

Whole-Genome-Sequence of Streptococcus Suis LSM178 with a Novel ST1005 Characterized the Hyper Pathogenicity in Human Infection

Yong Hu

Hubei University of Technology

Shiming Fu

Hubei University of Technology

Xingxing Dong

Huazhong Agricultural University

Lin Teng

University of Florida

Jinquan Li (✉ 15527430829@163.com)

Huazhong Agricultural University

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4
5 1 Key Laboratory of Fermentation Engineering (Ministry of Education), Hubei Key
6 Laboratory of Industrial Microbiology, National "111" Center for Cellular Regulation
7 and Molecular Pharmaceutics, Hubei Research Center of Food Fermentation
8 Engineering and Technology, Hubei University of Technology, Wuhan, 430068,
9 Hubei, People's Republic of China.

10 2 Key Laboratory of Environment Correlative Dietology, College of Food Science
11 and Technology, Huazhong Agricultural University, Wuhan, 430070, Hubei, People's
12 Republic of China.

13 3 Department of Animal Sciences, University of Florida, Gainesville, Florida, USA.

14 * Corresponding author. E-mail: lijinquan2007@gmail.com

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1 **Abstract**

2 *Streptococcus suis* (*S. suis*) has been well-recognized as a zoonotic pathogen
3 worldwide gearing up a great risk to the public health. In this study, an *S. suis*
4 LSM178 strain with serotype 2 and novel multi-locus sequence type of 1005, isolating
5 from a patient, was interpreted for the pathogenicity by its genetic information.
6 LSM178 was more efficiently invasive to Caco-2 cells than SC19 and P1/7.
7 Phylogenetic analysis showed that LSM178 clustered with highly virulent strains
8 including all human strains and epidemic strains. These serotype 2 *S. suis* from China
9 shared exclusively the typical virulence characteristics including the maximum (95/96)
10 virulent factors and type I-89 K Pathogenicity Island. Further, groups of genes were
11 identified to distinguish these highly virulent strains from other generally virulent
12 strains, emphasizing the key roles of genes modeling transcription, cell barrier,
13 replication, recombination and repair on the high pathogenicity for highly virulent
14 strains. Additionally, LSM178 contains a novel prophage conducive potentially to
15 pathogenicity. These characters would contribute to deeply studying the pathogenic
16 mechanism and virulence drift of human pathogenic *S. suis*.

17 **Key words:** *Streptococcus suis*; ST1005; Human infection; Virulence; Comparative
18 genomics; Pathogenicity

21 **Introduction**

22 *Streptococcus suis* (*S. suis*) is one of the most important swine pathogens leading to
23 severe economic losses to the porcine industry worldwide. However, *S. suis* has
24 emerged as an great zoonotic agent, causing fever, septicemia, meningitis, arthritis
25 and a variety of other symptoms in human. Since the first case of human *S. suis*
26 infection reported in 1968^[1], it had spread in more than 30 countries and/or regions,
27 particularly the southeast Asian countries where the pathogen represents a significant
28 public health concern^[2,3]. Seriously, 2 and 4 outbreaks have occurred in China
29 (Sichuan in 2005 and Jiangsu in 1998)^[4] and in Thailand (Phayao in 2007, Chiang
30 Mai and Lamphoon in 2008, Phetchabul in 2010 and Uttaradit in 2019)^[5] respectively.
31 Typing of *S. suis* strains is epidemiologically important to control the infection. The
32 most commonly used serotyping is not only used for identification and diagnosis of
33 clinical of *S. suis* isolates, but also its importance of on pathogenesis has been
34 suggested^[5]. Of the 35 serotypes (types 1-34 and 1/2) originally identified according

1 to the antigenicity of capsular polysaccharide (CPS), six *S. suis*-like strains (serotypes
2 20, 22, 26, 32, 33, and 34) have been taxonomically removed from the *S. suis* species
3 based on phylogenetic and/or sequence analyses^[6]. Additionally, new variants with
4 serotype Chz and novel cps loci were recently checked out, although their relation to
5 the virulence potential remain unclear^[7,8,9,10]. The prevalence of *S. suis* serotypes in
6 countries and regions is different. For instance, isolates associated with pigs disease
7 were predominantly identified as serotypes 2 and 9 in Europe^[11], and serotypes 2 and
8 3 in North America^[12]. However, serotype 2 is considered to be the most toxic and
9 prevalent serotype causing both pig and human infection worldwide^[13,14], although
10 other serotypes such as serotype 9 and 14 are increasingly urgent^[15]. A recent study
11 reported that, upon detection of raw pork and edible pig organs collected from 88
12 sales locations in central Thailand, the positive rate of *S. suis* was as high as 85.23%
13 and the positive rate of serotype 2 was 17.05%^[16].

14 Besides serotyping, genetically classification by multiple sequence locus typing
15 (MLST)^[17] has become increasingly important because of the higher resolution on
16 determining the strain evolution and on delineating the relationship between subtype
17 and the pathogenicity. For instance, while the serotype 2 ST1 strains present high
18 zoonotic potential worldwide, ST7 from serotype 2 and 14 is frequent to China^[5,18].
19 And, in Thai for human infections, ST104 are almost exclusively predominant in
20 serotype 2 and the main serotype 14 isolates was ST105^[5]. So far as to April 8, 2021,
21 2,808 STs have been recorded in the *S. suis* MLST database, showing that *S. suis* is
22 constantly evolving as the environment changes. Though only several STs have been
23 found to be mainly responsible for human infections, the increasing diversity brings
24 new risks and challenges, such as ST658 isolated in China^[19].

25 It is no doubt that virulence arsenal play more roles in human infection, since *S. suis*
26 was suggested to be a cause of community-acquired pathogen^[20]. It is difficult to
27 make any clear distinction about the virulence factors belonging exclusively to pigs
28 versus humans. In a recent study, no any defined genomic differences between human
29 strains and pig strains were suggested, although human disease isolates are limited to
30 a single virulent population whose origin nevertheless coincided with the first
31 intensification of pig production^[21]. Even, it couldn't definitely determine whether a
32 strain is a virulent one only by the presence of proposed virulence factors, making the
33 ambiguous definition for virulence factors^[22]. Virulence factors play key roles in
34 many aspects. One reason why serotype 2 strain showed a high zoonotic potential and

1 virulence in human was attributed to its better adherence to a human intestinal
2 epithelial cell^[5,23,24]. Deletion of these factors greatly attenuated virulence^[25,26,27,28,29].
3 As well, the ability to escape immune clearance is necessary for strain survival,
4 dissemination and pathogenesis. For example, CPS has been shown to enhance
5 bacterial resistance against the killing by host phagocytes^[30]. Many virulence factors
6 have potential to be vaccine candidates^[31]. It should be noted that a universal
7 cross-protective vaccine is highly challenging due to the diversity of *S. suis*. The
8 control of infection mainly depends on antibiotics therapy. And, the emergence of
9 antibiotic-resistant strains, especially multidrug-resistant strains, poses an intricate
10 problem^[32,33].

11 In this study, a human *S. suis* LSM178 with serotype 2 and novel ST1005 causing
12 fever, nausea and general malaise, was isolated and comprehensively assessed as a
13 hyper virulent strain based on toxicity tests and genomic analysis. With genome
14 sequencing, genetic features including mainly virulence factors, pathogenicity islands
15 (PAIs), prophages and core virulence genes were characterized to understand the
16 pathogenic potential. The analysis suggested that plastic genome contributes to
17 virulence evolution of *S. suis*, and special elements definitely model the virulence
18 change and the adaption of *S. suis* to human.

19

20 **Results**

21 **Zebrafish challenge**

22 In early a few hours, all three infection groups (LSM178, SC19 and P1/7) of infected
23 zebrafish showed no signs of disease except for a slight decrease of swimming ability.
24 After that, pathological changes appeared in challenged zebrafish such as systemic
25 hemorrhage or abdominal hemorrhage, abdominal swelling and dyspnea. The survival
26 rate did not show significant difference and stabilized at 10% for three groups (Fig. 1
27 A). However, the 50% and 90% lethal time by LSM178 (24-28 h and 34-38 h)
28 appeared always shorter than that by SC19 (26-32 h and 38-42 h) and P1/7 (30-36 h
29 and 36-40 h) in each of 3 independent repeated tests (Fig. 1 A, as one representative).
30 The main symptoms of the death caused by LSM178 infection are seriously
31 abdominal swelling, blood spots and ecchymosis (Fig. 1 B), which were similar with
32 those caused by SC19 and P1/7.

33

1 **Human whole blood resistance assay, cytotoxic assay and cell adhesion and**
2 **invasion test**

3 Though there was no significance in cytotoxicity to Caco-2 among three strains,
4 LSM178 appeared more cytotoxic than SC19 and P1/7 and showed almost twice as
5 toxic as P1/7 strain (Fig. 2 A). In human blood resistance assay, LSM178 survived
6 similarly to P1/7, but seemed grow better than SC19 (Fig. 2 B). Further, LSM178
7 showed significantly more invasion to Caco-2 cells than P1/7 (Fig. 2 C) , whereas the
8 two strains were similar adhesive (Fig. 2 D). And, with lower adhesion (Fig. 2 D), the
9 invasion of LSM178 was equal to that of SC19 (Fig. 2 C).

10
11 **Analysis of antimicrobial susceptibility profiles**

12 LSM178 genome consists of a single circular chromosome of 2,115,437 base pairs
13 (bp) with average GC content of 41.19% and 2,065 ORFs (Fig. 3). Sequence assays
14 designated LSM178 with serotype 2 and a novel ST (1005) up to now. With
15 goeBURST analysis, ST1005 was shown to be an individual ST (Supplementary Fig.
16 1).

17 The scanning results showed that LSM178 possesses 10 potential specific
18 antibiotic-resistant genes. Only two of them might get effects, those are a tetracycline
19 resistant gene (tetM, 04995) conferring the tetracycline resistance and a MSL
20 methylase (04940) encoding gene resulting in both erythromycin and clindamycin
21 resistance (Table 1). Others include five penicillin-binding proteins (PBPs, PBP1b
22 (00760), PBP1a (02025), PBP1a (09665), PBP2b (03000) and PBP2x (08260)), an
23 Aminoglycoside 6-adenylyltransferase (ANT(6)-Ia, 04805) and two primary targets of
24 quinolone (DNA gyrase (GyrA, 04275) and topoisomerase IV (ParC, 05695)). They
25 are inefficitive and were verificated to sensitive to penicillin, streptomycin and
26 levofloxacin (Table 1).

27
28 **Phylogenetic tree analysis**

29 A phylogenetic tree was generated based on 51,520 core-genome SNP sites using the
30 genome sequence of LSM178 and 52 published *S. suis* complete genomes (Fig. 4).
31 Within the 18 strains clustered with LSM178 (LSM178-branch), 7 out of 12 pig
32 isolates and 5 out of 6 human strains were from China. However, the closest 2 strains
33 (NCTC10234 and S735) were neither from China nor from human. Among the 53

1 strains, most ST1 (5/9) and ST7 (9/12) were clustered in LSM178-branch. It was
2 interesting that in LSM178-branch, the STs of human isolates (LSM178, LSM102,
3 05ZYH33, SC84, BM407 and 98HAH33 with ST1005, ST658, ST945, ST7, ST1 and
4 ST890 respectively) are more diverse than those of pig strains (just including ST1 and
5 ST7).

6

7 **Analysis of prophages and toxin-antitoxin (TA)**

8 Two prophages, pha17801 (1,417,367 bp - 1,458,272 bp) and pha17802 (1,871,779 bp
9 - 1,926,900 bp), were found in LSM178. The pha17801 featured by only integrase
10 (1,437,316 bp-1,437,858 bp / 1,446,215 bp - 1,447,357 bp), with 50 ORFs (1,417,367
11 bp - 1,458,272 bp) and GC content of 39.36%, is similar highly with partial complete
12 genome of *Streptococcus* phage 20617 (NC_023503). The prophage included two
13 overlapping GIs (one from 1,421,901 bp to 1,447,702 bp and the other from
14 1,437,316 bp to 1,447,702 bp). While these two GIs are shared almost by all 53
15 strains, the remaining sequence in pha17801 was only shared completely by 18 *S. suis*
16 (LSM178, 05ZYH33, 98HAH33, A7, BM407, CS100322, GZ1, JS14, LSM102, P1/7,
17 S10, SC070731, SC19, SC84, SS12, SS2-1, T15 and ZY05719). Excluding avirulent
18 T15^[34], these strains almost constituted the GZ1-branch covering the LSM178-branch
19 (Fig. 4).

20 In pha17802, the overlapped GI (1,866,902 bp - 1,904,229 bp) was characteristic to
21 LSM178 in comparison with other 52 *S. suis* (Fig. 5 A). The left part (1,871,862 bp -
22 1,892,563 bp) of the GI is only highly homologous (identity of 90%) with a region of
23 *S. suis* 6407, whereas the right part (1,892,563 bp - 1,903,867 bp) together with
24 sequence from 1,903,868 bp to 1,906,268 bp is not homologous with any *S. suis* but
25 with *Streptococcus agalactiae* Sag37 (identity of 91%) (Fig. 5 B). The prophage
26 comprises 55,121 bp with an average GC content of 41.87%, encoding a total of 68
27 ORFs including prophage core component genes coding lysin, tail, head, recombinase,
28 capsid, portal, integrase, portal, and Cro/C1-type repressor. The absence of
29 excisionase confirmed the non-plaques on several strains containing LSM178 (data not
30 shown). The short directly repeated sequences (5'GGTTTCAATTTTA3') located the
31 prophage between 09360 (adenylate kinase) and 09655 (preprotein translocase
32 subunit SecE). The sequence from 1,866,902 bp to 1,906,268 bp contained 23
33 function-known genes along with 29 hypothetical proteins and the unique sequence
34 (1,892,563 bp - 1,906,268 bp) involved 29 genes where 21 encode unknown proteins.

1 The TA of HicA (09375)-HicB (09370) is the only candidate virulent elements in this
2 phage.

3 Scanning the LSM178 genome, there are other 5 pairs of TA as follows, toxin
4 (09760)-antitoxin (09765), toxin (05185)-antitoxin (05180), toxin (06380)-antitoxin
5 (06385), toxin (07105)-antitoxin (07110) and SezA (04880)-SezT (04885) contained
6 in 84K-PAI. In addition, an orphan antitoxin-Xre (04820) was identified.

7

8 **Comparative analysis of virulence factors**

9 Examining the 96 published virulent factors among 53 *S. suis*, LSM178 is one of the 8
10 strains (LSM178, ZY05719, 98HAH33, SC84, 05ZYH33, SS2-1, SC19 and LSM102)
11 containing the maximum (95) virulence factors (Supplementary Table 1). The 95
12 factors contain 15 ones linking to immune evasion or systemic infection, including
13 SalKR, NisKR, Epf, Fhb, IgA1, Ide_{S. suis}, MRP, Sly, Nudp, SsnA, EndA, ScpA and
14 SsadS. Excluding GZ1 and BM407 with 87 and 93 virulent factors respectively, these
15 8 strains contain all human strains (LSM178, 98HAH33, SC84, 05ZYH33 and
16 LSM102) and epidemic strains (98HAH33, SC84, SC19, 05ZYH33 and ZY05719).
17 The Rgg is the only virulence factor missed in the 8 *S. suis* and it is found merely in
18 other 5 *S. suis* (D12, 0061, 1081, CZ130302 and HN105). Two (0061 and 1081) of
19 the 5 strains are probably avirulent due to their isolation from healthy pigs, suggesting
20 that the Rgg may not be a virulent determinant. The common characteristics of
21 virulence factors in the 8 highly virulent strains would mean the highly pathogenic
22 phenotype of LSM178.

23 An 84K (83,582 bp) PAI (926,718 bp - 1,010,299 bp) was found-highly homologous
24 with type I-89K-PAI of SC19 and both of them coline with ICESsu05SC260
25 belonging to ICESa2603 family (Fig. 5 C). The direct repeat sequences indicated the
26 location of the 84K-PAI just downstream of rplL (04665). The PAI includes
27 characterized virulence factors such as SalKR, NisKR and several type IV-like
28 secretion systems (Vir D4, Vir B6, Vir B1 and Vir B4). The presence of integrase,
29 transposase, excisionase and helicase support activity of transposition and propagation
30 of 84K-PAI. The major difference of LSM178 84K-PAI from SC19 89K-PAI is the
31 absence of PqqD family protein, asparagine synthetase and 4 ABC transporter units,
32 and addition of transposase and MSL methylase as described. Except the methylase
33 which may cause epigenetic changes associated with virulence, other proteins are not
34 defined as virulence factors and should not prompt virulent contribution.

1 The type I-89K-PAI appeared firstly in SS2-1 (diseased pig, 1998) and 98HAH33
2 (human, 1998), which related closely with SC84 (human, 2005), ZY05719 (pig, 2005)
3 and SC19 (pig, 2005) (Fig. 5 A and Fig. 4). From 2005, the PAI was reported in
4 almost all human strains (SC84 2005, 05ZYH33 2005, LSM102 2014 and LSM178
5 2016) but GZ1 (2005), but only in two (ZY0571 2005 and SC19 2005) rather than the
6 other 10 pig strains. The human strains not from China (861180/Netherlands/1986 and
7 BM407/vietham/2004) do not contain type I-89K-PAI (Fig. 5 A and Fig. 4). The
8 distribution of the type I-89K-PAI among the strains coincides interestingly with the
9 combination of maximum (95) virulent factors (Fig. 5 A and Supplementary Table 1).
10 Since LSM178 shares these important virulence markers with epidemic strains and
11 almost all human strains, these strains were referred here as the highly virulent strains
12 (HVS) at least for serotype 2 strains from China, those are LSM178, ZY05719,
13 98HAH33, SC84, 05ZYH33, SS2-1, SC19 and LSM102.

14

15 **Genomic comparative analysis**

16 Checking virulence factors between strains in GZ1-branch and closely related
17 avirulent T15, T15 have 87 virulence factors which were shared by GZ1-branch
18 strains except A7, P1/7, S10 and GZ1 (Supplementary Table 1). These four strains
19 also have 87 virulence factors, but they share Epf, NadR, RevS and SBP2 instead of
20 Trag, VirB1, VirB4 and VirD4 existed in the T15. However, there are no virulence
21 factors belonging exclusively to avirulent T15 or all GZ1-branch strains. To globally
22 find out the characteristic virulence factors, the difference was checked between
23 strains in GZ1-branch and closely related avirulent T15. Referring to T15, the
24 numbers of unique genes of each virulent strain were between 318 and 582, and 224
25 shared genes were found (Fig. 6 A). HVSs possess more core genes (332) than other
26 virulent strains (249, called generally virulent strain here) (Fig. 6 B and C). Addition
27 of any one generally virulent strain except BM407 (Vietnam) resulted sharp decreased
28 number of core high virulence genes (Fig. 6 D). However, a little changes were
29 observed when any one highly virulent strain was removed. It supported the HVSs as
30 a separated group in serotype 2 strains. The characteristic genes from various
31 categories encode a wide range of functions (Fig. 6 E-G). Compared with generally
32 virulent strains, HVSs show predominant increase on genes with functions of
33 transcription, cell barrier, replication, recombination, repair and mobile elements (Fig.
34 6 F and G). GIs of LSM178 occupy 10.4% of the genome (Fig. 6 H). While there are

1 3.7% of core virulence genes in GIs of LSM178, 23.5% of core high virulence genes
2 locate in the GIs (Fig. 6 H).

3

4 **Discussion**

5 Generally, *S. suis* infections in humans were restricted to workers in close contact
6 with pigs or swine byproducts. However, in southeast Asia, the bacterium has been
7 reported to affect the general population^[20]. The sporadic human *S. suis*, particular
8 those with serotype 2, should be get attention in epidemiological monitoring because
9 of their unpredictable adaptive potential, for instance of LSM178 here, a serotype 2
10 strain with novel ST1005. LSM178 was more efficiently invasive to Caco-2 cells
11 compared with P1/7 and SC84. However, no significant virulence were detected in
12 cytotoxic activity and challenging zebrafish which has been used as a model to
13 evaluate the virulence of *S. suis*^[35]. In deed, virulence of *S. suis* could not be
14 intensively evaluated and compared in other models^[36]. Probably, virulent strains
15 have their own outstanding virulence aspects, which can balance the weak ones and
16 eventually lead to a similar pathogenicity at least under the inoculation. For instance,
17 P1/7 and SC84 were prominent in biofilm formation (Supplementary Fig. 2) and
18 adhesion respectively.

19 Recent studies suggested that quinolones, beta-lactams, florfenicol and
20 trimethoprim/sulfamethoxazole could still treat well the clinic *S. suis* infection^[37,38,39].
21 However, the strains resistant to widely used effective beta-lactams have been
22 increasingly reported^[40,41]. LSM178 showed the sensitivity at least to beta-lactams and
23 quinolones, consistency with the features of resistance genes. The PBPs of LSM178
24 are entirely as same as those of sensitive A7, but harbor substitutions throughout the
25 sequence compared with the resistant R61
26 (Supplementary Fig. 3A-E)^[40]. Several mutations in the quinolone
27 resistance-determining region (QRDR) of both GyrA and ParC could increase the
28 resistance to fluoroquinolone^[42]. However, there revealed no amino acid changes in
29 the QRDRs between LSM178 and four quinolone-sensitive strains (A7, BM407, P1/7
30 and SC84)^[40] (Supplementary Fig. 4). Additionally, it is interesting that the P1/7
31 without ANT(6)-Ia conferred resistance against streptomycin, whereas ANT(6)-Ia
32 containing LSM178 could not^[38]. However, ANT(6)-Ia of LSM178 is only 49.5%
33 identical to the functional homologue (UniProtKB - P12055 (AADK_STAAU)) from
34 *Staphylococcus aureus*^[43].

1 Several factors are deemed to be important for the pathogenesis, such as CPS, Fbbs,
2 enolases, dipeptidylpeptidase DppIV and SrtA^[22], which were all found in LSM178. It
3 has been demonstrated that *S. suis* serotype 2 virulent strains are able to exacerbate
4 inflammatory activation scavenging bacteria. All the 15 known anti-immunity
5 factors^[19] exist in LSM178 and would modulate the immune responses improving its
6 survival at the inflammation area. Although it is hardly to define a virulent strain only
7 by proposed virulence genes, many avirulent strains isolated from healthy animal are
8 found with less virulence factors (less than 87), such as WUSS351, 0061, 1081,
9 HA1003, DN13, TL13, 05HAS68, YB51, LS9N, CZ130302, 90-1330 and T15. These
10 *S. suis* lack at least one of the marker virulence genes (Sly, Mrp, Efp and Cps2).
11 Notably, five of those strains contain less than 70 virulent factors (0061, 1081,
12 HA1003, LS9N and CZ130302). The 9401240 is an exception since it was isolated
13 from a diseased pig but has 69 virulence factors. Probably, the 9401240 is a
14 non-pathogenic strain occurred just in a case of co-infection. On the contrary, the
15 HVSs contain consistently the maximum (95/96) virulent factors. However, it should
16 be realized that the combination of various virulence factors may cause pathogenicity
17 despite the number of the virulence factors.

18 It is very clear that intermediately pathogenic strain could evolve to highly pathogenic
19 strain and then epidemic strain^[3]. In LSM178-branch, all 18 strains possessed the
20 serotype 2. However, while 6 human strains have their individual STs, 12 pig strains
21 were just ST1 (4 strains) or ST7 (8 strains). It may imply that while *S. suis* has been
22 purified to relatively stable genotype in pig, multiple evolutionary directions are in
23 progress to be epidemic during their adaption to human, at least for serotype 2
24 strains in China.

25 Type I-89K-PAI is specific to highly pathogenic *S. suis* linked to Chinese epidemics^[19]
26 and could horizontally transfer among strains^[44]. Humans can carry avirulent *S. suis*
27 without clinical signs^[2,45]. However, the 89K-PAI of human virulent strain should not
28 obtained from pig strain owing to the transfer, since all strains with 89K-PAI
29 presented an extremely short evolutionary distance from each other. It suggested that
30 human pathogenic infection was due to the interspecies transmission of a swine-origin
31 strain. Thinking of the gradually increasing of 89K-PAIs in human strains and
32 decreasing in pig strains, it may suggest that the 89K-PAI is one of the markers
33 adapting pig strain to human. On the contrary, the existence of PAI would not be
34 conducive to the adaptation of strains in pigs, that might explain the reason why the

1 pig strains loss it more and more rapidly. Under this consideration, the combinations
2 of 95 virulence factors maybe also regarded as one of the potential for the adaptation
3 to human. As well, the ST transition might play roles in adaption of strains to human,
4 since there is only ST7 in all 89K-PAI containing pig strains and just one human
5 strain (SC84) whereas other human strains possessed varied STs (ST1005, ST945,
6 ST890 or ST658).

7 In published *S. suis* genomes, many prophages were held as remnants^[46,47]. A few
8 intact prophages was described and one of them was reported to be induced to lyse *S.*
9 *suis*^[48]. The two prophages in LSM178 could not be induced to form plaques on
10 several *S. suis* strains. The reason might be that improper induction method was
11 adopted or that the phages produced are defective on infection even for the almost
12 intact pha17802^[48]. Alternatively, the lysis spectrum of the phages is very narrow, or
13 no phages were induced at all, which could be supported by the facts that pha17801
14 contains only integrase and pha17802 does not include excisionase. Genomic
15 comparative analysis suggested that the pha17802 may integrate two elements
16 horizontally transferred from *S. suis* 6407 and strain of *S. agalactiae* Sag37
17 respectively. The unique gene fragment from *S. agalactiae* Sag37 has not been found
18 in any other *S. suis* genome published so far. The empty target site might
19 accommodate potentially unpredictable integration of other mobile genetic elements.
20 Prophages make up a platform for the dissemination of virulence determinants
21 between intra- and inter-species, contributing evolution of pathogenic bacteria^[28,49].

22 For pha17802, except the HicAB located in the arm homologous to 6407, no known
23 genes contributing to virulence were identified. There were no evidences
24 demonstrating the contribution of the two transferring elements to virulence. However,
25 the rarity of pha17802 probably suggested that it would provide an advantage under
26 certain circumstances. Some clues may be provided by investigating the patient's
27 infection process. However, the lysogenic state would at least increase the survival in
28 the environment by resisting to infection of similar viruses. Furthermore, it may
29 increase the fitness of the bacteria by modulating host metabolism^[48].

30 While the core virulence genes were identified through the comparison between
31 avirulent T15 and virulent strains in GZ1-branch, they should be responsible for the
32 enhanced pathogenicity. Moreover, some genes could be used to make a distinction
33 between HVSs and other general virulent strains. Particularly, it should emphasize the
34 roles of genes with functions of transcription, cell barrier, replication, recombination

1 and repair in virulence enhancement, since their number increased obviously in HVSS
2 core genes. Thus, the importance of the mobilizable elements is beyond all doubt,
3 because it is the carrier responsible for genetic differences^[50]. In fact, GIs make up a
4 considerable part of the genome for LSM178 (10.4%). And, higher proportion of
5 virulence-enhancing genes are dispersed in GIs, for example that GIs of LSM178 are
6 colonized with 3.7% of core general virulence genes but with 23.5% of core high
7 virulence genes. However, there are less GIs in LSM178 (number of 23 and total
8 length of 21, 5087 bp) than T15 (29 and 31, 4245 bp) (Supplementary Fig. 5 B).
9 These suggested that fusion of specific PAIs increased the virulence. The
10 non-existence of CRISPRs defending against foreign invading elements should be one
11 of the reasons interpreting the rich GIs^[51]. In addition, assay showed that there are
12 more core avirulence genes (535) (Supplementary Fig. 5 A) than core virulence genes
13 (224). Probably, both the gain of virulence genes and loss of avirulence genes
14 contribute to the increased virulence. Other potential difference, such as SNPs and
15 patches of insertion and deletion, deserve also to be explored and should not be
16 ignored. These differences are small but numerous and they would definitely interpret
17 the change of strain virulence through just affecting the genes expression or protein
18 activity^[52].

20 MATERIALS AND METHODS

21 Strains and antimicrobial susceptibility testing

22 *S. suis* LSM178 was isolated at 2016 from a patient with clinical symptoms of fever,
23 nausea, and general malaise. The patient had contacted the pigs and handled raw pork
24 before admission. The *S. suis* SC19 and *S. suis* P1/7 were stored by our laboratory.
25 Antimicrobial susceptibility was tested by E-test (AB Biodisk, Sweden) with
26 *Streptococcus pneumoniae* ATCC49619 as a control. All protocols was approved by
27 committee of State Key Laboratory of Agriculture Microbiology and the ethics
28 committee of Huazhong Agricultural University. Experiments were performed in
29 ABSL 3 laboratory, Huazhong Agricultural University.

31 Human whole blood resistance assay

32 Blood assays were conducted according to an approval issued by the Medical Ethics
33 Committee of the Huazhong Agriculture University (Wuhan, China). Strain
34 suspension (100 μ L, 5.0×10^7 CFU/ml) was transferred into 900 μ L of fresh human

1 whole blood followed by incubation at 37°C. The sample was withdrawn every 1h and
2 diluted to incubate on TSA solid medium (containing 10% fresh FBS) at 37°C
3 overnight to count colony-forming units (CFU). Growth index (%) = (CFU_{at a certain time}
4 point - CFU_{original inoculum}) / CFU_{original inoculum} × 100%.

5

6 **Zebrafish challenge**

7 Zebrafish was feeded as previous description. Inoculum was collected at the end of
8 the logarithm period, cleaned twice with phosphate buffered saline (PBS), and
9 adjusted to the appropriate dose (2.5×10^9 CFU/ml)^[35]. Adult zebrafish was infected
10 by intraperitoneal inoculation with 20 µL of bacterial solution per tail. Each
11 group contained 10 zebrafishes. The symptoms of zebrafish were recorded every 2
12 hours. Euthanasia of zebrafish was conducted at 60 h after challenge using
13 Tris-buffered tricaine at a concentration of 320 µg/ml.

14

15 **Biofilm formation assay**

16 Strains (20 µL, 5.0×10^7 CFU/ml) were inoculated into 2 mL TSB medium
17 (containing 10% fresh FBS) and cultured in a 24-well cell plate at 37°C for 3 days,
18 and the un-inoculated medium was used as the control. After that, the strains were
19 wased twice with sterilized PBS, and fixed with 500 µL methanol for 30 min to attach
20 the strains to the wall. Then, methanol was removed and the plate was air-dried at
21 room temperature. Next, 500 µL of 0.1% crystal violet dye solution was added in and
22 removed out until 30 min later. After drying at 56°C, 500 µL of 33% acetic acid
23 solution was added and placed on a shaker for 30 min to release the crystal violet
24 bound to the biofilm. The released solution (200 µL) from each well was measured at
25 a wavelength of 600 nm.

26

27 **Cell experiments**

28 Strains at log phase was used in the experiments. For cytotoxic assay, Caco-2 cells ($1 \times$
29 10^4)^[53] in 96 well plate was used to detect cytotoxicity of the strains (2×10^5 CFU)
30 with lactate dehydrogenase kit (Beyotime, Beijing, China). The percentage of
31 cytotoxicity was calculated referring to the protocol of the kit: cytotoxicity (%) =
32 (LDH release from infected cells –spontaneous release of LDH from uninfected cells)
33 / (maximum LDH release from cell lysate–spontaneous release of LDH from

1 uninfected cells) $\times 100\%$.
2 For cell adhesion and invasion, single layer Caco-2 cells in the 24 well culture plate
3 were inoculated with 500 μL bacterial suspension (1×10^6 CFU). After washing to
4 remove unadhesive strain, cells were then treated by trypsin digestion for 2 hours. In
5 invasion, extracellular bacteria were treated with gentamicin (100 $\mu\text{g}/\text{ml}$) and
6 penicillin G (5 $\mu\text{g}/\text{ml}$) before trypsin treatment. The digested cells were lysed using
7 1% saponin and the lysis was inoculated on THB plate. The rate of adhesion (Ra) and
8 invasion (Ri) was expressed as $(\text{CFU}_{\text{determined from plate}} / \text{CFU}_{\text{original inoculum}}) \times 100\%$. The
9 relative invasion rate was expressed as $\text{Ri}_{\text{strain}} / \text{Ri}_{\text{P1/7}} \times 100\%$.

10

11 **Plaque assay**

12 LSM178 culture in exponential growth phase was induced by mitomycin C (500
13 ng/ml) (Sigma, St. Louis, USA) for 5-15 min. The culture (100 μL) was mixed with 3
14 ml TSA (45°C) containing 10% fresh FBS to prepare sandwich plaque assay at 37°C.
15 Plaque formation was observed after 12 h.

16

17 **Genomic analysis**

18 The genomic DNA was extracted using a DNA extraction kit (TaKaRa DNAiso;
19 TaKaRa Biotechnology Co., Ltd., Dalian, China). The genome of LMS178 was
20 sequenced using combined platforms of Illumina Miniseq and PacBio sequel. After
21 correction of the results using Pilon (Walker B J, Abeel T, Shea T, et al. Pilon: an
22 integrated tool for comprehensive microbial variant detection and genome assembly
23 improvement), the complete circular genome was constructed (NCBI-SRA accession:
24 PRJNA596999).

25 According to the published studies, virulence factor database of *S. suis* was
26 established (Supplementary Table 1) and used to detect the potential virulence genes
27 in genome. Antibiotic resistance genes were predicted with the comprehensive
28 antibiotic resistance database (CARD) with default settings^[54]. The ST was
29 determined using MLST typing scheme (<https://pubmlst.org/ssuis/>). Serotyping
30 strategy was executed based on the homology and coverage (80%, $\text{evalue} \leq 1\text{e-}10$)
31 between WZY amino acid sequence of the LSM178 and 33 standard strains with
32 known serotypes. The sequence of CpsK was used to discriminate the serotype 2
33 (W161) from 1/2 (C161) for all strains with serotype 2 or 1/2^[55,56]. ST complexes

1 were analysed by goeBURST^[54] program (<http://goeburst.phyloviz.net>). Prediction of
2 gene islands (GI) and prophages were performed using IslandViewer 4^[57] and
3 PHAST^[58] respectively. Clustered Regularly Interspaced Short Palindromic Repeats
4 (CRISPRs) were predicted by CRISPR recognition tool (CRT)^[59]. Open reading
5 frames (ORFs), tRNA and rRNA were predicted with Prokka. The proteins in
6 prophage and PAI were integrally annotated by databases of NR, eggNOG, KEGG,
7 Swiss-Prot and GO. Genome mapping with informations was generated by CGView^[60].
8 To identify the unique regions in the genome of LSM178, the whole-genome
9 sequence was used as a reference to compared with that of the other 52 strains using
10 BLAST Ring Image Generator (BRIG)^[61]. The 84K PAI in LSM178, 89K PAI in SC
11 19 and ICESsu05SC260 were compared to determine their similarity using BLASTn
12 embedded in Easyfig. Similarly, the characteristics of the prophages in LSM178 were
13 evaluated. The differential genes between two strains were extracted with Roary^[62] to
14 create *Venn* diagrams and their COG functions were classified. Circos^[63] was
15 conducted to map the distribution of GIs and interesting genes in genomes of
16 LSM178 and T15.

17

18 **Phylogenetic analysis**

19 Complete genomic sequences of 52 *S. suis* sequences were downloaded from NCBI
20 database (<https://www.ncbi.nlm.nih.gov/genome/genomes/199>) (Supplementary Table
21 2). The chromosomal sequences were aligned using Parsnp program generating
22 core-genome single-nucleotide polymorphisms (SNPs). Phylogenetic tree based on
23 core SNP of 53 complete *S. suis* genome (including LSM178) was constructed using
24 maximum-likelihood phylogenetic trees by FastTree embedded in the Parsnp. The
25 bootstrap value was set at 1000 times. The phylogenetic tree was displayed using the
26 online website iTOL (<http://itol.embl.de/>).

27

28 **Statistical analysis**

29 Each experiment was repeated 3 times or more and the significance was analyzed
30 with unpaired student's test using GraphPad Prism 5. The $P < 0.05$ and $P < 0.01$ were
31 represented as * and ** respectively.

32

33 **Ethics statement**

1 This study was carried out in compliance with the ARRIVE guidelines. The study was
2 approved by ethics committee of Huazhong Agricultural University and all
3 experiments were performed in accordance with guidelines of State Key Laboratory
4 of Agriculture Microbiology. The informed consent was obtained from all participants
5 and/or their legal guardians.

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7
8
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11 **Authors' contributions**

12 J.L designed the study. Y.H, S.F, X.D and L.T performed experiment and analyzed
13 data. Y.H, S.F wrote the manuscript. All authors provided critical review and
14 commentary and agree to the final version to be published.

15 **Conflict of Interest**

16 The authors declare no conflicts of interest and no competing financial interests.

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3
4
5
6
7

8 **Table**

9

10 Table 1. Minimal inhibitory concentration (MIC) of 12 antimicrobial agents for
 11 LSM178 and the resistance phenotype (RP).

	Antibiotics MIC (µg/mL)											
	PEN	TET	VAN	CRM	CHR	CLI	LVX	ERY	STR	IPM	LZD	RFP
ATCC 49619	0.25	≤0.5	≤0.12	0.5	2	≤0.06	0.5	≤0.06	0.12	0.12	≤0.06	0.25
LSM178	≤0.06	>3	0.25	≤0.25	<3	>0.5	0.75	>0.5	≤0.12	≤0.06	0.12	0.25
RP	-	+	-	-	-	+	-	+	-	-	-	-

12 Abbreviations: PEN, penicillin; TET, tetracycline; VAN, vancomycin; CRM,
 13 cefuroxime; CHR, chloramphenicol; CLI, clindamycin; LVX, levofloxacin; ERY,
 14 erythromycin; STR, Streptomycin; IPM, Imipenem; LZD, Linezolid; RFP, rifampici;
 15 +, resistance; -, sensitivity.

16

17 **Figure Legends**

18

19 **Figure 1.** Evaluation of the virulence of the LSM178 in zebrafish. (A) Survival rate
 20 of zebrafish infected with LSM178, SC19, P1/7 and placebo. (B) Images of
 21 zebrafish infected with LSM178 or treated with placebo.

22 **Figure 2.** The resistance of LSM178 to human whole blood and experiments with
 23 Caco-2 cells. (A) The cytotoxicity of LSM178 to cells. (B) The growth index of LSM
 24 in human whole blood. (C) The relative invasion of LSM178 to cell. (D) The adhesion
 25 of LSM178 to cell. P<0.05 *, P<0.01 **.

26 **Figure 3.** The circular diagram of the LSM178 genome. From inside to outside, the
 27 first circle, the scale of genome; the second circle, GC skew; the third circle, GC
 28 content; the fourth and seventh circles, the COG category of coding sequence (CDS)
 29 on two strands; the fifth and sixth circles, the position of CDS, tRNA and rRNA on
 30 two strands.

1 **Figure 4.** Phylogenetic tree of 53 *S. suis* based on core genome SNP.

2 **Figure 5.** Genome analyse of LSM178. (A) Genome comparison among 52 *S. suis*
3 and LSM178. Each circle showed the variations of strain relative to LSM178. From
4 inside to outside, the genomes was as follows: 61, 05HAS68, 05ZYH33, 1081, 6407,
5 861160, 90-1330, 9401240, 98HAH33, A7, AH681, BM407, CS100322, CZ130302,
6 D12, D9, DN13, GD-0001, GD-0088, GZ0565, GZ1, HA0609, HA1003, HN105,
7 HN136, INT-01, ISU2812, JS14, LS9N, LSM102, NCTC10234, NCTC10237,
8 NSUI002, NSUI060, P1/7, S10, S735, SC070731, SC19, SC84, SH0104, SH1510,
9 SRD478, SS12, SS2-1, ST1, ST3, T15, TL13, WUSS351, YB51 and ZY05719. The
10 variable colors in each circle stand for sequence identity with the inset of *S. suis* 61 as
11 an example. (B) Comparison of the 84K-PAI with the 89K-PAI from SC19. The
12 84K-PAI were marked with key genes (black) including virulence-related factors
13 (bold), antibiotic resistance factors (bold), major differential genes and core
14 transposition elements. Green box, GIs; Red box, Tn916. (C) Comparison of the
15 pha17802 with the homologous region located in *S. suis* 6407 and *S. agalactiae* Sag37.
16 Genes rather than hypothetical protein-coding ones were given predictable functions
17 (black). Green box, GI. The functional regions were designed with red line.

18 **Figure 6.** Chromosomal features of LSM178. (A) Venn diagram of the special genes
19 of each strain from GZ1-branch. The special genes for each strain were extracted with
20 T15 as the reference. The overlap was named core virulence genes. (B) Venn diagram
21 describing the special genes of HVs. (C) Venn diagram describing the special genes
22 of generally virulent strains. (D) Changes of core high virulence genes. $P < 0.01$ **.
23 (E-G) COG function classification of the core genes from (A-C) respectively. (H)
24 Locations of GIs (blue), core general virulence genes (black) and core high virulence
25 genes (red) in LSM178 genome.

26
27

Figures

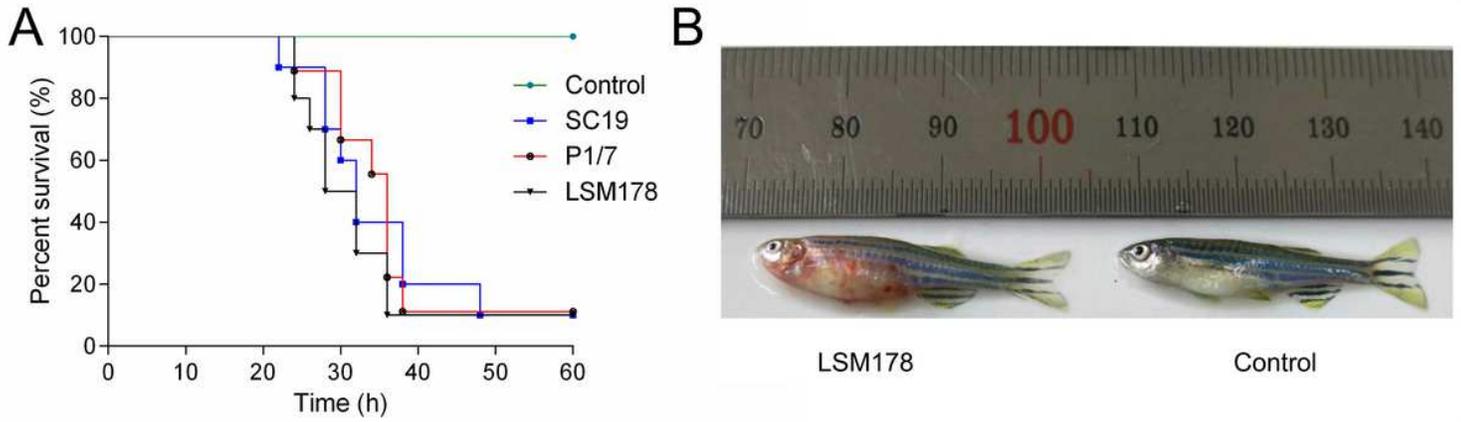


Figure 1

Evaluation of the virulence of the LSM178 in zebrafish. (A) Survival rate of zebrafish infected with LSM178, SC19, P1/7 and placebo. (B) Images of zebrafish infected with LSM178 or treated with placebo.

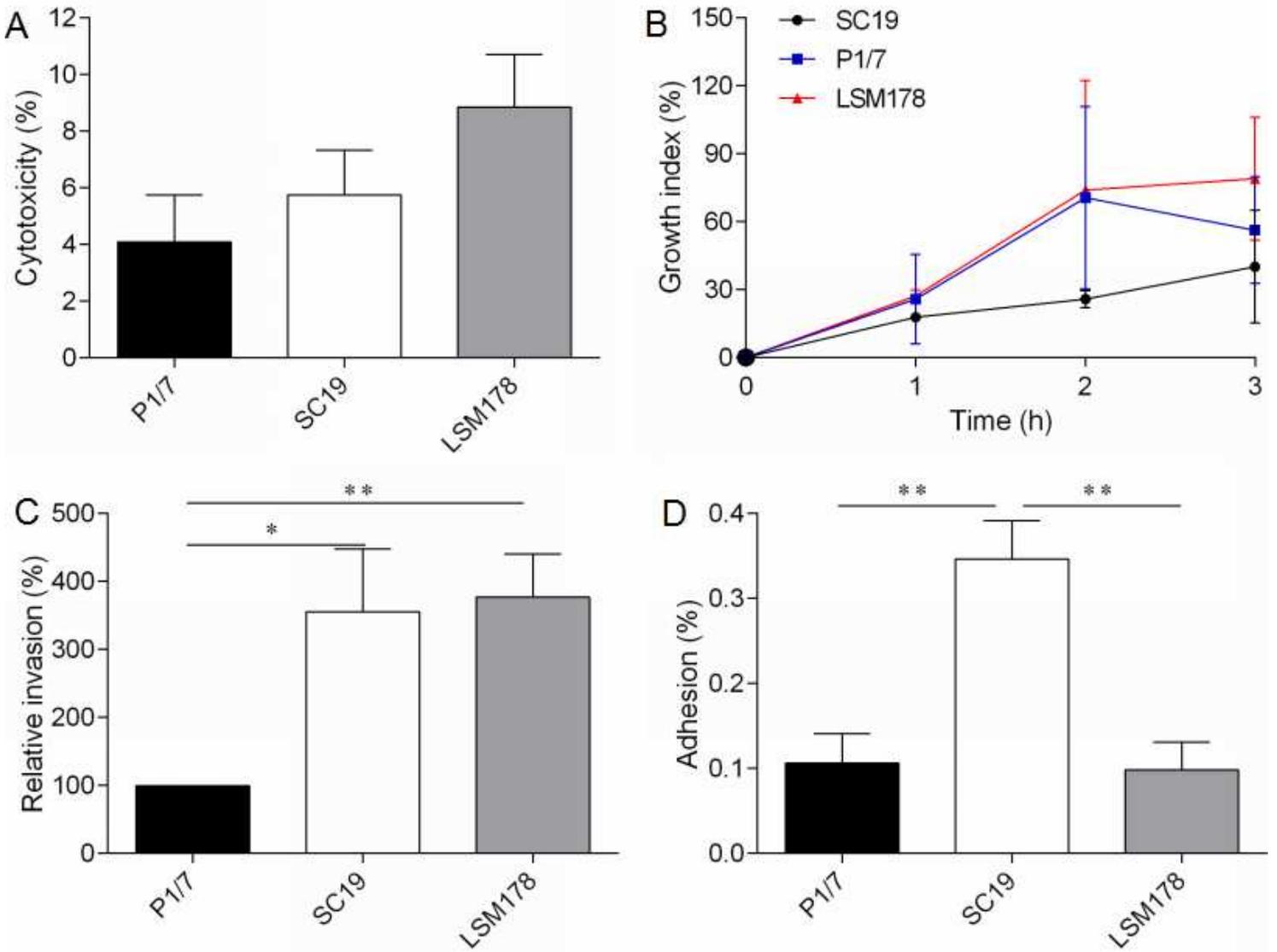


Figure 2

The resistance of LSM178 to human whole blood and experiments with Caco-2 cells. (A) The cytotoxicity of LSM178 to cells. (B) The growth index of LSM in human whole blood. (C) The relative invasion of LSM178 to cell. (D) The adhesion of LSM178 to cell. $P \geq 0.05$ *, $P \geq 0.01$ **.

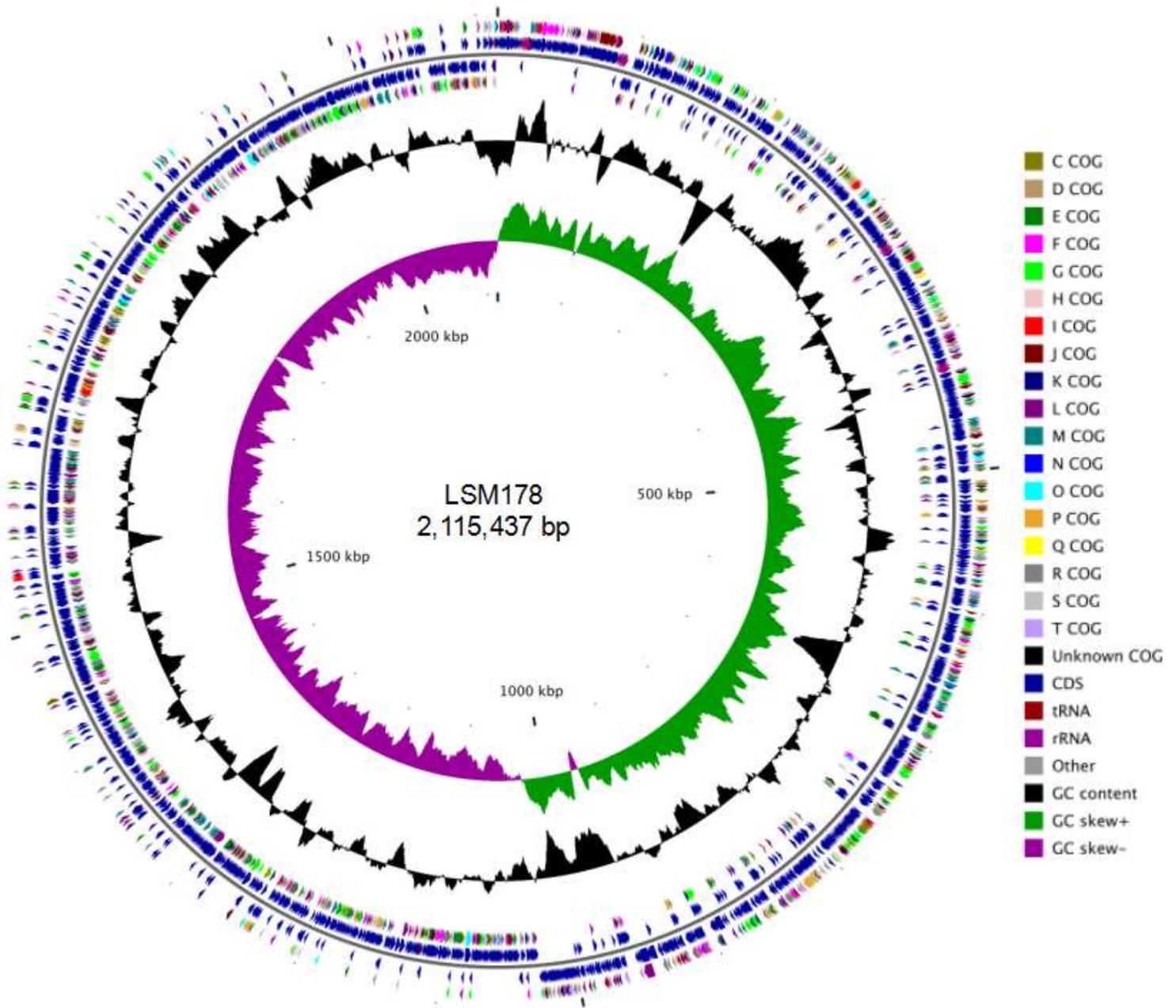


Figure 3

The circular diagram of the LSM178 genome. From inside to outside, the first circle, the scale of genome; the second circle, GC skew; the third circle, GC content; the fourth and seventh circles, the COG category of coding sequence (CDS) on two strands; the fifth and sixth circles, the position of CDS, tRNA and rRNA on two strands.

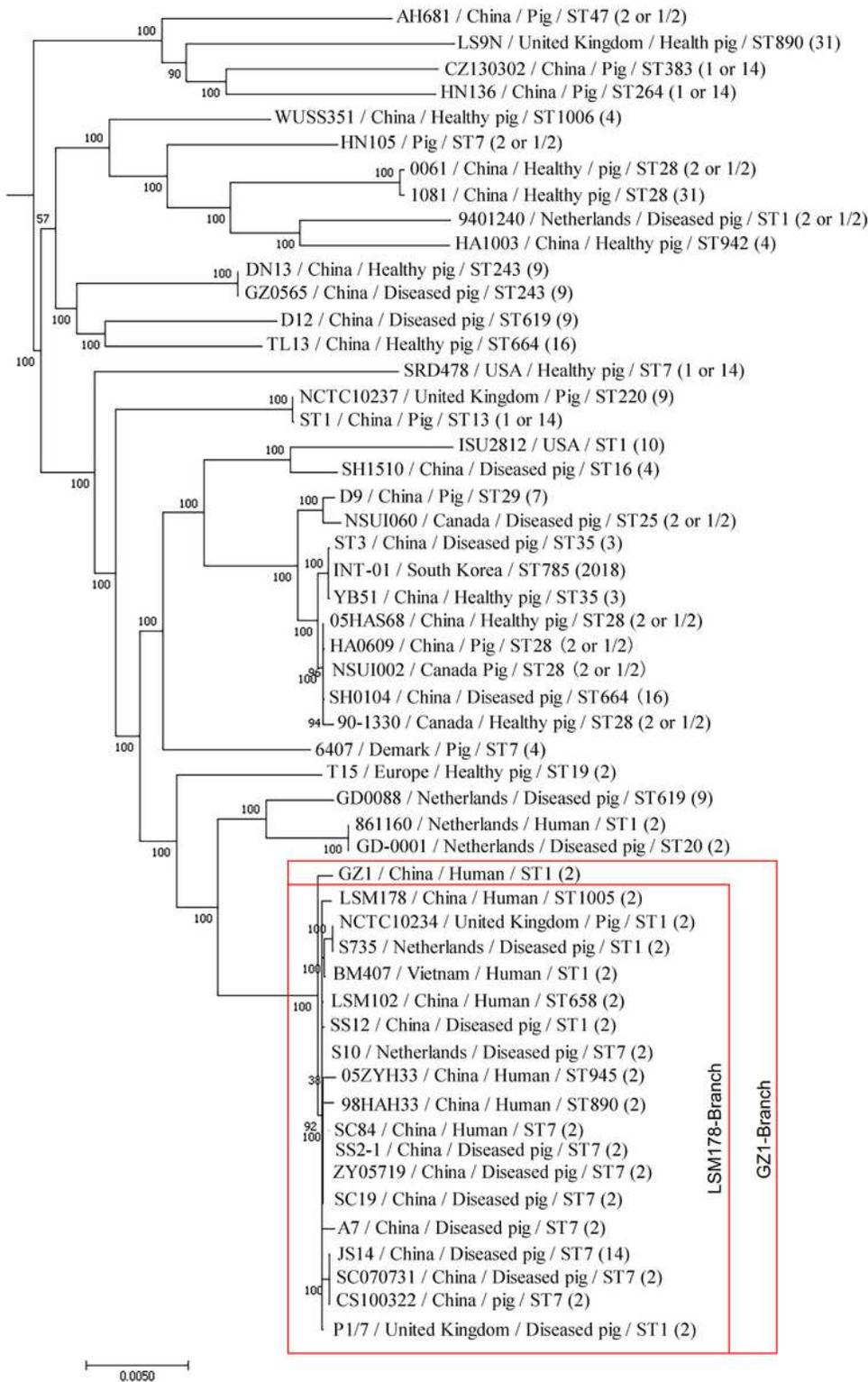


Figure 4

Phylogenetic tree of 53 *S. suis* based on core genome SNP.

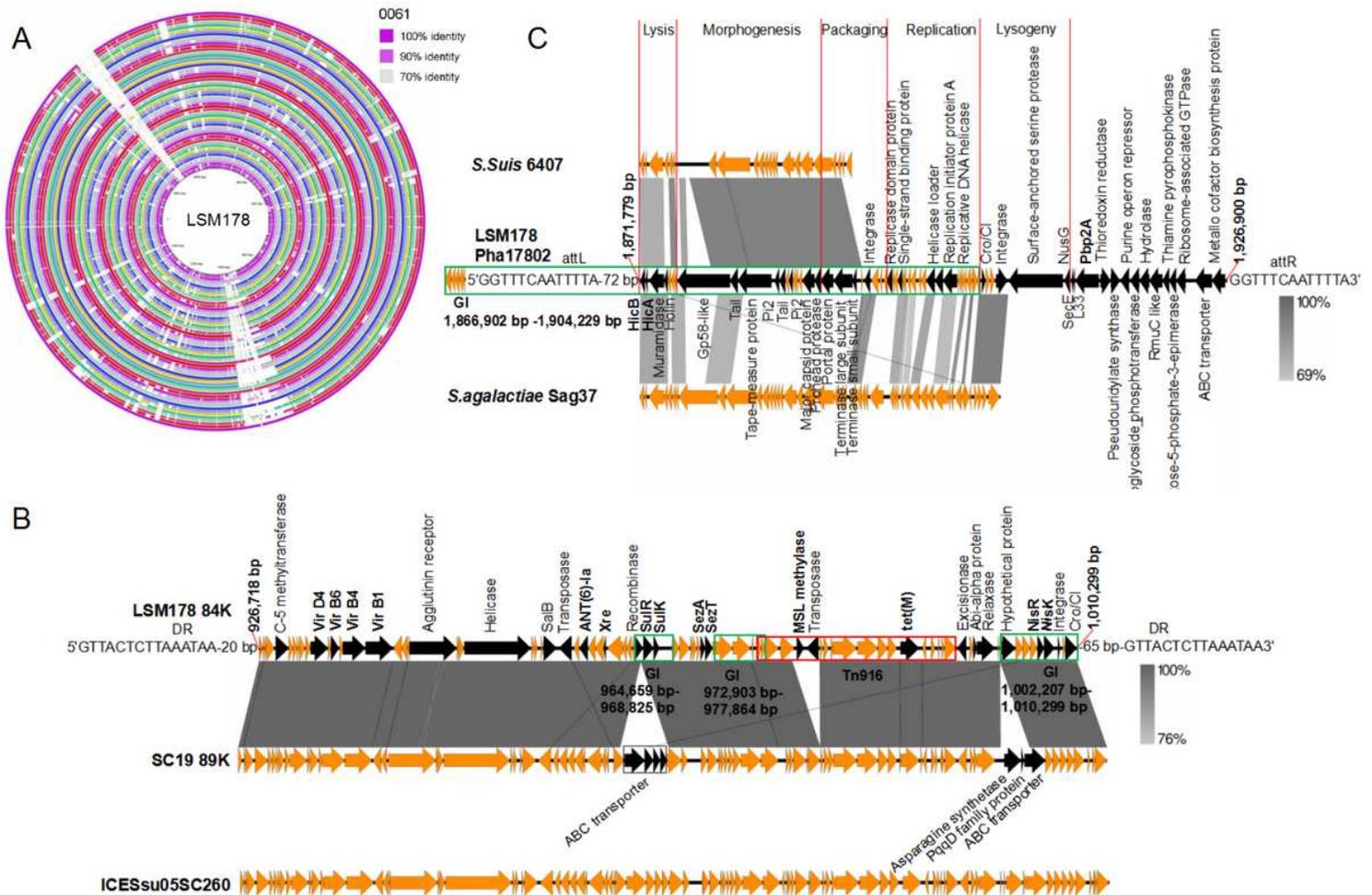


Figure 5

Genome analysis of LSM178. (A) Genome comparison among 52 *S. suis* and LSM178. Each circle showed the variations of strain relative to LSM178. From inside to outside, the genomes was as follows: 61, 05HAS68, 05ZYH33, 1081, 6407, 861160, 90-1330, 9401240, 98HAH33, A7, AH681, BM407, CS100322, CZ130302, D12, D9, DN13, GD-0001, GD-0088, GZ0565, GZ1, HA0609, HA1003, HN105, HN136, INT-01, ISU2812, JS14, LS9N, LSM102, NCTC10234, NCTC10237, NSUI002, NSUI060, P1/7, S10, S735, SC070731, SC19, SC84, SH0104, SH1510, SRD478, SS12, SS2-1, ST1, ST3, T15, TL13, WUSS351, YB51 and ZY05719. The variable colors in each circle stand for sequence identity with the inset of *S. suis* 61 as an example. (B) Comparison of the 84K-PAI with the 89K-PAI from SC19. The 84K-PAI were marked with key genes (black) including virulence-related factors (bold), antibiotic resistance factors (bold), major differential genes and core transposon elements. Green box, GIs; Red box, Tn916. (C) Comparison of the pha17802 with the homologous region located in *S. suis* 6407 and *S. agalactiae* Sag37. Genes rather than hypothetical protein-coding ones were given predictable functions (black). Green box, GI. The functional regions were designed with red line.

