A serine/threonine phosphatase 1 of *Streptococcus suis* type 2 is an important virulence factor

Lihua Fang¹,², Jingjing Zhou¹, Pengcheng Fan¹, Yunkai Yang¹, Hongxia Shen¹, Weihuan Fang¹,*

¹Zhejiang University Institute of Preventive Veterinary Medicine and Zhejiang Provincial Key Laboratory of Preventive Veterinary Medicine, Hangzhou 310058, China
²Center for Synthetic Biology Engineering Research, Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences, Shenzhen 518055, China

*Streptococcus suis* is regarded as one of the major pathogens of pigs, and *Streptococcus suis* type 2 (SS2) is considered a zoonotic bacterium based on its ability to cause meningitis and streptococcal toxic shock-like syndrome in humans. Many bacterial species contain genes encoding serine/threonine protein phosphatases (STPs) responsible for dephosphorylation of their substrates in a single reaction step. This study investigated the role of *stp1* in the pathogenesis of SS2. An isogenic *stp1* mutant (Δ*stp1*) was constructed from SS2 strain ZJ081101. The Δ*stp1* mutant exhibited a significant increase in adhesion to HEp-2 and bEnd.3 cells as well as increased survival in RAW264.7 cells, as compared to the parent strain. Increased survival in macrophage cells might be related to resistance to reactive oxygen species since the Δ*stp1* mutant was more resistant than its parent strain to paraquat-induced oxidative stress. However, compared to parent strain virulence, deletion of *stp1* significantly attenuated virulence of SS2 in mice, as shown by the nearly double lethal dose 50 value and the lower bacterial load in organs and blood in the murine model. We conclude that Stp1 has an essential role in SS2 virulence.

**Keywords:** *Streptococcus suis* type 2, serine/threonine protein phosphatase, virulence

**Introduction**

*Streptococcus suis* type 2 (SS2), which causes septicemia, meningoencephalitis, and arthritis, is an important pathogen of pigs and humans [15]. Strains with high pathogenicity can even cause toxic shock syndrome in humans [10]. Incidence of SS2-related diseases in humans working with pigs is increasing around the world [12]. The bacterium enters the swine host through the respiratory tract and after colonization in the tonsils reaches the central nervous system via blood circulation, leading to fatal septicemia or meningoencephalitis [35]. Considerable progress has been made in recent years in deciphering the SS2 virulence factors, such as muramidase-releasing protein, suilysin, s-ribosylhomocysteinase, SalK/SsalR (a two-component system of the 89K PAI) and CovR (orphan response regulator) [4,10]. The results of such studies have led to a better understanding of the pathogenesis of SS2.

Regulation of protein function or enzyme activity by reversible phosphorylation is important for maintenance of cellular responses to dynamic internal or external environmental conditions in both prokaryotes and eukaryotes [26]. Serine/threonine protein phosphatases (STPs) dephosphorylate their substrate proteins in a single reaction step [24]. The STPs are divided into two large families, the phosphoprotein phosphatase P (PPP; PP1, PP2A, and PP2B) and the phosphoprotein phosphatase M family (PPM; PP2C), on the bases of sequence similarity, metal ion dependency, and inhibitor sensitivity [28]. Based on sequence similarities, *Streptococcus* (*S*.) *suis* STP belongs to the PP2C subfamily of the PPM [3].

Studies on prokaryotic STPs have increased in number in recent years. The STP secreted from *S. pyogenes* is apoptotic in HEp-2 cells [1]. *Mycoplasma genitalium* expresses STP critical for its virulence [21]. In *Staphylococcus aureus*, STP1 contributes to reduced virulence and susceptibility to vancomycin [7]. *SipI* of group B *Streptococcus* mediates post-transcriptional regulation of hemolysin, autolysis, and virulence [24]. A *Streptococcus suis* serotype 9 (SS9) strain contains *stp* gene that was involved in virulence [37]. There are two STPs in SS2 (encoded by *stp1* and *stp2*) that belong to the PPM and PP families, respectively [25]. Homology of the amino acid sequences of *stp1* between SS9 and SS2 is 99.0%, while it is 23.1% between SS9 *stp1* and...
SS2 stp2. Functions of STP in SS2 infection remain unreported to date. This study examined whether Stp1 of SS2 is a virulence factor by testing the effects of a stp1 deletion mutant in cultured cells and mice.

Materials and Methods

Bacterial strains and culture conditions

The strains of S. suis and Escherichia (E.) coli used for cloning and mutant construction are listed in Table 1. The SS2 strain ZJ081101 was isolated from the lungs of a diseased pig. Brain heart infusion (BHI; Oxoid, UK) was used for culturing S. suis at 37°C, while Luria-Bertani broth or Luria-Bertani agar was used for culturing E. coli. For cloning and the selection of mutants, the following antibiotics (Sigma-Aldrich, USA) were mixed with the culture media: spectinomycin at 100 mg/mL for SS2 and 50 mg/mL for E. coli; chloramphenicol at 4 mg/mL for SS2 and 8 mg/mL for E. coli; and ampicillin at 100 mg/mL for E. coli.

Table 2. Primers used for PCR amplification

| Primers | Primers sequence (5’ to 3’) | Functions |
|---------|-----------------------------|-----------|
| L1-Sphl | ACA GCATGC ACG TCTATCCGTTCCAGTATTC | Region upstream of stp1 |
| L2-Sall | CGC GTCGAC GAA CGTTTTCTGTTACCTACA | Region downstream of stp1 |
| R1-BamHI | ACG GGATCC TCGTTCACATTACGAGG | CatR gene cassette |
| R2-EcoRI | CGC GAAATTC GAACTGTTGTATGGCCGTACT | An internal fragment of stp1 |
| Cat1-Sall | CGC GTCGAC CACCGAACTAGACTGAGG | Mutant identification |
| Cat2-BamHI | ACG GGATCC TAA ATCGGATGCGTCCGAGG | For expressing Stp1 |
| In1-Stp1 | TGATGGAATGGGTGGTC | Mutant identification |
| In2-Stp1 | TGAGGGAATGGGTGGTC | |
| Out1-Stp1 | TTAAGGGAATGGGTGGTC | |
| Out2-Stp1 | GGACTGTAACGTCGCTGGGA | |
| ES1-Stp1-EcoRI | GGTTGAAATGCGAATGCTATTACTG | |
| ES2-Stp1-BglII | TCGAGATCTTACCGTCCGTTACCGTAAATG | |
| Gdh-F | CCAAGCAAGATACAAAGATGG | |
| Gdh-R | GCAAGGTATCTGGTTCAACAG | |
| Cps2j-F | TGAGTCTTTATACACCTGTT | |
| Cps2j-R | AGAAAATTCATATTGTCCACC | |
Serine/threonine phosphatase 1 is critical for SS2

Cloning, expression, and purification of recombinant Stp1

The ORF of stp1 was amplified from the genome DNA of SS2 strain ZJ081101 by PCR with the primer pairs ES1-Stp1/ES2-Stp1 (Table 2). The verified complete gene was cloned, using the EcoRI and BglII sites, into pET-32a (Invitrogen, USA) which contains a 22 kDa His-tag-encoding sequence. The recombinant plasmid pET-Std1 was transformed into E. coli Rosetta (DE3) for expression of the recombinant Stp1 (rStp1). E. coli pET-Stp1 cells in their log phase of growth were induced with 1 mmol/L of isopropyl-β-D–thiogalactopyranoside at 37°C for 4 h. The rStp1 protein was purified by Ni2+-nitrilotriacetic acid affinity chromatography and concentrated by ultrafiltration (Millipore, USA). The filtrates were passed through a 0.22-μm membrane (Millipore) and held at −80°C until use.

Phosphatase assays

p-Nitrophenyl phosphate (pNPP) was used as a substrate as described [16] in 500 μL of phosphatase buffer [50 mM Tris HCl (pH 8.0), and 2 mM MnCl2] containing 20 mM pNPP and 2 μg of Stp1. Hydrolysis of pNPP in the samples was measured after incubation for 10 min by determining the increase in absorbance of p-nitrophenol (pNP) at 405 nm (SpectraMax M2; Molecular Devices, USA).

Preparation of cytoplasmic and cell-wall proteins from whole bacterial lysates

The SS2 strains, both wild-type and mutant, were incubated at 37°C for 6 to 7 h and used to prepare cytoplasmic and cell-wall proteins from whole bacterial cells for SDS-PAGE and western blotting. Whole bacterial lysates were prepared by treating the bacterial suspensions in a Precellys 24 homogenizer (Bertin Instruments, France) in the presence of 0.1 mm ceramic beads at 3,360 × g repeated 5 times for 30 sec each. The supernatant samples were collected as whole cell cytoplasmic proteins (CPP, cytoplasmic proteins; CWP, cell-wall proteins).

Western blot

The Bradford method was used to quantify the concentrations [29] from 30% to 100% and transferred to absolute acetone for 20 min. Specimens were then placed at room temperature in a mixture of absolute acetone and resin at a 1:1 ratio for 1 h, transferred to a 1.3 ratio of absolute acetone to resin mixture for 3 h, and to a Spurr’s resin mixture for overnight. Before thin sectioning, specimens were embedded in capsules containing the embedding medium and heated at 70°C for approximately 9 h. The bacterial sections were stained by uranyl acetate and alkaline lead citrate, each for 15 min, and inspected via transmission electron microscopy (Model H-7650; Hitachi, Japan).

Bacterial morphology by transmission electron microscope

This was carried out essentially as described by Neiss et al. [22]. Briefly, the bacterial cells were subjected to fixation in 2.5% glutaraldehyde in phosphate buffer (pH 7.0) and treatment with 1% OsO4 in phosphate buffer, each followed by washing. Specimens were then dehydrated by a graded series of ethanol
of the protein samples. Thirty micrograms of each protein sample were subjected to SDS-PAGE on 12% gels and transferred onto PVDF membranes (Millipore). The membranes were blocked with Tris-buffered saline containing 0.1% Tween 20 and 5% non-fat milk and subjected to probing with the antisera to rStp1 or rGAPDH and to HRP-conjugated anti-rabbit IgG [Multisciences (Lianke) Biotech, China]. Chemiluminescence substrate (SuperSignal West Femto Maximum Sensitivity Substrate; Thermo Fisher Scientific, USA) was used to visualize the protein bands.

**Resistance to peroxides in vitro**

The mutant strain Δstp1 and its parent strain were grown in BHI containing gradient concentrations of H2O2 or paraquat (a chemical generator of superoxide anions) [6] and incubated at 37°C. The cultures were examined at 620 nm after 4 and 8 h of incubation on SpectraMax M2 microplate reader (Molecular Devices).

**In vitro adhesion assay**

Adhesion of the Δstp1 mutant and its parent strain to murine endothelial cell line bEnd.3 and human epithelial cell line HEp-2 was determined as previously described [17]. Bacterial cultures at the mid-log phase (10⁶ CFU/well; CFU, colony-forming unit) were added to the cells in 24-well culture plates at 100 multiplicity of infection (MOI). The plates were spun at 800 × g for 10 min and subjected to incubation for 1 h incubation at 37°C and 5% CO₂. The infected cells were washed with PBS and disrupted by repeated pipetting with sterile distilled water. The cell lysates were transferred to Eppendorf tubes and vortexed to release the bacteria. The samples were then 10-fold diluted in PBS and plated on BHI agar plates for bacterial counting. Adhesion (%) was defined as (CFUadh/CFUtotal) × 100 [33].

**Phagocytosis and intracellular survival**

The murine macrophage cell line RAW264.7 was used to examine the effect of stp1 deletion on phagocytosis and intracellular survival according to a previously reported protocol [8]. Briefly, the cells were incubated in RPMI 1640 medium containing 10% fetal calf serum (Gibco, USA) in 24-well plates. Wild-type and mutant SS2 strains were incubated in BHI broth for 6 h at 37°C, pelleted by centrifugation, and resuspended in sterile PBS. The cells were incubated for 30 min at 37°C and 100 MOI, washed, and incubated for 1 h in fresh RPMI 1640 medium containing gentamicin (100 mg/mL) and penicillin G (5 mg/mL) to inactivate extracellular bacteria. Cells were then washed and incubated in the fresh medium containing 20 mg/mL of gentamicin. The cells were subjected to further incubation at 37°C and 5% CO₂ for 1 or 2 h. After incubation, the cells were trypsinized and disrupted by repeated pipetting for bacterial enumeration on BHI agar plates. Survival (%) was calculated as CFU₂h/CFU₁h × 100.

**Animal infections and determination of bacterial load in organs**

BALB/c mice were used in experimental infections to evaluate the role of stp1 in SS2 virulence [36]. For determination of lethal dose 50 (LD₅₀), eleven groups of 4-week-old female BALB/c mice (10 per group) were randomly allocated. Five groups were assigned to each strain (wild-type or its mutant Δstp1), and the last group of each strain received PBS as a sham control treatment. Five gradient inoculum sizes were used for intraperitoneal infection. Mortality was recorded twice a day for 7 days post-infection. The improved Karber’s method was used to calculate LD₅₀. The bacterial burdens in blood and organs were examined in mice receiving intraperitoneal inoculation of the wild-type strain or its mutant (10 mice per strain) at 6.0–8.0 × 10⁸ CFU/mouse. Mice were euthanized at 24 and 48 h post-infection (hpi), and their brain, kidney, liver, and spleen were removed and homogenized individually in 1 mL PBS at pH 7.4. The homogenates were 10-fold diluted in PBS and appropriate dilutions were plated onto BHI agar plates. Anti-coagulant blood samples (100 µL) were collected at 24 and 48 hpi. Animal experiments were conducted following the guidelines and approved protocols of the Laboratory Animal Management Committee of Zhejiang University (approval No.2015029).

**Statistical analysis**

All data are expressed as mean ± SD values of three independent experiments, each performed in triplicate in wells or tubes. The two-tailed Student’s t-test was used to determine the statistical significance of differences between the stp1 deletion mutant and its parent strain.

**Results**

**Construction of stp1 defective mutant**

A stp1 knockout mutant was produced by electroporating suicide vector pSET4s containing a CatR cassette (panel A in Fig. 1) into wild-type competent SS2 cells and was used to evaluate the role of stp1 in pathogenesis. The SS2 Δstp1 mutant was generated by allelic change of stp1 with cat, which was verified by sequencing of the PCR product. Null expression of Stp1 in the mutant was confirmed by absence of its band on the western blot probed with anti-Stp1 polyclonal antibody. The results confirmed successful inactivation of stp1 in the SS2 strain (Fig. 1).

**Morphology of the mutant strain Δstp1 and its parent strain**

The Δstp1 mutant had longer chains than its parent strains (panel A in Fig. 2). The two strains showed difference on their surfaces as revealed by transmission electron microscope: the
Serine/threonine phosphatase 1 is critical for SS2

\( \Delta stp1 \) mutant had much fewer ‘pilus-like’ surface structures than its parent strain (panels B and C in Fig. 2).

**Phosphatase activity of rStp1**

To determine whether Stp1 of SS2 is a functional phosphatase, we expressed Stp1 in *E. coli*. The results showed that rStp1 was successfully expressed as a 41 kDa His-tagged fusion protein. Hydrolysis of pNPP was measured by assessing an increase in absorbance of pNP at 405 nm over 10 min. We observed that rStp1 hydrolyzed pNPP in the presence of Mn^{2+} (Fig. 3).

**Effect of stp1 inactivation on adhesion to cultured cells**

The bEnd.3 and HEP-2 cell lines were used to determine the effects of *stp1* deletion on adhesion of SS2 to host cells. Fig. 4 shows that the \( \Delta stp1 \) mutant exhibited better adhesion to both cell lines than that of its parent strain: 1.92 ± 0.07 vs. 0.30 ± 0.014 (\( p < 0.01 \)) in bEnd.3 cells and 1.92 ± 0.86 vs. 0.40 ± 0.047 (\( p < 0.05 \)) in HEP-2 cells.

---

**Fig. 2.** Cell morphology of *Streptococcus suis* type 2 wild-type (WT) and its isogenic \( \Delta stp1 \) mutant. Transmission electron micrographs of bacteria (A–C). Scale bars = 5 \( \mu \)m (A), 0.2 \( \mu \)m, 100 nm, 50 nm (B, from left to right), 0.2 \( \mu \)m, 100 nm, 100 nm (C, from left to right).

**Fig. 3.** Phosphatase activity of Stp1 of *Streptococcus suis* type 2. (A) Purification of protein Stp1. (B) Dephosphorylation of p-nitrophenyl phosphate (pNPP) with Stp1 in the Mn^{2+} buffer. (C) Kinetics of pNPP hydrolysis by Stp1 as measured at different substrate concentrations over 10 min. The \( K_m \) and \( V_{max} \) were determined to be 5.95 (± 1.265) mM and 1.586 (± 0.09) nmol/\( \mu \)g per min, respectively. OD, optical density.

**Fig. 4.** Comparison of *Streptococcus suis* type 2 wild-type (WT) and its isogenic \( \Delta stp1 \) mutant effects on adhesion to bEnd.3 and HEP-2 cell monolayers. \* \( p < 0.05 \), \** \( p < 0.01 \).
Deletion of stp1 led to increased resistance to killing by macrophages

Fig. 5 shows that there was no marked difference in phagocytosis between Δstp1 mutant and its parent strain. However, the mutant was more resistant than the wild-type strain to killing by macrophages (0.79 ± 0.08 vs. 0.16 ± 0.037, \( p < 0.05 \)). By using the chemical peroxides H\(_2\)O\(_2\) or paraquat in BHI broth, we observed that the Δstp1 mutant was more resistant than its parent strain to paraquat treatment at 5 to 10 mM (\( p < 0.01 \)), but not to hydrogen peroxide (Fig. 6). These results suggest that deletion of stp1 enhanced resistance to phagocytic killing, probably by increasing the resistance to reactive oxygen species (ROS).

Role of stp1 in virulence to mice

The role of SS2-stp1 in virulence to BALB/c mice was examined by assessing responses to inoculation of 2.44–2.47 × 10\(^9\) CFU of the relevant strains per mouse. Death rate was 80% for mice challenged with the wild-type strain and 20% when Δstp1 mutant-challenged. At day 5 post-infection, doubling the inoculum level led to 100% death (10/10) in mice receiving the wild-type strain, but only 60% (6/10) death in mice inoculated with the Δstp1 mutant. Compared to its parent strain, deletion of stp1 decreased the virulence of SS2 (LD\(_{50}\): 2.7 × 10\(^9\) CFU for Δstp1 strain vs. 1.51 × 10\(^9\) CFU for parent strain; Table 3). Bacterial burdens in blood and internal organs were lower in the Δstp1 mutant than in its parent strain. At 48 hpi, bacterial load in organs of mice receiving the parent strain remained high, while the load at 48 hpi in mice inoculated with Δstp1 mutant was below the detection limit (2 log, i.e., 100 CFU) of the method used (Fig. 7). These findings indicate that inactivation of stp1 reduces virulence of SS2 in mice.

### Table 3. Virulence of Streptococcus suis type 2 wild-type (WT) and its isogenic Δstp1 mutant in mice

| Strains | Inoculum size | Death (%) | LD\(_{50}\) |
|---------|---------------|-----------|-----------|
| WT      | 4.93 × 10\(^9\) | 100       | 1.51 × 10\(^9\) |
|         | 2.47 × 10\(^9\) | 80        |           |
| Δstp1   | 4.87 × 10\(^9\) | 60        | 2.70 × 10\(^9\) |
|         | 2.44 × 10\(^9\) | 20        |           |

LD\(_{50}\), lethal dose 50.
Serine/threonine phosphatase 1 is critical for SS2

Discussion

In pigs and humans in Europe and Asia, SS2 is the serotype most frequently associated with disease [13]. A recent review indicated that there are more than 60 bacterial components that have been identified in relation to SS2 virulence, including surface and secreted factors, proteinases/enzymes, and transcriptional regulators [10]. Bacteria adapt to changing environments by regulating their gene expression through signal transduction systems. In SS2, SalK/SalR [20], IhK/Irr [19], and an orphan response regulator CiaRH [14] were reported to contribute to its virulence. Early reports have indicated that STPs have important roles in bacterial physiology, cell division, and growth by modulating phosphorylation states of their partner kinases (the serine/threonine kinases) or other substrates important in metabolism or virulence [3,11,23,34]. Herein, we present evidence that Stp1 of SS2 has phosphatase activity and is a virulence factor in a mouse model.

A S. suis infection starts by colonizing the nasopharyngeal tissue. Bacterial interaction with the epithelial cells in the respiratory tract is central to the initiation of infection and in further spread from the respiratory tract to the bloodstream [9]. Once in the bloodstream, phagocytic cells have a pivotal role against the invading pathogens. In this study, we reveal that deletion of stp1 enhances adhesion of SS2 to both the HEp-2 and bEnd.3 cell lines, suggesting that Stp1 might suppress the adhesiveness of SS2 to host cells. This may indicate that Stp1 has a negative regulatory effect on expression of adhesins on the bacterial surface. Since the Δstp1 mutant exhibited increased survival in macrophages, we suspect that this might be due to an increased resistance to ROS. In the paraquat-induced superoxide radical (O$_2^-$) system, the stp1 deletion mutant was more resistant than its parent strain. We propose that this might be related to regulation of SodA (a superoxide dismutase) by Stp1, which is capable of protecting SS2 from damage by ROS [32]. In Listeria monocytogenes, a superoxide dismutase is post-translationally controlled by phosphorylation of its STP [2].

Theoretically, enhanced resistance to macrophage killing as well as increased adhesion of the stp1 deletion mutant would lead to increased virulence. However, we found that the virulence of the Δstp1 mutant was in fact attenuated in mice, as shown by the high LD$_{50}$ value as well as the reduced bacterial burden in internal organs and blood. This indicates that phenotypic changes in cultured cells in vitro are not correlated with in vivo situations in this particular phosphatase Stp1. This could be due to multiplicity of target protein substrates that are regulated post-translationally by Stp1 and to changes in several functions other than adhesion and phagocytic killing. In prokaryotes, there are fewer STP than serine/threonine kinases, indicating that STPs have multiple substrates [18]. In S. agalactiae, deletion of skl or double deletion of stp-sk1 has resulted in virulence attenuation [26]. However, the role of stp1 on the virulence of S. agalactiae remains undescribed [27]. The stp deletion mutant of S. aureus N315 exhibited increased resistance to antimicrobials, but it was not evaluated for its role in virulence [5].

Taken together, our data show for the first time that stp1 is important in the virulence of SS2. Further studies are needed to analyze the dephosphorylation profiles of its potential substrates (including SodA) and their relationship to ensuing changes of bacterial functions; thereby resolving the discrepancy between virulence attenuation in mice and the increased adhesion and resistance to macrophage killing in vitro as a result of stp1 deletion.
Acknowledgments

This work is part of research funded by the National Science Foundation of China (Project 31572472) and the Ministry of Agriculture (Project 201303041).

Conflict of Interest

The authors declare no conflict of interest.

References

1. Agarwal S, Pancholi P, Pancholi V. Serine/threonine phosphatase (SP-STP), secreted from Streptococcus pyogenes, is a pro-apoptotic protein. J Biol Chem 2012, 287, 9147-9167.
2. Archembault C, Nahori MA, Pizarro-Cerda J, Cossart P, Dussurget O. Control of Listeria superoxide dismutase by phosphorylation. J Biol Chem 2006, 281, 31812-31822.
3. Barford D, Dus AK, Egloff MP. The structure and mechanism of protein phosphatases: insights into catalysis and regulation. Annu Rev Biophys Biomol Struct 1998, 27, 133-164.
4. Baums CG, Valentin-Weigand P. Surface-associated and secreted factors of Streptococcus suis in epidemiology, pathogenesis and vaccine development. Anim Health Res Rev 2009, 10, 65-83.
5. Burnside K, Lembo A, de Los Reyes M, Iliuk A, Binh Tran NG, Bus JS, Gibson JE. The structure and mechanism of protein phosphatases: insights into catalysis and regulation. Annu Rev Biophys Biomol Struct 1998, 27, 133-164.
6. Bus JS, Gilson JE. Pararquat: model for oxidant-initiated toxicity. Environ Health Perspect 1984, 55, 37-46.
7. Cameron DR, Ward DV, Kostoulas X, Howden BP, Moeller RC Jr, Eliopoulos GM, Peleg AV. Serine/threonine phosphatase Stp1 contributes to reduced susceptibility to vancomycin and virulence in Staphylococcus aureus. J Infect Dis 2012, 205, 1677-1687.
8. Cybulski RJ Jr, Sanz P, Alem F, Stibitz S, Bull RL, O'Brien AD. Four superoxide dismutases contribute to Bacillus anthracis virulence and provide spores with redundant protection from oxidative stress. Infect Immun 2009, 77, 274-285.
9. Dong W, Ma J, Zhu Y, Zhu J, Yuan L, Wang Y, Xu J, Pan Z, Wu Z, Zhang W, Lu C, Yao H. Virulence genotyping and population analysis of Streptococcus suis serotype 2 isolates from China. Infect Genet Evol 2015, 36, 483-489.
10. Feng Y, Zhang H, Wu Z, Wang S, Cao M, Hu D, Wang C. Streptococcus suis infection: an emerging/reemerging challenge of bacterial infectious diseases? Virulence 2014, 5, 477-497.
11. Gaidenko TA, Kim TJ, Price CW. The PrpC serine-threonine phosphatase and PrkC kinase have opposing physiological roles in stationary-phase Bacillus subtilis cells. J Bacteriol 2002, 184, 6109-6114.
12. Grenier D, Bodet C. Streptococcus suis stimulates ICAM-1 shedding from microvascular endothelial cells. FEMS Immunol Med Microbiol 2008, 54, 271-276.
13. Haas B, Vaillancourt K, Bonifait L, Gottschalk M, Grenier D. Hyaluronate lyase activity of Streptococcus suis serotype 2 and modulatory effects of hyaluronic acid on the bacterium’s virulence properties. BMC Res Notes 2015, 8, 722.
14. Han H, Liu C, Wang Q, Xuan C, Zheng B, Tang J, Yan J, Zhang J, Li M, Cheng H, Lu G, Gao GF. The two-component system Ihk/Irr contributes to the virulence of Streptococcus suis serotype 2 strain 05ZYH33 through alteration of the bacterial cell metabolism. Microbiology 2012, 158, 1852-1866.
15. Houde M, Gottschalk M, Gagnon F, Van Calsteren MR, Segura M. Streptococcus suis capsular polysaccharide inhibits phagocytosis through destabilization of lipid microdomains and prevents lactosylceramide-dependent recognition. Infect Immun 2012, 80, 506-517.
16. Jin H, Pancholi V. Identification and biochemical characterization of a eukaryotic-type serine/threonine kinase and its cognate phosphatase in Streptococcus pyogenes: their biological functions and substrate identification. J Mol Biol 2006, 357, 1351-1372.
17. Ju CX, Gu HW, Lu CP. Characterization and functional analysis of atl, a novel gene encoding autolysin in Streptococcus suis. J Bacteriol 2012, 194, 1464-1473.
18. Kennelly PJ. Protein kinases and protein phosphatases in prokaryotes: a genomic perspective. FEMS Microbiol Lett 2002, 206, 1-8.
19. Li J, Tan C, Zhou Y, Fu S, Hu L, Hu J, Chen H, Bei W. The two-component regulatory system CiaRH contributes to the virulence of Streptococcus suis 2. Vet Microbiol 2011, 148, 99-104.
20. Li M, Wang C, Feng Y, Pan X, Cheng G, Wang J, Ge J, Zheng F, Cao M, Dong Y, Liu D, Wang J, Lin Y, Du H, Gao GF, Wang X, Hu F, Tang J, Saik/SalR, a two-component signal transduction system, is essential for full virulence of highly invasive Streptococcus suis serotype 2. PLoS One 2008, 3, e2080.
21. Martinez MA, Das K, Saikolappan S, Materon LA, Dhandayuthapani S. A serine/threonine phosphatase encoded by MG.207 of Mycoplasma genitalium is critical for its virulence. BMC Microbiol 2013, 13, 44.
22. Neiss WF. Electron staining of the cell surface coat by osmium-low ferrocyanide. Histochemistry 1984, 80, 231-242.
23. Osaki M, Arcondéguy T, Bastide A, Touriol C, Prats H, Trombe MC. The StkP/Php signaling couple in Streptococcus pneumoniae: cellular organization and physiological characterization. J Bacteriol 2009, 191, 4943-4950.
24. Pereira SFF, Goss L, Dworkin J. Eukaryote-like serine/threonine kinases and phosphatases in bacteria. Microbiol Mol Biol Rev 2011, 75, 192-212.
25. Pullen KE, Ng HL, Sung PY, Good MC, Smith SM, Alber T. An alternate conformation and a third metal in PstP/Ppp, the M. tuberculosis PP2C-family Ser/Thr protein phosphatase. Structure 2004, 12, 1947-1954.
26. Rajagopal L, Clancy A, Rubens CE. A eukaryotic type
Serine/threonine phosphatase 1 is critical for SS2

27. Rajagopal L, Vo A, Silvestroni A, Rubens CE. Regulation of purine biosynthesis by a eukaryotic-type kinase in Streptococcus agalactiae. Mol Microbiol 2005, 56, 1329-1346.

28. Rajala RVS, Kanan Y, Anderson RE. Photoreceptor neuroprotection: regulation of Akt activation through serine/threonine phosphatases, PHLPP and PHLPP1. In: Rickman CB, LaVail MM, Anderson RE, Grimm C, Hollyfield J, Ash J (eds.). Retinal Degenerative Diseases: Mechanisms and Experimental Therapy. pp. 419-424, Springer, London, 2016. (Advances in Experimental Medicine and Biology; Vol. 854).

29. Segura M, Gottschalk M. Streptococcus suis interactions with the murine macrophage cell line J774: adhesion and cytotoxicity. Infect Immun 2002, 70, 4312-4322.

30. Takamatsu D, Osaki M, Sekizaki T. Construction and characterization of Streptococcus suis- Escherichia coli shuttle cloning vectors. Plasmid 2001, 45, 101-113.

31. Takamatsu D, Osaki M, Sekizaki T. Thermosensitive suicide vectors for gene replacement in Streptococcus suis. Plasmid 2001, 46, 140-148.

32. Tang Y, Zhang X, Wu W, Lu Z, Fang W. Inactivation of the sodA gene of Streptococcus suis type 2 encoding superoxide dismutase leads to reduced virulence to mice. Vet Microbiol 2012, 158, 360-366.

33. Tang Y, Zhao H, Wu W, Wu D, Li X, Fang W. Genetic and virulence characterization of Streptococcus suis type 2 isolates from swine in the provinces of Zhejiang and Henan, China. Folia Microbiol (Praha) 2011, 56, 541-548.

34. Ulijasz AT, Falk SF, Weisblum B. Phosphorylation of the RitR DNA-binding domain by a Ser-Thr phosphokinase: implications for global gene regulation in the streptococci. Mol Microbiol 2009, 71, 382-390.

35. van Samkar A, Brouwer MC, Schultsz C, van der Ende A, van de Beek D. Streptococcus suis Meningitis: a systematic review and meta-analysis. PLoS Negl Trop Dis 2015, 9, e0004191.

36. Zhao Y, Liu G, Li S, Wang M, Song J, Wang J, Tang J, Li M, Hu F. Role of a type IV-like secretion system of Streptococcus suis 2 in the development of streptococcal toxic shock syndrome. J Infect Dis 2011, 204, 274-281.

37. Zhu H, Huang D, Zhang W, Wu Z, Lu Y, Jia H, Wang M, Lu C. The novel virulence-related gene stp of Streptococcus suis serotype 9 strain contributes to a significant reduction in mouse mortality. Microb Pathog 2011, 51, 442-453.