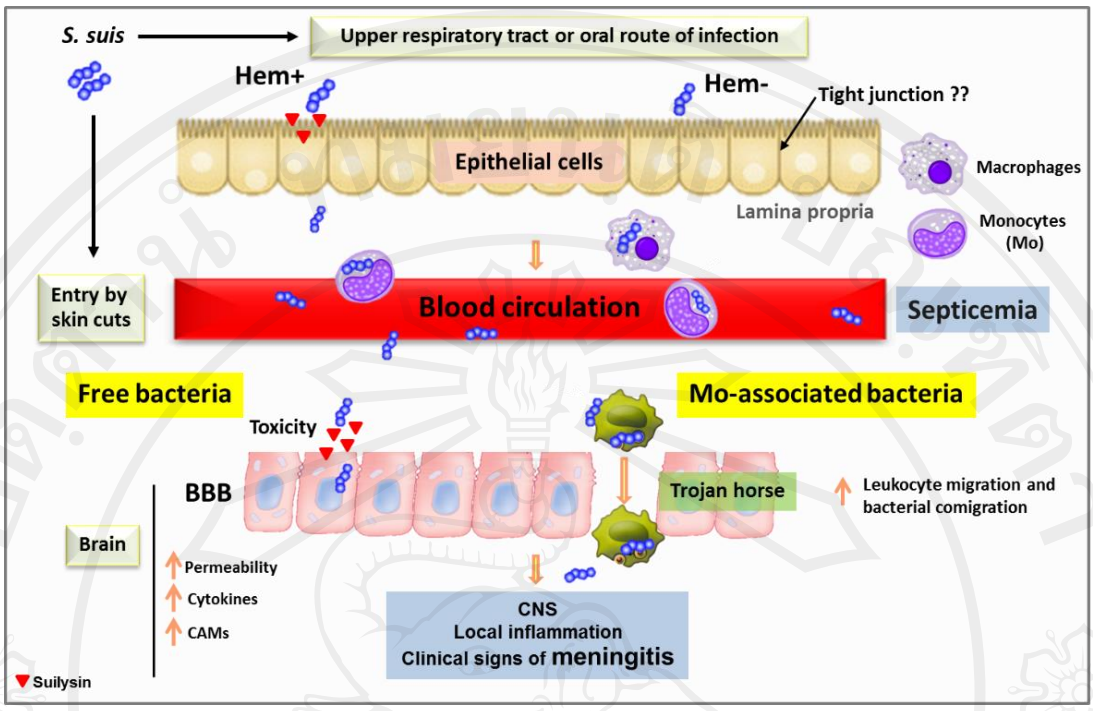


Figure 5 Proposed model for steps in the pathogenesis of *S. suis* serotype 2 infection in human (adapted from Gottschalk and Segura, 2010).

2.2 Interactions of *S. suis* with mammalian cells

The pathogenesis of the *S. suis* infection is unclear and is also possibly the multistep process. In fact, the first steps of *S. suis* to colonize the host cells are poorly understood. The current most accepted hypothesis is that the pathogen breaches the mucosal epithelium in the upper respiratory tract of pigs (Gottschalk and Segura, 2000). Similarly, in humans, *S. suis* may interact with epithelial cells either at the epidermal surface or in the intestine (oral route of infection) (Wertheim *et al*., 2009; Gottschalk *et al*., 2010; Kerdsin *et al*., 2011).

Many studies are available regarding the interactions between *S. suis* and epithelial cells. For example, *S. suis* serotype 2 interactions with human brain microvascular endothelial cells (BMEC) forming the blood-brain barrier (BBB) and human umbilicalvein endothelial cells (HUVEC). Adherence assays showed that *S. suis* adhered only to BMEC, whereas group B streptococci (GBS) adhered to both types of cell. The adherence levels of the acapsular *S. suis* mutant 2A and GBS (COH1-13) were similar to those of wild-type strains S735-SM and GBS (COH1), respectively (Charland *et al*., 2000).

In addition, different interactions of *S. suis* serotype 2 with epithelial cell lines (LLC-PK1: kidney of a normal juvenile pig, PK(15): kidney of an adult pig, A549: human lung carcinoma, HeLa: human cervix carcinoma and MDCK: kidney of normal adult dog) were studied in comparison to GBS. The results showed that *S. suis* serotype 2 was able to adhere to epithelial cells (uncapsulated mutant 2A adheres epithelial cells at higher degree than the parent strains) but not to invade all epithelial cells. In contrast, GBS strain COH1 was able to invade the human epithelial cell lines A549 and HeLa. (Lalonde *et al*., 2000). Therefore, it is possible that the attachment of bacteria to host cells and tissue and their subsequent invasion and spreading are key processes during pathogenesis. Then, a systematic identification of the surface proteins of *S. suis* serotype 2 that may interact with host cells is necessary for understanding the pathogenesis of *S. suis* serotype 2 (Chen *et al*., 2011).

2.3 Proteomics of *S. suis*

Recently, the many studies of bacterial proteomes is being conducted to discover virulence factors of pathogenic bacteria in order to understand pathogenesis or identify drug targets or vaccines. All protein components are usually identified by special techniques such as Mass spectrometry (matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) and Liquid chromatography–mass spectrometry (LC-MS)). Proteome or proteomics is the large-scale study of proteins, particularly their structures and functions (Anderson NL and Anderson NG, 1998; Blackstock WP and Weir MP, 1999). Proteomics is interesting because it gives a much better understanding of an organism than genomics. In this literature review, the types of protein were divided into 3 groups: group 1, cell-associated proteins; group 2, extracellular and secretory proteins; and group 3, other novel proteins.

2.3.1 Cell -associated proteins

Generally, the cell walls of gram-positive bacteria contains variety of proteins with several functions including the transport of substances and sensing the surrounding environment of the cell. Many virulence determinants in *S. suis* are surface proteins, such as MRP, Sao protein, which provide the organisms to be able to resist to various compounds as well as interfere with host immune defense mechanisms.

2.3.1.1 Membrane-associated proteins

Examples of membrane-associated proteins and extracellular proteins of *S. suis* serotype 2 were studied in the strains isolated from a pig with septicemia (strain HA9801) by immunoproteomic assay (western blot combined with MALDI-TOF MS). The membrane-associated proteins that were specific to virulent strain and recognized by the immune sera (three specific pathogen free (SPF)) of minipigs identified in that study were endothelin-converting enzyme 1 (ECE-1), elongation factor Tu (EF-Tu), extracellular solute-binding protein, hypothetical protein, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), arcA, pyridine nucleotide-disulphide oxidoreductase, E1α

subunit of pyruvate dehydrogenase complex, l-lactate dehydrogenase, cysteine synthase K/M: cysteine synthase A/S, extracellular solute-binding protein (belong to family 3).

Amongst these identified proteins, ECE has been reported to be a potent vasoconstrictor that exhibits mitogenic activity in vascular smooth muscle cells (SMCs) and has been implicated in the pathophysiology of cardiac diseases including hypertension, vascular hypertrophy and atherosclerosis. EF-Tu is a very conservative surface protein of bacteria, and it was also frequently detected by immunoproteomics in other bacteria, such as *Helicobacter pylori* (Haas *et al.*, 2002) and *Shigella flexneri* (Ying *et al*., 2005). The pyridine nucleotide-disulphide oxidoreductase of SS2 may play a role in catalyzing the NAD+-dependent reoxidation of dihydrolipoamide (DLA) in many multienzyme complexes. The E1α subunit of the pyruvate dehydrogenase complex (PDC) is similar to the E1 components of the PDC that occupies a critical position in energy metabolism. In addition, GAPDH was reported to be involved in the first step of bacterial adhesion to host cells and exhibits a high affinity for plasmin and a significantly lower affinity for plasminogen (Bergmann *et al.,* 2004). L-lactate dehydrogenase was identified as one of the predominant immunogenic proteins in pigs naturally or experimentally infected with *Mycoplasma hyopneumoniae* (Frey *et al*., 1994). The newly identified membrane-associated antigens of SS2 are of great interest in terms of understanding the pathogen–host interaction and as novel vaccine candidates (Zhang and Wu, 2007a).

2.3.1.2 Cell wall- asoociated proteins

The cell wall components of *S. suis* SS2 that were identified as immunogenic proteins which were recognized by hyperimmune sera and convalescent sera of minipig including MRP, GAPDH, Sao, amylase binding protein B, Endothelin-converting enzyme 1, and putative 5'-nucleotidase (Zhang and Lu, 2007b). MRP proved to be immunogenic and can induce high antibody titers after pig immunization which conferred protection in pig challenge experiments (Okwumabua *et al*., 2005). Sao elicited a significant humoral antibody response and proved to be a promising vaccine candidate (Li *et al*., 2006).

Other cell wall proteins, which had been reported to be immunogenic, had significant sequence similarity to known proteins whose functions have been biochemically demonstrated in other organisms including molecular chaperone (similar to *S. pneumonia* (Kim *et al*., 1998) and *S. pyogenes* (Lemos *et al*., 2000)), translation EF-Tu similar to that found in *Chlamydia trachomatis* (Bini *et al*., 1996) and *S. flexneri* (Ying *et al*., 2005), basic membrane lipoprotein, putative cyclo-nucleotide phosphodiesterase, ABC transporter substrate-binding protein-maltose/maltodextrin, and ABC-type phosphate transport system, ATPase component, and RiGE which was 83% homologous with Sao.

The cell wall components unique to SS2 that had been identified including hypothetical protein SSU98_0197 and translation initiation factor 2 which has a LPXTG anchor motif. Both proteins have no reports of the immunogenicity (Zhang *et al*., 2008).

2.3.1.3 Surface proteins

In addition to, *S. suis* serotype 2, proteomic studies of *S. suis* serotype 9 (SS9) have been reported. In 2008, Wu and colleagues investigated the surface proteins of three SS9 strains (GZ0565, GD0606, SH0626) that were isolated from the organ of diseased pigs. It was found that 8 surface proteins, such as arginine deiminase, extracellular solute-binding protein, translation elongation factor Ts, neprilysin, peptide ATP-binding cassette transporter peptide-binding protein, pyruvate kinase, phosphate acetyltransferase and fructose-bisphosphate aldolase, were immunogenic.

Of the proteins identified, arginine deiminase (AD) is a member of the arginine deiminase system, found in many bacteria including streptococci and enterococci (Curran *et al*., 1998). Ef-Ts is essential for the elongation of the polypeptide chain during protein synthesis. Ef-Ts expressed by all reference strains of all *S. suis* serotypes are antigenically similar, as determined by Western blot analysis (Martinz *et al*., 2003). Thus, Ef-Ts would be a promising candidate antigen for the development of a universal vaccine.

Some of the proteins identified in SS9, of which the immunogenic nature of is a novel observation including neprilysin, peptide ATP-binding cassette (ABC) transporter peptide-binding protein, pyruvate kinase, phosphate acetyltransferase, and fructosebisphosphate aldolase. Amongst those proteins, the *S. suis* neprilysin is a Zn^{2+} metallopeptidase involved in the degradation of biologically active peptides (Dion *et al*., 1995). The role of the bacterial enzyme is uncertain while the mammalian enzymes have been shown to play essential roles in events such as inflammatory response phenomena and in pain and cardiovascular regulation (Froeliger *et al*., 1999).

Rodriguez-Ortega and colleagues also identified a set of surface proteins of *S. suis* serotype 2 strain 235/02 (isolated from infected pig) by combining proteomics with computational analysis (LC-MS/MS). Examples of identified proteins from theirs study were MRP, subtilisin-like serine protease, DNA nuclease, hypothetical protein SSU05_0196, hypothetical protein SSU05_1311, agglutinin receptor, type II secretory pathway, ribonucleases G and E, hypothetical protein SSU05_1295, ABC-type xylose transport system.

All of them corresponding to the 4 categories of surface proteins of gram-positive bacteria (Navarre and Schneewind, 1999). For examples, **LPXTG-cell wall proteins** that containing a peptidoglycan-anchoring motif in the *C*-terminus of the protein (the LPXTG motif); **lipoproteins**, which linked to the underlying plasma membrane through a lipid covalently bound at their *N*-terminus; **secreted proteins**, which can bind to the surface by charge/ hydrophobic interactions; and **membrane proteins** that embedded in the plasma membrane underlying the wall through at least one transmembrane helix (TMH).

In 2010, Mandanici and colleagues have identified a cell wall anchored protein of *S. suis* serotype 2, showing protective capacity in immunized mice against infection by a virulent strain (P1/7). Their results are in accordance to the proteins that had been reported previously by Geng and colleagues (Geng *et al*., 2008), for example, LPXTG cell wall proteins, lipoprotein, secreted proteins, membrane and multitransmembrane proteins.

In addition, a novel method for identifying bacterial surface proteins was used in the study of Zhang and colleagues. This novel method that combined immunoproteomic and immunoserologic techniques was established for identifying bacterial cell surface proteins. The *S. suis* surface proteins identified in their study included MRP and ABC transporter protein. MRP is an important protective antigen in SS2, while a periplasmic ABC transporter protein is a new immunoreactive protein from *S. suis* identified for the first time. Moreover, ABC transporters are associated with virulence in bacteria. They import various nutrients required for survival in different niches and export substances toxic to the cell (Garmory and Titball, 2004). Thus, this transporter may be involved in the pathogenesis of *S. suis* by facilitating bacterial attachment to host cells (Zhang *et al*., 2011).

Later, Chen and colleaques (2011) have identified the potential surface interacting protein (SIPs) of *S. suis* serotype 2 (SS2) which can interact with the surface molecules of Hep-2 cells. The total of 40 SIPs were identified and suggested to be involved in the adherence of SS2, and most of them are predicted to be cytoplasmic proteins (non-LPXTG proteins). Notably, non-LPXTG proteins from SS2 have been reported to play a major role in the adherence of SS2 to ECM protein (Vanier *et al*., 2008). Amongst these, 3 SIPs (enolase, FBPS and GAPDH) that had been previously reported as surface proteins for SS2 adherence.

A previous study by Gomez-Gascon and colleagues had analyzed the proteomics of a collection of 39 strains obtained from infected pigs (in farms of Spain and the Netherlands). The global "pan-surfome", which is the set of all the proteins found in the whole collection, corresponded to 113 proteins, of which 33 corresponded to the lipoproteins category (out of 42 predicted in the P1/7 genome, representing 78.6% of total) where as cell wall proteins identified represented 85% of those predicted in the genome. Of all proteins identified, SsnA appears to be the best candidate for further vaccination studies. SsnA is a surface-located protein in most of the serotypes of the zoonotic pathogen *S. suis*. This protein has an attractive potential for a vaccine, as it is well exposed and highly accessible to antibodies (as demonstrated by flow cytometry), being in addition very immunogenic (Gomez-Gascon *et al*., 2012).

2.3.2 Extracellular and Secretory proteins

The extracellular or secretory proteins of bacterial pathogen have been shown to play an important role in pathogenesis. In *S. suis*, a number of extracellular or secreted proteins have been identified, which most likely represent only a small part of the secretome of this pathogen.

2.3.2.1 Extracellular proteins

The immunogenic extracellular proteins identified in both virulent (ZY05719, HA9801) and avirulent (T15) strains of SS2 were performed by 2D gel and MALDI-TOF-MS. Those proteins, which were DNA nuclease, serine-type-D-Ala-D-Ala carboxypeptidase, could react with convalescent serum of a specific pathogen free (SPF) mini-pig (Zhang and Lu, 2007b). Whereas the proteins that were differentially expressed only in virulent strains had also been identified. Those proteins were extracellular protein factor (EF), muramidase released protein (MRP), DNA nuclease, enolase, suilysin (SLY) serine, ECE-1, GroEL, AbpB, CHAP, heat shock protein (Hsp70). Of all the proteins identified, EF protein and MRP from SS2 were proved to play a role in the pathogenesis of SS2 infections in pigs and human patients (Vecht *et al*., 1991).

Other extracellular proteins that were immunogenic including ECE-1 (ZY12) and enolase (ZY16). (Bergmann *et al*., 2001; Carson *et al*., 2002). Enolase may contribute to pathogenicity by binding the plasminogen of an infected host, potentially allowing the bacteria to acquire surface-associated proteolytic activity (Bergmann *et al*., 2001; Pancholi and Fischetti, 1998).

2.3.2.2 Secretory proteins

The secretory proteins of SS2 strain (strains 98012, 200505, NJ and 606) was identified using IPG strips of pH 4-7 (2-DE). Among the 77 proteins identified, 5 known secretory proteins containing extracelluar factor (SSU0171), streptolysin O (SSU1231), putative endopeptidase (SSU0152), hyaluronate lyase (SSU1053), and putative amidase (SSU0020) were observed, which were predicted to have a signal peptide. Moreover, they identified a novel protein putative endopeptidase (SSU0152)

proposed as putative virulence-associated factor in other microorgnisms (Ansai *et al*., 2003; Slomiany and Slomiany, 2005).

Besides, proteomic studies of *S. suis* serotype 9 (SS9) strain GZ0565 have been reported by Wu and colleagues. A comparative proteomics between 2 SS9 isolates from diseased (GZ0565) and healthy pigs (SH040917) were performed using 2-DE combined with MALDI-TOF-MS and MALDI-TOF/TOF-MS. Of 13 metabolism-related proteins identified, 5 were proposed as putative virulence-associated factors such as DNA nuclease, *o*-acetylserine lyase, peptidoglycan-binding LysM, phosphoglycerate mutase, and putative 50-nucleotidase. Interestingly, DNA nuclease is also found as a potential virulence factor in *Streptococcus pyogenes* (Matsumoto *et al*., 2005). The nuclease has been reported to digest DNA released from dead cells, reduces the viscosity of pus, and allows the organism greater motility (Bisno *et al*., 2003). In *S. suis*, DNaes was reported to contribute to degradation of neutrophil extracellular traps (NETs) and evasion of NET-mediated antimicrobial activity.

In 2011, secreted proteins of SS9 strain GZ0565 were screened again. Eleven out of 16 protein spots were successfully identified immunoreactive such as sortases, ABC transporter substrate-binding protein–maltose/maltodextrin, ABC transporter periplasmic protein, CHAP domain containing protein, peptidoglycan-binding LysM, elongation factor Tu, elongation factor G, thymidine kinase, molecular chaperone DnaK, hypothetical protein SSU98_2184. Interestingly, sortases, an enzyme involved in the covalent linkage of surface proteins to the peptidoglycan (Race *et al.* 2009). They play critical roles in the virulence of Gram-positive bacterial pathogens such as SS2 (Wang *et al.* 2009), *Streptococcus agalactiae* (Lalioui *et al*. 2005), *Streptococcus pneumonia* (Paterson and Mitchell, 2006), and *Listeria monocytogenes* (Bierne *et al*. 2002).

Furthermore, proteomic analysis of *S. suis* serotype 2 strain 98012 determined using two-dimensional gel electrophoresis (2-DE) combined with mass spectrometry showed that most of the proteins identified were located in the cytoplasm and involved in energy metabolism, protein synthesis and cellular processes (Jing *et al*. 2008). Some virulence-associated proteins of SS2 were found, including arginine

deiminase, ornithine carbamoyl-transferase, carbamate kinase, muramidase-released protein precursor, extracellur factor, and suilysin.

Some of the proteins identified in previous study were also found in the whole-cell protein expressions of SS grown under either biofilm or planktonic conditions. Those proteins that were up-regulated under conditions of biofilm growth including GAPDH, UDP-N-acetylglucosamine 1-carboxyvinyltransferase 2, putative pyruvate dehydrogenase E1 component alpha subunit, ornithine carbamonyltransferase whereas some was down-regulated including ABC transporter periplasmic-binding protein, fructose-bisphosphate aldolase.

Interestingly, the common immunogenic proteins (hemolysin, GAPDH, PDH and DnaK) were identified under both planktonic and biofilm growth condition. Notably, GAPDH is a SS surface protein and mediates cell adhesion and plays an important role in bacterial infection and invasion (Brassard *et al*., 2004; Wang and Lu, 2007). It has also been reported that GADPH is an immunogenic protein found on the cell wall of SS (Zhang *et al*., 2008). It had been suggested that the proteins common to both growth conditions could be promising vaccine candidates to prevent both biofilm infections and acute infections (Wang *et al*., 2012).

2.3.3 Other novel proteins

In 2013, Zhang and colleagues identified a novel collagen type I-binding protein from SS2 or called Cbp40, which mediates the adhesion of SS2 to collagen. as demonstrated by western blotting and ELISA. This protein also confered good protection against highly pathogenic *S. suis* infection in zebrafish, which indicated that the protein has the potential to be developed into a vaccine. Conversely, deletion of the *cbp40* gene leading to the reduction of collagen binding activity may affect the formation of *S. suis* biofilm, reduce bacterial adhesion to HEp-2 cells and reduce virulence in a zebrafish infection model. Moreover, their results showed that Cbp40 contributed to cytokine/chemokine induction likely to result neutrophil recruitment and activation. Collectively, these data suggest that Cbp40 plays an important role as an ECM adhesion protein that interacts with host cells during infection (Zhang *et al*., 2013a).

Later, a putative Lmb protein (CDS 0330) of a highly virulent strain of *S. suis* serotype 2 was identified by Zhang and colleagues (2013). Their results indicated that Lmb is a laminin binding protein. It was found to expressed on the surface of several pathogenic streptococci. This protein could mediated attachment of *S. agalactiae* to human laminin (Spellerberg *et al*. 1999). In addition to laminin binding, Lmb has also been identified as a zinc-binding protein (Linke *et al*. 2009; Ragunathan *et al*. 2009; Bayle *et al*. 2011). Importantly, they have detected the *CDS 0330* gene in all sequenced *S. suis* 2 strains of different geographic origins. Furthermore, they and several other researcher groups have reported that Lmb expressed *in vivo* could elicit an immune response against infection (Wahid *et al*. 2005). Vaccination with recombinant CDS 0330 protected mice from infection of the highly pathogenic *S. suis* serotype 2. Taken together, their data suggested that CDS 0330 is the *lmb* gene of *S. suis* serotype 2 and confers protection against infection of a highly virulent strain (Zhang *et al*., 2013b).

From the literature review, significant progress has been made in the characterization of *S. suis* using a proteomic approaches. Pathogenic determinants are identified through proteomics among virulent and avirulent isolates *S. suis* serotype 2 and serotype 9. Those proteins included specific surface proteins, secreted proteins, extracellular proteins, membrane-associated proteins, cell wall-associated proteins, whole cell proteins and novel proteins. None of the studies have yet been investigated proteins of *S. suis* during exposure to murine or human macrophages. Thus, this study was aimed to investigate the protein profiles of *S. suis* serotype 2 and serotype 14 ,isolated from healthy pig and patient in Northern Thailand, while exposure to murine macrophage (RAW 264.7) and human macrophage (U 937) cell line. Comparative proteomic analyses of relevant strains may lead to the improved understanding of how this *S. suis* interact with host macrophages.

Table 3 Examples of Proteomic studies in *S. suis* serotype 2 and serotype 9

SS	Strains	Sources	Components of *S. suis*	Techniques	No. of proteins #	Ref.
2	HA9801	Diseased pig	Membrane-associated proteins	2DE, MALDI-TOF MS	11	Zhang and Lu, 2007a
2	ZY05719 HA9801 T-15	Diseased pig Diseased pig Healthy pig	Extracellular proteins	2DE, SDS-PAGE, MALDI-TOF MS	9	Zhang and Lu, 2007b
2	98012 200505 NJ, 606	Patient Diseased pig Diseased pig	Whole-cell proteins Secretory proteins	2DE, MALDI-TOF MS, ESI-MS/MS	373 77	Jing *et al*., 2008
9	GZ0565 GD0606 SH0626	Diseased pig Diseased pig Diseased pig	Surface proteins	2D-gel, SDS-PAGE, MALDI-TOF MS	8	Wu *et al*., 2008a
9	GZ0565 SH040917	Diseased pig Healthy pig	Secretory proteins	2D-gel, MALDI-TOF MS	13	Wu *et al*., 2008b
2	ZYS	Diseased pig	Cell wall-associated proteins	2DE, Western blot, MALDI-TOF, LC-MS/MS	34	Zhang *et al*., 2008
2	235/02	Infected pig	Surface proteins	LC-MS/MS	28	Rodriguez-Ortega *et al*., 2008

SS: *S. suis* serotype; Ref.: reference

Table 3 (Continued)

SS	Strains	Sources	Components of *S. suis*	Techniques	No. of proteins#	Ref.
2	P 1/7	Diseased pig	Surface proteins	LC-MS/MS	32	Mandanici *et al*., 2010
2	HA9801 T15	Diseased pig Healthy pig	Surface proteins	2DE, Western blot, MALDI-TOF MS	2	Zhang *et al*., 2011
9	GZ0565	Diseased pig	Secreted protein	2DE, Western blot, MALDI-TOF MS	10	Wu *et al*., 2011
2	ZYS	Diseased pig	Surface proteins	ACSP*, LC-MS/MS	40	Chen *et al*., 2011
2	HA9801	Diseased pig	Whole-cell proteins	2DE, Western blot, MALDI-TOF/TOF MS	13	Wang *et al*., 2012
1-19	39 strains	Infected pig	Surface proteins	LC-MS/MS	113	Gomez-Gascon *et al*., 2012

Table 3 (Continued)

SS	Strains	Sources	Components of *S. suis*	Techniques	No. of proteins[#]	Ref.
2	HA9801 T15 ZY05719	Diseased pig Healthy pig Diseased pig	Cbp40 protein	Collagen-binding assay, ELISA	1	Zhang *et al*., 2013a
2	05ZYH33	Patient	laminin-binding protein (Lmb: CDS 0330)	SDS-PAGE, Western blot	1	Zhang *et al*., 2013b

SS: *S. suis* serotype; Ref.: reference

[#] Number of proteins were identified in each studies; Lmb: laminin-binding protein

* ACSP: affinity chromatography and shotgun proteomics

2DE: two-dimensional gel electrophoresis; MS: mass spectrometry

SDS-PAGE: Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis

MALDI-TOF: matrix-assisted laser desorption ionization time-of-flight

ESI-MS: electrospray tandem mass spectrometry

LC-MS: Liquid chromatography–mass spectrometry