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Effect of the glycosyltransferases on the capsular polysaccharide synthesis of *Streptococcus suis* serotype 2

Yanyan Zhang[a,c,d], Dandan Ding[a,c,d], Manli Liu[b], Xiaopei Yang[a,c,d], Bingbing Zong[a,c,d], Xiangru Wang[a,c,d], Huanchun Chen[a,c,d], Weicheng Bei[a,c,d], Chen Tan[a,c,d,*]

[a] *State Key Laboratory of Agricultural Microbiology, College of Veterinary Medicine, Huazhong Agricultural University, Wuhan 430070, Hubei, China*
[b] *Center of Bio-Pesticide Engineering Research, Hubei Academy of Agricultural Science, Wuhan 430064, Hubei, China*
[c] *The Cooperative Innovation Center for Sustainable Pig Production, Huazhong Agricultural University, Wuhan 430070, Hubei, China*
[d] *Key Laboratory of Development of Veterinary Diagnostic Products of Ministry of Agriculture, Huazhong Agricultural University, Wuhan 430070, Hubei, China*

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ABSTRACT

Streptococcus suis serotype 2 (*S. suis* 2) is a serious zoonotic pathogen causing septicemia and meningitis in piglets and humans. The capsular polysaccharide (CPS) is an essential virulence factor for *S. suis* 2 to infect the host. The synthesis of CPS repeating units involves multiple glycosyltransferases. In this study, four *genes* (*cps2E, cps2G, cps2J* and *cps2L*) encoding different glycosyltransferases involved in CPS synthesis were researched in *S. suis* 2. Four deletion mutants (Δ*cps2E*, Δ*cps2G*, Δ*cps2J* and Δ*cps2L*) with their CPS incomplete and their sialic acid content significantly decreased were constructed in *S. suis* 2 SC19. All these four mutant strains showed enhanced adhesion to Hep-2 cells and increased sensitivity to phagocytosis. Flow cytometric analysis also revealed that these four mutants were more susceptible to the attack by the complement system. In a mouse model of infection, the mutant strains were rapidly cleared by the immune system, compared with the wild-type strain. In summary, this study characterized four genes (*cps2E*, *cps2G*, *cps2J* and *cps2L*) involved in CPS synthesis of *S. suis* 2 SC19 and it revealed that these *genes* were all crucial for SC19 to invade and survive in the host.

Contents

* Corresponding author at: State Key Laboratory of Agricultural Microbiology, Laboratory of Animal Infectious Diseases, College of Animal Science & Veterinary Medicine, Huazhong Agricultural University, Wuhan 430070, Hubei, China. Fax: +86 27 87282608.
E-mail address: tanchen@mail.hzau.edu.cn (C. Tan).

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1. Introduction

Streptococcus suis is one of the most prevalent pathogens in swine causing a range of disease syndromes including arthritis, meningitis, pneumonia, septicemia and endocarditis, etc., which resulted in serious economic losses in the pig industry (Staats et al., 1997). It is also an emerging zoonotic agent able to induce meningitis, endocarditis, and streptococcal toxic shock-like syndrome in humans (Sriskandan and Slater, 2006). Among the 33 *S. suis* serotypes identified on the basis of antigenic differences in their CPS (Liu et al., 2013; Hill et al., 2005), *S. suis* 2 is most commonly isolated from pigs and humans and is the most frequently reported worldwide (Liu et al., 2007). *S. suis* 2 mainly infects people who have direct contact with carrier pigs, sick pigs, or raw pork via wounds on the skin, or the mucosa of the mouth, or nasal cavity (Francois et al., 1998). A recent report showed that a total of 1642 cases of *S. suis* human infection had been reported worldwide until December 31, 2013 (Guillaume et al., 2014).

The CPS is an essential virulence factor for the pathogenesis of *S. suis* 2, and the virulence of unencapsulated mutants has been shown to be significantly decreased in pig and mouse models (Charland et al., 1998; Smith et al., 1999; Feng et al., 2012). The CPS plays a role in protecting *S. suis* 2 against phagocytosis and being killed by macrophages, mononuclear cells, and neutrophils, by impairing lipid microdomain stability and signal transduction (Benga et al., 2008; Houde et al., 2012). A recent study, using a new intranasal animal model, showed that CPS can impair the function of complement system by interfering with the deposition of C3b on the *S. Suis* 2 surface (Seitz et al., 2014).

Multiple genes are reported to be involved in the biosynthesis of the repeating unit of the native CPS. Smith et al. (1999) identified a cluster of genes, the *cps* gene cluster, essential for the biosynthesis of CPS and found that CPS is comprised of glucose, galactose, *N*-acetylglucosamine, rhamnose, and sialic acid (Van Calsteren et al., 2010). Van Calsteren et al. (2010) elucidated CPS structure of *S. suis* 2 and predicted the *cps* genes responsible for the synthesis of CPS repeating units. Furthermore, the function of these *genes* have been predicted. Gene *cps2E* was predicted to be the initiating gene in the process of the synthesis of CPS repeating units, and the deletion of this gene led to the complete absence of CPS, as was reported in *Streptococcus pneumoniae* (Cartee et al., 2005). Gene *cps*2L was reported to be responsible for the synthesis of sialyltransferase mediateing the transfer of sialic acid to a side chain of CPS repeating units (Van Calsteren et al., 2010). The sialic acid played an important role in interfering with the activation of the alternative complement cascade and the mediation of bacterial attachment to the surface of phagocytes (Charland et al., 1996; Lewis et al., 2012; Segur and Gottschalk, 2002). Genes *cps2G* and *cps2J* were predicted to be responsible for the synthesis of a short side chain and a long side chain of CPS repeating units (Van Calsteren et al., 2010). However, the effect of these genes' deletion in *S. suis* 2 on CPS synthesis and virulence remains to be examined. Given the function of these *genes* for the synthesis of *S. suis* 2 CPS, a more detailed understanding between *S. suis* 2 CPS and these *genes* encoding different glycosyltransferases will provide a more effective strategy to cure and eliminate *S. suis* 2 disease.

In this study, four genes (*cps2E*, *cps2G*, cps2J and *cps2L*) encoding different glycosyltransferases responsible for the synthesis of CPS repeating units will be investigated, and four mutant strains, namely, Δ*cps2E*, Δ*cps2L*, Δ*cps2J* and Δ*cps2G* will be constructed in *S. suis* 2 SC19. It is hypothesized that the deletion of these *genes* may affect the completeness of CPS. This incompleteness will result in the significant decrease in the virulence of these mutant strains, compared with that of *S. suis* 2 SC19 strain both in vitro and in vivo. The findings may suggest that the single deletion of all these genes severely could damage the integrity of the CPS of *S. suis* 2 SC19, and that the virulence of these mutants will be obviously attenuated.

2. Material and methods

2.1. Bacteria strains, growth condition, and plasmids

The bacterial strains and plasmids used in this study are listed in Table 1. The *S. suis* 2 strain SC19 was cultivated in TSB or plated on TSA (Difco Laboratories, Detroit, MI, USA) with 10% newborn bovine serum (Sijiqing Biological Engineering Materials Co., Ltd., Hangzhou, China). *Escherichia coli* strain DH5α was grown in Luria-Bertani liquid medium or plated on Luria-Bertani agar at 37 °C. If required, spectinomycin (Sigma, St Louis, MO, USA) was added to the growth media at the following concentrations: 100 μg/mL for *S. suis* 2 and 50 μg/mL for DH5α. As a thermosensitive suicide vector, the shuttle vector pSET4s was used for gene replacement in *S. Suis* 2 (Tan et al., 2011).

2.2. Construction of mutant strains with gene deletion

Gene *cps2E* was deleted from *S. suis* 2 SC19 by the previously described allelic exchange method with minor modifications (Faulds-Pain and Wren, 2013; Takamatsu et al., 2001). The alternative allele was a DNA sequence including two flanking regions of gene *cps2E* from *S. suis* 2 SC19 genome carrying restriction enzyme sites on either side of the target gene. The digested PCR fragment was directly cloned into pSET4s vector to generate a vector, pSET4s::*cps2E*, for purpose of the deletion of gene *cps2E*. This vector was transformed into *S. suis* 2 SC19. The mutagenesis included two steps: the first step was the recombination event generating a single-crossover clone deriving from the integration of pSET4S::*cps2E* into the genome at 28 °C by spectinomycin selection, a second step was the recombination generating a double-crossover clone with the deletion of pSET4s vector sequence, and this deletion brought about the desired mutation or its reverting to wild-type strain (WT). The deletion was confirmed by using specific primers listed in Table 2 and DNA sequencing. In a similar manner, the other three mutant strains (Δ*cps2G*, Δ*cps2J* and Δ*cps2L*) were constructed.

2.3. RNA isolation and quantitative PCR (qPCR)

Total RNA was isolated according to the protocol recommended by the manufacturers (Promega) with some modification. 50 μL of overnight cultured *S. suis* 2 SC19 was added to five milliliter of TSB with 10% (vol/vol) newborn bovine serum and was inoculated to exponential growth phase (OD_{600} = 0.6) at 37 °C for 4 h. Supernatant