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Research progress in China on the virulence factors of *Streptococcus suis* serotype

Xiuwei YUE[1], Yixuan HOU[1], Xiuguo HUA[1], Shujie WANG[2], Zhibiao YANG[1,*], Xuehui CAI[2]

[1]Shanghai Key Laboratory of Veterinary Biotechnology, School of Agriculture and Biology, Shanghai Jiao Tong University, Shanghai, P.R. China

[2]Division of Swine Infectious Diseases, National Key Laboratory of Veterinary Biotechnology, Harbin Veterinary Research Institute of Chinese Academy of Agricultural Sciences, Harbin, P.R. China

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Abstract: *Streptococcus suis* serotype 2 (*S. suis* 2) is an important pathogen of zoonoses, which causes meningitis in pigs and represents a high health risk for humans related to porcine industry. Two outbreaks causing severe acute diseases in humans with high morbidity and mortality were reported in China, in 1998 and in 2005, respectively, which were clinically featured with streptococcal toxic shock syndrome. Some genes (or encoded products) were known as virulence factors, such as capsular polysaccharide, suilysin, muramidase-released protein, and extracellular factor. However, these were not enough to detect many potential cases of *S. suis* 2. Scientists in China have done a lot to discover more novel virulence factors. Up to now, some new putative virulence-related factors found in China have been reported. The purpose of this paper is to provide a complete summary of the research of virulence factors of *S. suis* 2 in China.

Key words: *Streptococcus suis* serotype 2, virulence factors, China

1. Introduction

Streptococcus suis is a gram-positive coccus that causes clinical disease syndromes in swine and other domestic animals, even in humans. Thirty-five capsular serotypes (types 1–34 and type 1/2) have been identified (1–4). Among these, *Streptococcus suis* serotype 2 (*S. suis* 2) is the most important pathogen. The disease syndromes caused by *S. suis* 2 in swine include toxic shock, permanent hearing loss, and colon carcinoma (5–9).

Some main virulence factors of *S. suis* 2 were found in earlier years, including capsular polysaccharides (10–14), muramidase-released proteins (MRP), extracellular factors (EFs) (15–17), and suilysin (13,18,19). However, these virulence factors were not adequate to identify even half of the *S. suis* 2, so more virulence factors were desired to be found.

There were 2 large-scale outbreaks of human *S. suis* 2 epidemic in China, bringing about a lot of disasters. One broke out in Jiangsu Province in 1998, with a porcine mortality rate of 39.7%. Human infections were also found in the endemic area. The other occurred in Sichuan Province in 2005, with 214 persons infected and 30 people dead. Thus, scientists in China are giving more and more attention to the research of *S. suis* 2. Many virulence-related factors have been found, which can be listed as follows.

2. Genes within the 89-kb pathogenicity island

A specific 89-kb DNA fragment was found by Chen et al. (5) in the chromosomes of 98HAH12 and 05ZYH33, which were highly virulent strains and have only been detected in China. It shares the universal properties of pathogenicity islands (PAIs), such as distinct GC content (36.8%), which is obviously lower than that of the genome (42.3%). Some putative virulence factors were then identified within the 89-kb PAI.

2.1. Gene 0969

Gene 0969, encoding the components of the type IV protein secretion system, is located in the 89-kb PAI. The type IV protein secretion system is the main passageway of pathogenic protein released by highly pathogenic microorganisms, such as unit toxins. Guo et al. (20) observed that the gene 0969 mutant strain had the same bioactivity and equal ability to adhere to the Hep-2 cell as the wild strain. However, in the mice infection model, the growth of the gene 0969 mutant was inhibited,

* Correspondence: xinfeiyang3808@163.com

demonstrating that gene 0969 may be a putative virulence factor of *S. suis* 2, reflecting in the transportation of some macromolecular substances.

2.2. Gene 0906-0907

The mutant strain of the 2-component signal transduction system gene 0906-0907, located in the candidate 89-kb PAI, was constructed by Li et al. (21) to evaluate its biological activity. The experiment results showed that mice from 2 different groups became ill 2 h after respectively being invaded by the wild *S. suis* 2 strain and the gene 0906-0907 mutant strain and then died after 6 or 7 h, without a marked difference in pathogenicity. It was demonstrated that gene 0906-0907 may not be a virulence factor of *S. suis* 2. However, further study carried out with the piglet model is desired to verify whether gene 0906-0907 is really not a virulence factor.

2.3. SalK/SalR

According to the bioinformatics analysis, the GC content of the 2-component signal transduction system SalK/SalR (salKR), 29.9%, is dramatically less than that of both the 89-kb PAI and the whole genome of *S. suis* 2 at 36.8% and 41.1%, respectively. Li et al. (22) showed that the SalKR mutant strain had no pathogenicity in the piglet infection experiment. On the contrary, functional complementation of salKR into the isogenic salKR mutant strain restored its high pathogenicity. Furthermore, knocking out salKR could decrease the ability of colonization and decrease the resistance to polymorphonuclear leukocyte-mediated killing. These findings demonstrated that salKR was necessary for the full virulence of highly pathogenic *S. suis* 2.

2.4. ATP-binding cassette transporters

Through homologous recombination, gene 0910, located within the 89-kb PAI and encoding ATP-binding cassette transporters (ABCTs), was replaced by the chromosomal resistance cassette. Pian et al. (23) showed that there were no significant differences in biological characteristics and virulence in mice between the ABCT mutant strain and the wild *S. suis* 2 strain. ABCT may not be a virulence factor for *S. suis* 2.

Moreover, until now, the 89-kb PAI associated with the Chinese outbreak strain of *S. suis* 2 has still not been detected in the United States. To see whether the 2005 highly pathogenic Chinese *S. suis* 2 was spreading in the United States, Schmid et al. (24) designed 3 different PCR primer pairs on the basis of the nucleotide sequences surrounding and internal to the unique PAI of strain 05ZYH33 (the Chinese outbreak strain) to screen 290 swine isolates of *S. suis* obtained from different regions. The results showed that the 05ZYH33 strain was absent in the United States.

3. Genes encoding different enzymes

According to previous research, in addition to the 89-kb PAI, some genes encoding specific enzymes were presumed to be potential virulence factors of *S. suis* 2.

3.1. Sortase A and sortase C5

A sortase A (srtA) mutant of *S. suis* 2 was obtained by Wang et al. (25) by homologous recombination, with 2 known virulence factors, MRP and surface antigen 1, absent, as analyzed by immunofluorescence. Piglet infection tests showed that knockout of srtA weakened the full virulence of the 05ZYH33 strain and meanwhile impaired its localization ability in specific tissues or organs, especially the brain and lungs. Furthermore, the srtA mutant strain displayed significant reduction in adherence to Hep-2 cells and human umbilical vein endothelial cells (26). Sortase C5 (srtC5) is located in a putative gene of pili. The knockout of srtC5 can reduce the adherence ability and full virulence, compared with the wild *S. suis* 2 strain (ZY458) (27). All in all, srtA and srtC5 play an important role in the full virulence of pathogenic *S. suis* 2.

3.2. Inosine 5-monophosphate dehydrogenase

Inosine 5-monophosphate dehydrogenase (IMPDH) plays an important role in bacteria passing through the cytomembrane to multiply inside the host. It can change the cell cycle of Hep-2 cells (28). In the experiment processed by Zhang et al. (29), the IMPDH mutant was found to be less qualified for 6 carbohydrates' metabolism and less acidic materials and to be less virulent in porcine and experimental models than the wild *S. suis* 2 strain. This may support the idea that the deletion of IMPDH had a negative effect on the expression of the main virulent protein.

3.3. Dipeptidyl peptidase IV

The enzymatic activity of dipeptidyl peptidase IV (DPP IV) on DPP IV protein product was checked by enzymatic assays by Ge et al. (30) and found to be functional. DPP IV can also interact with human fibronectin, as proven by ELISA analysis. The DPP IV mutant showed lower virulence than the wild strain and debilitated adhesion capability. In addition, the complement strain of the DPP IV mutant recovered its impaired pathogenicity. This demonstrated that DPP IV contributed to the full virulence of *S. suis* 2.

3.4. NeuB gene

Sialic acid plays an important role in the adhesion ability and the signal transduction process (31). The NeuB gene, encoding sialic acid synthase, was deleted by homologous recombination. Dong et al. (32) showed that the NeuB gene mutant strain displayed no differences in mycelial morphology, hemolytic activity, and dyeing properties compared with the wild *S. suis* 2 strain (05ZYH33), as analyzed by biological characteristics assays. However, the capsule of the NeuB mutant strain was thinner and

more tight than that of the wild *S. suis* 2 strain. In animal infection models, 8/10 mice were killed by the wild *S. suis* 2 strain in 48 h. Four mice restored their energy after 24 h of infection with the NeuB mutant strain. This indicated that the NeuB gene plays an important role in the pathogenesis of *S. suis* 2.

4. Genes coding regulatory factors

Some genes encoding regulatory factors were also assumed as putative virulence factors of *S. suis* 2.

4.1 Transcription regulator Rgg

De Greeff et al. (33) proved that the deletion of the orphan response regulator RevS gene can decrease the pathogenicity of *S. suis* 2. Pan et al. (34) demonstrated that response regulator CovR is a negative modulator of virulence. The transcription regulator Rgg played divergent roles in 2 experiments. Wang et al. (35) insisted that Rgg may be unrelated to the virulence of *S. suis* 2. However, Zheng et al. (36) deemed the deletion of Rgg in *S. suis* 2 to reduce the utilization of lactose and maltose. In addition, Rgg might have a role in the adherence of *S. suis* 2. They also observed that the Rgg mutant strain had significantly lower lethality than the wild *S. suis* 2 strain in the piglet infection model. Therefore, further tests are required to confirm whether transcription regulator Rgg is really a virulence factor for *S. suis* 2.

4.2 Lin0523

Lin0523 (also called virA) is isogenous to the S subunit of restriction endonuclease. The Lin0523 mutant strain was constructed by homologous recombination by He et al. (37). The results showed that the Lin0523 protein may contain a signal peptide and a transmembrane region, as analyzed by bioinformatics methods. In addition, it was detected extracellularly. In another experiment (38), rabbits infected with the wild *S. suis* 2 strain or with the complement strain of the virA mutant strain both exhibited obvious severe clinical symptoms and died within 6 days. However, rabbits infected with the virA mutant strain gained weight normally and no clinical symptoms were observed. This demonstrated that the Lin0523 protein may be a secretory protein, and gene Lin0523 is necessary for the full virulence of *S. suis* 2.

Table. Novel virulence factors of *Streptococcus suis* serotype 2 found in China.

Gene (or encoded product)	Animals used in the experiments	Invasiveness	Others
Gene 0969/components of type IV protein secretion system	Mice (13)	+	May be associated with the transportation of macromolecular substances.
Gene 0906-0907	Mice (18)	–	May not be a virulence factor. Piglet infection model is needed.
SalK/SalR, 2-component signal transduction system	Piglets (17)	+	Resistance of mutant to PMN-mediated killing was greatly decreased.
Gene 0910 (ABCT)	Mice (24)	–	Piglet infection model is needed.
Sortase A	Piglets (7,33)	+	Resistance of mutant to PMN-mediated killing did not decrease significantly.
Sortase C5	Rabbits (38)	+	Virulence of mutant strain was impaired sharply.
IMPDH	Rabbits, piglets, mice (37)	+	Knock-out of IMPDH weakened the virulence to sensitive animals.
Dipeptidyl peptidase IV	Mice (9)	+	Mice immunized with protein DPP IV showed 100% survival.
NeuB Gene	Mice (6)	+	O
Transcription regulator Rgg	Mice and piglets (34) Piglets (36)	– +	Further tests are required to confirm whether Rgg is functional.
Lin0523 (virA)	Rabbits (14,19)	+	Lin0523 protein may be a secretory protein.

+: positive; –: negative; O: not mentioned.

5. Conclusions

Homologous recombination provides an available way to analyze the function of some gene segments. Therefore, more and more virulence factors of *S. suis* 2, an important and worldwide pathogen that causes streptococcal toxic shock syndrome (STSS), are being discovered in the laboratory. However, the known virulence factors, such as capsular polysaccharide, MRP, EF, and suilysin, were not sufficient to detect most cases of *S. suis* 2. Close attention to the research on new virulence factors of *S. suis* 2 is desirable. In this paper, the Table sums up these 11 genes recently discovered in China to see their functions more clearly. The search for preventative approaches to *S. suis* 2-related disease is always of much interest worldwide. These findings may enrich the knowledge of *S. suis* 2 pathogenesis, and especially of Chinese virulent strains, and facilitate development of new strategies against the challenge of deadly *S. suis* 2 infections.

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