Microbial Pathogenesis 104 (2017) 137–145

Contents lists available at ScienceDirect

Microbial Pathogenesis

journal homepage: www.elsevier.com/locate/micpath

The 1910HK/RR two-component system is essential for the virulence of *Streptococcus suis* serotype 2

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ARTICLE INFO

Article history:
Received 28 October 2016
Received in revised form
3 December 2016
Accepted 31 December 2016
Available online 14 January 2017

Keywords:
Streptococcus suis
1910HK/RR
Two-component system
Virulence

ABSTRACT

Streptococcus suis serotype 2 is a major zoonotic pathogen, and the two-component system plays an important role in bacterial pathogenesis. The present study targeted the 1910HK/RR two-component system of *S. suis* 2. A 1910HK/RR deletion mutant (Δ1910HK/RR) and the corresponding complementation strain (CΔ1910HK/RR) were constructed in *S. suis* 2 strain 05ZYH33. 1910HK/RR deletion had no effect on *S. suis* 2 growth, but significantly inhibited the adherence and invasion of *S. suis* 2 to HEp-2 cells. Analysis of the role of 1910HK/RR in murine and pig infection models demonstrated that 1910HK/RR played a distinct role in the virulence of *S. suis* 2. In addition, deletion of 1910HK/RR significantly impaired the survival of 05ZYH33 in human blood. These data provided important insights into the pathogenesis of *S. suis* 2.

1. Introduction

Streptococcus suis (*S. suis*) is a major swine pathogen that causes severe economic losses in the swine industry worldwide [1]. To date, 33 serotypes of *S. suis* (types 1–31, 33, and 1/2) have been described based on the composition of the capsular polysaccharide (CPS) [2]. Among these serotypes, *S. suis* serotype 2 is thought to be the most virulent and is the most frequently isolated in association with diseases in most countries [3]. *S. suis* 2 also causes serious infections in humans who come in contact with infected swine or pork-derived products [4,5], and can induce meningitis, septic shock, and permanent hearing loss [5,6]. Many cases of human *S. suis* 2 infection have been reported in China, the United States of America, Canada, New Zealand, Australia, Korea, and Greece [7–9]. The first human case of *S. suis* 2 infection was reported in Denmark in 1968 [10]. Two large outbreaks of *S. suis* 2 occurred in China in 2005, resulting in 25 human cases with 14 deaths and 215 human cases with 38 deaths, respectively [6]. Patients affected in these two outbreaks showed prevalent features of *Streptococcus* toxic shock-like syndrome (STSLS) [11]. Although many studies of S. suis 2 have been published in recent years, our understanding of the pathogenesis and virulence factors of this microorganism remains limited [12]. The epidemic strain occurred in China in 2005 is unusual in that it contains a unique 89-kb (89K) pathogenicity island (PAI). We observed the loss of genes from the 89K PAI in sporadic cases in southern China in 2007, implying the dynamic evolution of this PAI. Therefore, 89K PAI might be able to be used to monitor prevalent strains of *S. suis* in China [13]. *Streptococcus suis* infection may be an emerging/reemerging challenge of bacterial infectious diseases in the future [14].

Bacterial cell division is strictly regulated in the formation of equal daughter cells. This process is governed by a series of spatial and temporal regulators, and several new factors of interest to the field have recently been identified. Gluconate 5-dehydrogenase (Ga5DH) participates in *Streptococcus suis* cell division [14]. Very recently, the researcher observed that regulation of the D-galactosamine (GalN)/*N*-acetyl-D-galactosamine (GalNAc) catabolism pathway is linked to its infectivity. It seems likely that the regulatory network of bacterial metabolism is complicated into SS2 virulence. Given the fact that biotin metabolism and utilization is

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http://dx.doi.org/10.1016/j.micpath.2016.12.026
0882-4010/

associated with Francisella pathogenesis, it is much interest to define the biotin utilization pathway in the humanpathogen *S. suis* 2 [15]. Functional definition of BirA suggests a biotin utilization pathway in the zoonotic pathogen *Streptococcus suis* [16]. Bacterial pathogens can exploit metabolic pathways to facilitate their successful infection cycles, but little is known about roles of D-galactosamine (GalN)/*N*-acetyl-*d*-galactosamine (GalNAc) catabolism pathway in bacterial pathogenesis. Zhang et al. reported that Two novel regulators of *N*-acetyl-galactosamine utilization pathway and distinct roles in bacterial infections [17].

The virulence of *S. suis* 2 is associated with the polysaccharide capsule, which is rich in sialic acid and confers antiphagocytic properties on *S. suis* 2. In murine and porcine infection models, the *S. suis* 2 capsule was found to act as a virulence factor because capsular mutants were nonpathogenic and were more rapidly cleared from the bloodstream than wild-type (WT) cells [18]. The virulence of *S. suis* 2 has also been shown to be associated with the pathogen-host interaction.

In the infected host, *S. suis* 2 uses two-component systems (TCSs) as the common regulatory mechanism to response to environmental signals. TCSs control the expression of virulence factors in a wide range of bacterial species in response to external stimuli. In many cases, multiple virulence genes that are essential for bacterial survival in a host are controlled by TCSs [19–21]. Thousands of TCSs have been identified in more than 100 sequenced bacterial genomes [22], demonstrating the ubiquitous nature of these systems in bacteria. Two *S. suis* 2 strains isolated in China (strains 98HAH12 and 05ZYH33) have been sequenced. The *S. suis* 2 genome sequence contains 15 putative TCSs, along with single, unpaired response regulator [23]. Among them, the SalK/SalR system [24], two orphan response regulators, RevS and CovR [25,26], CiaRH [27], NisK/NisR [28], Ihk/Irr [29] and VirR/VirS [30] have been reported. The pathogenesis mechanism of some TCSs mainly due to regulation of ABC amino acid transport, physiological metabolism, virulence and capsular synthesis genes. However, thers are some TCSs, such as CovR, can represses the synthesis of many virulence factors of bacteria [26]. The 1910HK/RR is one of the TCSs in *S. suis* 2. However, its role in the pathogenesis of *S. suis* 2 has not been studied.

In this study, we constructed a 1910HK/RR-knockout mutant (⊿1910HK/RR) and the corresponding complementation strain (C⊿1910HK/RR) in strain 05ZYH33. The role of the 1910HK/RR TCS in *S. suis* 2 virulence was explored. Our findings revealed that the 1910HK/RR TCS is involved in the virulence of *S. suis* 2.

2. Material and methods

2.1. Ethics statement

Animal experiments were approved by the Laboratory Animal Monitoring Committee of Huazhong Agricultural University and performed strictly according to the recommendations in the Guide for the Care and Use of Laboratory Animals of Hubei Province, China. Heparinized venous blood samples were provided by healthy donors and collected in accordance with the approved guidelines. Approval was obtained from the Institutional Medical Ethics Committee of Huazhong Agricultural University and the healthy donors provided written informed consent in accordance with the Declaration of Helsinki.

2.2. Bacterial strains, plasmids and culture conditions

The bacterial strains and plasmids used in this study are listed in Table 1. *S. suis* 2 strain 05ZYH33, isolated from Chinese patients with STSLS in Si Chuan province, China [11], is a virulent strain showing strong invasiveness and high pathogenicity. Primers are listed in Supplementary Table 1. The *S. suis* 2 strain was selected on the basis of clinical relevance and available genome sequence information. *S. suis* 2 strains were grown in Tryptic Soy Broth (TSB), or on tryptic soy agar (TSA) plates (Difco, Detroit, MI, USA) with 10% newborn bovine serum (Sijiqing Biological Engineering Materials Co. Ltd., Hangzhou, China) at 37 °C. *Escherichia coli* DH5a was cultured in Luria Broth (LB) liquid medium or plated on LB agar. In addition, spectinomycin (Spc, 100 μg/mL, Sigma) was used to screen *S. suis* 2 transformants, and ampicillin (Amp, 50 μg/mL, Sigma) was used to screen *E. coli* transformants.

2.3. Construction of the mutant strain and functional complementation of 1910HK/RR deletion

The thermosensitive suicide vector pSET4s was used to delete the 1910HK/RR gene in S. suis 2 strain SC19. To delete 1910HK/RR TCS from S. suis 2 strain 05ZYH33, two DNA fragments flanking the 1910HK/RR locus were amplified using two primers P1(5′-GCGAGCTCTGTTACTGAAATAGTTTAC-3′), P2(5′-GCCCCGGGGATG-GAGGCGAGATGAG-3′) and P3(5′-GCCCCGGGAAGTAATCCTTGT-CAACTTTC-3′), P4 (5′-GCGTCGACTCCCTACGCTAGAAAGATTC-3′). The PCR products were purified, digested with the appropriate restriction enzymes, and Simultaneously cloned into pSET4s [31]. The resulting plasmid, pSET4s-Δ1910HK/RR, was transformed into S. suis 2 strain 05ZYH33. After allelic replacement, the mutants were selected and identified by PCR using primersP7(5′-TCGGA-TACGTGGCCTACAT-3′) and P8(5′-TGATTCAATTGCCCCAGAC-3′). The 1910HK/RR gene was confirmed by reverse transcription PCR (RT-PCR) using primers P9(5′- GGGAGATAGCTTTACGGGC-3′) and P10 (5′-CAGAAGAACTTTCATTTTGCG-3′) and DNA sequencing analysis.

For the 1910HK/RR complemented strain, a DNA fragment containing the 1910HK/RR gene and its predicted promoter were amplified by primers P5(5′- TGCACTGCAGTTGAATTTAGTC-TAAAAACAGGCT-3′) and P6 (5′-

Table 1
Bacterial strains and plasmids used in this study.

Strain or plasmid	Relevant characteristics[a]	Source or reference
Strains		
S. suis		
05ZYH33	Virulent strain isolated from Chinese patients; serotype 2	Laboratory collection
Δ1910HK/RR	1910HK/RR TCS deletion mutant of strain 05ZYH33	This study
C⊿1910HK/RR	Complemented strain *of* Δ1910HK/RR; Spc^R	This study
E. coli Trans5α	Cloning host for recombinant vector	TransGen
Plasmids		
pSET4s	*E. coli-S. suis* shuttle vector; Spc^R	31
pSET4s-Δ1910HK/RR	Knockout vector designed for deletion of *0350*	This study
pSET2	*E. coli-S. suis* shuttle vector; Spc^R	32
pSET2-1910HK/RR	pSET2 containing the 1910HK/RR TCS gene and its promoter	This study

[a] Spc^R, spectinomycin resistant.