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Streptococcus suis in Omics-Era: Where Do We Stand?

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Abstract

Streptococcus suis (*S. suis*), a group of heterogeneous species with thirty-five serotypes, is considered a previously-neglected but recently-emerging human pathogen. Lack of full understanding of molecular mechanism by which this group of pathogenic bacteria infects both swine and humans greatly hampered the availability of specific/effective therapeutics against severe *S. suis* infections. The release of the first genomic sequence of *S. suis* announced the arrival of its "Omics-Era". This review concentrates on recent highlights of *S. suis* studies in aspects of Omics. Also, we discussed remaining research gaps and future perspectives.

Keywords: *Streptococcus suis*; Genomics; Proteomics

Introduction

Streptococcus suis (*S. suis*) is recognized a complex bacterial population with heterogeneous species [1]. Thirty-five different serotypes (1, 1/2, 3, 4-34) are classified according to their varied capsule antigens, of which serotype 2 is most virulent and frequently isolated from clinically diseased piglets [2,3]. Not only is *S. suis* a leading swine pathogen, but has also been emerging into a serious zoonotic agent esp. in Southeast Asia [1,4]. Human *S. suis* infections have been recorded in more than 30 countries and/or regions (such as Thailand [5], Vietnam [6,7], China [8,9], etc.) [6], and over 850 cases of human *S. suis* infections have been accumulated since its first discovery of human SS2 meningitis in Denmark, in 1968 [1-3,6]. *S. suis* infections can result in a variety of clinical diseases including meningitis, septicemia, arthritis, etc. [2].

In past decades, genetic studies have identified a collection of bacterial virulence associated factors with contribution to *S. suis* pathogenesis [1,3,6]. Unfortunately, an effective/efficient therapeutics against SS2 infection is still not available thus far, which is somewhat due to our fragmented understanding *S. suis* serotype 2, a notorious bug. While it seemed that this situation is being changed now. In particular, a big outbreak of human SS2 infections in China in 2005 challenged seriously to the public health, and also attracted great interests from scientific community worldwide [8,9]. After *S. suis* genomes firstly appeared in 2007 (Figure 1) [10], comparative genomics and proteomics of this bacteria have been extensively carried out, which led to fruitful findings, esp. in aspects of *S. suis* infections. In this review, we attempted to show an updated overview of *S. suis* research in Omics-Era.

Comparative/Functional Genomics of *Streptococcus suis*

Increasingly accumulated genomic sequences of *S. suis* have been constituting a powerful platform and solid basis for further functional studies on this group of pathogenic bacteria. Currently, majority of the *S. suis* strains with known genomes belong to SS2 [10-13], excepted two genomic sequences are from SS3 [14], and SS14 [15]. Although the average GC percentage of all the sequenced *S. suis* genomes is ~41% (Table 1), the size of their genomes varies markedly (1.6~2.1 Mb), indicating presence of genomic diversity and flexibility of *S. suis* populations (http://www.ncbi.nlm.nih.gov/sites/entrez?db=genome&cmd=DetailsSearch&term=streptococcus+suis&save_search=false). In general, the genome of *S. suis* consists of a circular chromosome of about 2.0 Mb, but with an extra 24Kb plasmid in the strain BM407 isolated from a human case of meningitis in Vietnam [12]. However, the chromosomal structure is not conserved throughout all the sequenced strain (Figure 2). The strains 98HAH12, 05ZYH33 and SC84 isolated from patients featuring streptococcal toxic shock syndrome in China have a highly similar genome and the genomes of strains GZ1 is very similar to European strain P1/7, which is in good agreement with their epidemiological history. GZ1 was isolated in 2005 from a patient in China who had septicemia and is representative of most strains isolated from humans in Europe and Asia before the 2005 Sichuan outbreak [13]. Overall, those five serotype 2 strains are highly synthetic. However, the strain BM407 is divergent. A 0.8Mb large inversion makes it distinct from other serotype 2 strains, which is due to the recombination events between identical IS3 elements on opposite replichores [12] .

In 2007, our research group decoded whole genomes of three Chinese SS2 isolates, two of which are virulent (98HA12 & 05ZYH33), and another one is avirulent (05HAS68) [10]. Comparative genomics analysis revealed that a putative pathogenicity island (PAI) of about 89 kb (termed as 89K in the latter) is exclusively present in the epidemic strains in the two Chinese SS2 outbreaks but not in other clinical isolates (Figure 2) [10] . *S. suis* strain BM407 contains two regions in the inversion with high similarity to 89K. In addition, analysis of the

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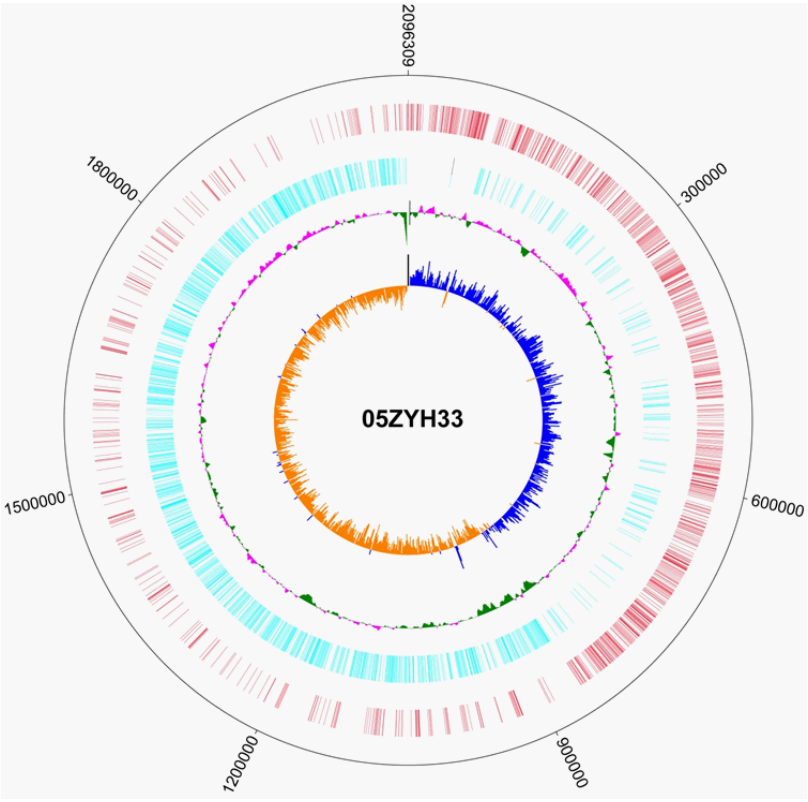

Figure 1: Circular representation of the reference genome 05ZYH33. The outer circle shows the genome scale. The second and third circles display predicted coding regions on the plus and minus strands, respectively. The fourth circle shows the GC content, Values >41.1% (average) are plotted outward (pink) and values < 41.1% are inward (green). The inner circle shows the GC skew, Blue indicates values>0, and orange indicates values<0.

unfinished genomic sequence of SS2 strain 89/1591 revealed that a partial 89K sequence (~30 kb) is present in this typical North American virulent strain. Similarly, Ye et al. [13] also compared genomes of three kinds of virulent *S. suis* 2 strains (intermediately pathogenic, highly pathogenic, epidemic), and concluded that the epidemic SS2 strain in China has acquired a collection of genomic islands, some of which are pathogenic islands. Follow-up studies from our former collaborative groups approved our initial idea that 89K is a functional PAI [16, 17]. As we anticipated before, 89K PAI carried at least two sets of genetic elements with requirements for SS2 virulence, which are salK-salR two component system [17] and a type IV-like secretion system, virD4-virB4 [16], respectively. Meanwhile, Holden and coworkers [12] reported the extensive analysis of comparative genomics of three virulent SS2 strains with different geographic origins (one is a European swine isolate, the other two are human isolates from Vietnam and China) that gave genomic evidence that rapid evolution of virulence and antibiotics resistance among pathogenic *S. suis* species.

Using the tilling microarray (Nimble Gen), two independent research groups in China (one is Zhu's lab [11], and the other is Xu's lab [18]) both demonstrated that significant genomic diversities are present among the *S. suis* population. In the former study, Wu and coworkers [11] performed pathogenicity-related genomics analysis of 18 SS2 strains with different geographic origins, in which 05ZYH33, an epidemic SS2 strain in China was reference control. An updated knowledge on 89K PAI was showed, i.e., partial relics of 89K can be observed in some other virulent and avirulent strains [11]. In particular, a working model for SS2 microevolution was proposed which is mainly based on the regions with differences (RD) across the entire SS2 genomes. In the latter, Zheng et al. [18] used GZ1, another human SS2 isolate with known genomic sequence as reference to conduct assays of comparative genomic hybridizations. 31 isolates in total were subjected for this extensive study, which represent 23 serotypes and 25 sequence types (STs) [18]. After systemic analysis, they drew several major conclusions as follows: 1) The size of core genome of *S. suis* was estimated to account for 68% of the GZ1 genome; 2) 26 out of 62 region of difference probably are genomic islands; 3) 6 putative genomic islands might be related with bacterial high pathogenicity; and 4) Gain and loss of regions with difference occurred amongst *S. suis* species with different sequence types [18]. Unlike an observation by Zheng et al. [18], de Greeff and coworkers [19] suggested that core genome contains 78% (rather than 68%) of open reading frames (ORFs) in P1/7 strain, using comparative genomic hybridizations of 55 *S. suis* strains. We believed that this kind of inconsistency is understandable, which is somewhat due to t at least following three possibilities: 1) The genomic size of GZ1 and P1/7 is quite different; 2) The conception "68% of genome size" does not mean to "68% of ORF" (vice versa); 3) Algorithm used in above two studies could be not exactly the same. More importantly, the major findings from Smith's group complemented somewhat the observations from other groups, esp. offering important information on genetic similarity, and virulence traits of *S. suis* populations [19].

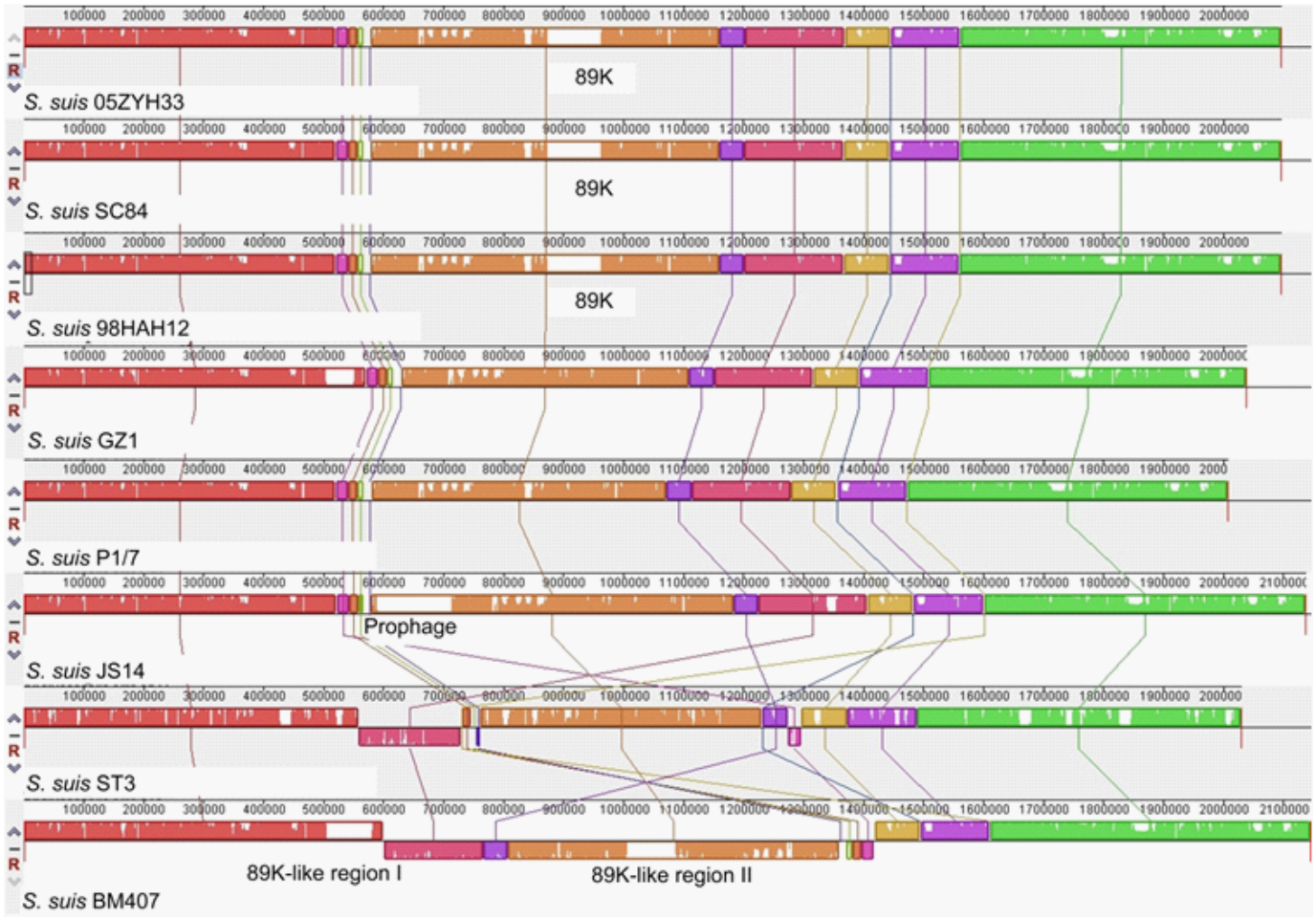

Figure 2: Genome Alignment of *Streptococcus suis* strains. Each strain chromosome is depicted as a series of ordered LCBs (Locally Collinear Blocks). Vertical lines connect homologous LCBs across the genomes. LCBs identically present in the eight genomes are given the same colors and horizontally flipped LCBs identify chromosomal inversions. Gaps or white spaces in LCB order represent strain-specific regions.

Strains	Serotype	Length (bp)	GC Contents	No. of Genes	Accession No.	Year	Ref.
P1/7	SS2	2,007,491	41%	2011	AM946016	2009	[12]
89/1591	SS2	2,143,253	41%	2162	AAFA00000000	2004	/
05HAS68	SS2	1,640,446	41%	1607	AARD00000000	2010	/
98HAH12	SS2	2,095,698	41%	2253	CP000408	2007	[10]
05ZYH33	SS2	2,096,309	41%	2254	CP000407	2007	[10]
SC84	SS2	2,095,898	41%	2068	FM252031	2009	[12]
BM407	SS2	2,146,229	41%	2118	FM252032	2009	[12]
GZ1	SS2	2,038,034	41%	1987	CP000837	2009	[13]
ST3	SS3	2,028,815	41%	2053	CP002633	2011	[14]
JS14	SS14	2,137,435	41%	2137	CP002465	2011	[15]

/: Not published

Table 1: A collection of *S. suis* strains with known genomes.

In the first half of this year, Jin's research group reported two non-SS2 genomic sequences (Table 1): one is SS3 [14], and the other is SS14 [15]. Although at that time they failed to show further dissection of the genomic difference/diversity of these three serotypes (SS2, SS3 & SS14), the availability of whole-genome sequences of these two non-SS2 Chinese isolates might give some clues to their specific geographic tropism and environmental adaptation [14,15]. As we estimated, serotype 14 strain JS14 is collinear to the serotype 2 strains, but Serotype 3 strain ST3 is different from serotype 2 strains by three inversions and translocations (Figure 2). Although the genome of strain JS14 is similar to those of strains P1/7 and GZ1, a prophage region was found to be specific to *S. suis* JS14 compared to serotype 2 strains (Figure 2) [15]. Very recently, the same research group sequenced genomes of four more non-SS2 Chinese strains (serotype 1, 7, 9 & 1/2), which partially closed this gap of knowledge on genomic diversity among different serotypes [20]. Additionally, Jin and co-worker [21] conducted

comparative genomics analyses of two *S. suis* strains with an opposing phenotype in antibiotics sensitivity (one is strain R61 with multi-drug-resistance, and the other is strain A7 with full sensitivity to all tested antibiotics), and then proposed that gene horizontal transfer would be an important evolutional force to determine an ability of *S. suis* acquire multiple-drug resistance.

Proteomics approaches to *Streptococcus suis*

Most of our knowledge of *S. suis* proteome is derived from studies of SS2 strains. Given the relevant literatures available to date, the total number of *S. suis* protein discovered by proteomics analysis (~400) is estimated to be less than 20% of gene products encoded by *S. suis* genome (~2200). The current situation indicated that most of the putative coding sequences of proteins are in great demand to be experimentally approved, which in turn might require an improved proteomics-based approach with much higher sensitivity. On the other hand, bacteria sampled from different growth phases in different cultivation conditions esp. its naturally-infecting status could contribute to fine identification of protein with low abundance.

Jiang and coworkers reported, for the first time, a large scale of proteomics-aided identification of *S. suis* proteins [22]. Totally, 373 different proteins out of 834 processed spots were determined. Functional category suggested that 1) most of the identified proteins are localized in bacterial cytoplasm, and involved in central metabolisms: energy metabolism, protein synthesis, and cellular processes; 2) the proteins in high abundance in 2-dimentional electrophoresis gels are correlated mainly to housekeeping gene products associated with carbohydrate metabolism, protein quality control and translation [22]. Intriguingly, a bunch of known virulence-associated factors were revealed, including muramidase-released protein (MRP) [23,24], extracellular factor (EF) [23,24], suilysin [25,26], etc. Additionally, two putative infection-related new proteins (enolase and endopeptidase) were pinpointed. Among them, enolase have been demonstrated by three different research groups to be a protective antigen protein that can be exported to bacterial surface via an unknown atypical mechanism [27-30].

Comparative analysis of immune-proteomics of two virulent Chinese SS2 strains (ZY05719 and HA9801) and an avirulent strain (T15) identified eight out of nine antigenic proteins with differential expression [31]. Among them, only six genes were determined, and found to be common to virulent /avirulent strains [31]. It suggested that these genes' expression level can be correlated to virulence, although they are not indicator genes specific to virulent strains. Using similar strategy, Sun et al. [32] revealed 11 secreted proteins with antigenicity from another Chinese virulent SS2 strain, SC84. Further analysis indicated that they are all localized on cell wall (or referred to as extra-cellular protein). In consistency with Jiang et al.'s observations [22], a couple of known antigenic factors, like MRP, EF, and Sly, were also visualized in this improved proteomics-aided assay [31]. Four new antigenic proteins were also proposed that include putative 5'-nucleotidase, ribo-nucleases G and E, and predicted metal-loendo-peptidase, respectively [31]. To further address this issue, Wang and co-authors [33] supplemented two more new cell wall-associated proteins, catabolite control protein A and leucylaminopeptidase. Although these newly-determined antigens still needed further experimental verification, somehow they feature potential to develop new detection method and subunit vaccine candidates specific to *S. suis* 2.

As we expected, several antigen molecules revealed by proteomics have been confirmed to be promising vaccine candidates (Table 2). Sat, a newly-discovered cell wall protein was found to confer around 80% protection of mice against severe SS2 challenge. Undoubtedly, it suggested that *S. suis* Sat could be an immune-protective antigen [34]. PAP1-2b, a surface protein was believed to function as an ancillary pilus subunit [35]. Similar to observations with Sat [34] and Enolase [28,30], PAPI-2b also exerted potent protective effects on mice against systematic SS2 infections [35]. Immunization of SsPepO protein initially reported in earlier immuno-proteomics [31] was found to elicit a strong humoral immune response and confer effective protection against SS2 challenge in two different models of experimental animals (mice and SPF-piglets) [36].Very recently, Lu's research group developed a new protocol "pre-absorbed immuno-proteomics" for determination of *S. suis* surface proteins, and its efficacy was validated by identification of MRP and an ABC transporter protein, bacterial surface antigen [37].

Antigens	Traits	Animal models	Year	Ref.
Sat	Surface protein	Mice	2010	[34]
PAPI-2b	Pilus subunit	Mice	2010	[35]
SsPepO	A secretary immunogenic protein	Mice/piglets	2011	[36]

Table 2: Protective antigens determined by assays of animal infections following proteomics-guided screening.

Although SS2 is a prevalent serotype responsible for clinical infections of piglets and humans, SS9 is also frequently isolated from diseased swine. Unlike SS2, proteomics knowledge on SS9 is pretty limited. Recently approaches of immuno-proteomics were applied for identification of immunogenic proteins of this kind of serotype (GZ0565 and SH040917). First, eight immunogenic proteins were recognized from cell wall samples of GZ0565, a virulent SS2 strain. They are 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-bisphosphatealdolase, respectively [38]. Second, comparative proteome analysis of secreted proteins of virulent SS9 strain GZ0565 and an avirulent SS9 isolate SH040917 led to the identification of 13 candidate proteins, five of which are putative virulence-related factors: DNA nuclease, o-acetylserinelyase, peptidoglycan-binding LysM, phosphorglyceratemutase, and putative 5'-nucleotidase [39]. It provided critical clues to better understand the SS9 pathogenesis.

Structural biology of *Streptococcus suis*

Although structural biology is an efficient approach to develop new antibacterial therapeutics against severe bacterial infection via targeting virulence-associated factors, current situation of its application into *S. suis* research remains relatively lagged. Compared with the totally 2200 putative proteins encoded by the whole genome of a representative strain of *S. suis* serotype 2, 05ZYH33 [10], the proteins with known structures is less than 0.5% [40-47]. As of writing this review, crystal structures of only nine proteins have been determined (Table 3). Among them, only two proteins are suggested to be involved in bacterial virulence: one is suilysin, belonging to a family of thiol-activated, membrane damaging toxins [41]; the other is the Dps-like peroxide resistance protein (Dpr) that is proposed to be a metal-binding protein with a role in bacterial iron uptake [45]. Four proteins are bacterial enzymes related to central metabolisms, which

Proteins	Functions	Resolution	Year	Ref.
Dpr	Dps-like peroxide resistance protein involved in iron uptake	1.95 Å	2004	[45]
RmlB	dTDP-d-glucose dehydratase	1.5 Å	2003	[43]
RmlC	dTDP-6-deoxy-D-xylo-4-hexulose 3,5-epimerase in the rhamnose pathway	1.3 Å	2003	[44]
ManD	Mannonate dehydratase	2.9 Å	2009	[46]
Ga5DH	Gluconate 5-dehydrogenase	2.0 Å	2009	[47]
TroA	ABC)-type transporter associated with Zn2+/Mn2+ uptake	2.6 Å	2011	[42]
SrtC	A surface-anchoring enzyme that specifically recognizes and cleaves the LPNTA motif of its substrates	2.4 Å	2011	[40]
Suilysin	Toxin/virulence factor	2.85 Å	2011	[41]

Table 3: Summary of protein structures determined from *S. suis* species.

are dTDP-d-glucose dehydratase (RmlB) [43], dTDP-6-deoxy-D-xylo-4-hexulose 3,5-epimerase (RmlC) from the rhamnose pathway [44], Mannonatedehydratase (ManD) [46], Gluconate 5-dehydrogenase (Ga5DH) [47], respectively. The remaining two proteins are consisted of TroA, an ATP-binding cassette (ABC)-type transporter required for Zn^{2+}/Mn^{2+} uptake [42] and a predicted Sortase C [40], which is supposed to be a surface-anchoring enzyme that specifically recognizes and cleaves the LPNTA motif of its substrates. Structural information of numerous proteins involved in interface between bacteria and its infected host remains elusive. It is also in great demand to determine structures of all the other known/partially confirmed virulence factors. It is a reasonable/practical way to facilitate molecular design of small molecular drugs used for therapeutics combating human SS2 infections.

Experimental models for *Streptococcus suis* infections

Ideally, all the information on *S. suis* pathogenesis acquired via the approaches of genomics/proteomics should be experimentally verified. Thereby establishment of experimental models of bacterial infections is perquisite for evaluation of contribution of putative virulence factors to bacterial virulence in vivo. Four kinds of infection models in total have been reported, which are separately Piglets [1,3], Mice [2,48,49], Zebrafish [39] and Amoeba [50]. Because that the swine is a natural host for *S. suis*, an observation in experimental infection of piglets is much more reliable relative to other animals. However, the major disadvantage of this model featured the expensiveness and inconvenience of piglets' maintenance. Balb/c mouse is an alternative mammalian model for *S. suis* infections, while the results in some cases can't be quite repeated [2]. Fortunately, CD1 mice seemed to have developed a satisfied mini-animal model in the replacement of Balb/c mice [48,49]. Wu and co-workers reported that Zebra fish is somehow comparable to piglets, and can function as an infection model to test *S. suis* virulence [39]. Very recently, Bonifeit et al. [50] found that Amoeba is an alternative host model which is susceptible to *S. suis* challenges.

Concluding remarks and Perspectives

The knowledge on *S. suis*, an emerging human pathogen, have been significantly accumulated in Omics-Era. In this brief review, we aim to show an updated (but not complete) picture of *S. suis* studies centering in advanced/integrated approaches, such as functional genomics, comparative proteomics, and structural biology. Although sequence information on tens of serotypes have been shown using the technology of Tilling microarray, the accuracy is mainly dependent on the reference genomic sequence. Thereby, at least to this point somewhat it is not comparable to that short gun genome DNA sequencing. In fact, only two whole-genomes are derived from the non-SS2 serotypes, SS3 and SS14 [14,15], suggesting that it is in demand to sequence all the other non-SS2 serotypes. Once all the genomes of 35 different serotypes are completed, the core genome and pan-genome of *S. suis* populations could be drew much more exactly. I am quite sure that it might significantly contribute to better understanding of *S. suis* pathogenesis. The current situation that the number of identified proteins by proteomics is only 1/5 of the predicted genes of *S. suis* 2 can be explained by at least 3 reasons: 1) Expressions of some genes are tightly controlled by growth environments (such as carbon/nitrogen sources, growth temperature); 2) Low level expression of auxiliary genes failed to be detected; 3) Some infection-related genes are only turned on when entry of SS2 into hosts. Thereby, it seemed to be a challenge to describe a relatively complete proteomic network picture of *S. suis*. The knowledge on structural biology of *S. suis* is very little, esp. in the structures of virulence factors. We believed that it is a promising direction in the near future, which can provide structural basis for drug design targeting virulence factors necessary for *S. suis* infection and pathogenicity. In particular, much attention should be paid to regulatory networks of *S. suis* virulence at different levels (such as small RNA-mediated post-transcriptional regulation), which might return unexpected new insights into *S. suis* pathogenesis.

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