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Running Title (within 10 words)	Genomic sequence of Streptococcus suis INT-01
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8 Abstract

9 Streptococcus suis is a major pig pathogen causing severe economic losses to the swine industry. This 10 study aimed to analyze the genome of S. suis strain INT-01 isolated from a domestic pig in Korea. We 11 found that the genome of strain INT-01 contains 2,092,054 bp, with a G + C content of 41.3%, and the 12 capsular polysaccharide synthesis locus of this strain is almost identical to that of serotype 3 S. suis strain 13 4961 isolated from China, suggesting that these isolates can be classified as serotype 3. Genomic analyses 14 revealed that strain INT-01 is an $epf/mrp^+/sly^- S$. suis, which is the most prevalent genotype in Korea, 15 and several virulence-related genes associated with the pathogenicity of S. suis were also detected. The 16 genomic information of strain INT-01 may provide important insights into the development of control 17 strategies against S. suis infections in Korea. 18 19 Keywords (3 to 6): Streptococcus suis, Pathogen, Genotype, Control strategy, Swine industry 20 21



23 Streptococcus suis is a major pig pathogen causing severe economic losses to the swine industry and is 24 considered a prominent zoonotic agent [1]. Thus far, 35 capsular serotypes have been reported for this 25 species, and its distribution among pigs geographically varies [2]. Several virulence factors have been 26 reported for S. suis, and extracellular protein factor (epf), muraminidase-released protein (mrp), and 27 suilysin (sly) are considered to be the major virulence-associated genes underlying the pathogenesis of 28 this bacterium [3]. Although a recent study has reported that serotype 3, with the $epf/mrp^+/sly^-$ genotype, 29 is the most prevalent serotype among Korean isolates [1], its genomic characteristics remain unknown. 30 In this study, strain INT-01 was isolated in 2018 from the tonsillar swab of a growing pig with 31 respiratory clinical signs that had been reared at a private farm in Yesan (Chungcheongnam-do, Korea). 32 The α-hemolytic isolate displayed 99.7% 16S rRNA identity to S. suis S735^T (AY585196), thus 33 suggesting that strain INT-01 is a member of S. suis. The antimicrobial susceptibility of strain INT-01 34 was evaluated according to the testing guidelines and interpretive breakpoints in the M100S document of 35 the Clinical and Laboratory Standards Institute [4]. The isolate was resistant to tetracycline, erythromycin, 36 gentamicin, lincomvcin, and levofloxacin. Its genome was sequenced using the PacBio RS II system 37 (Pacific Biosciences, CA, USA) with P6-C4 chemistry by constructing a 20 kb SMRTbellTM template 38 library, using the DNA/polymerase binding kit P6 (Pacific Biosciences, CA, USA) in accordance with the 39 manufacturer's instructions. Genome assembly of the filtered reads (1,245,264,540 bp, 153,790 reads, N₅₀, 40 and 10,696 bp) was performed using the PacBio HGAP (v.2.3) pipeline with default settings, using the de 41 novo assembly protocol, and the reads $(2,113,334 \text{ bp}, 409 \times)$ were polished using Ouiver. Annotation was 42 performed using the Prokaryotic Genome Annotation Pipeline (National Center for Biotechnology 43 Information, http://www.ncbi.nlm.nih.gov/books/NBK174280/). 44 The genome of INT-01 was found to comprise 2,092,054 bp (41.3% G + C content), encoding 2,05445 coding sequences, 12 rRNAs, 57 tRNAs, and 4 non-coding RNAs (Table 1). Genome similarities among 46 INT-01 and other S. suis strains were assessed using ANI Calculator [5], and the genome was found to be 96.9% similar to that of S. suis S735^T (NC 018526, serotype 2); furthermore, this strain is most similar to 47

48 *S. suis* ST3 (NC_015433, serotype 3) [6,7] (**Figure 1**). Moreover, the capsular polysaccharide synthesis

49 (*cps*) locus of INT-01 is almost identical (> 99.9%) to that of strain 4961 (JF273646, serotype 3), thus
50 suggesting that INT-01 can be classified as serotype 3 [8].

51 Major virulence-associated genes in INT-01 were manually compared with those of the S. suis strains 52 available in the GenBank database, and other virulence-associated and antibiotic-resistant genes were 53 screened as previously described [9]. Consequently, strain INT-01 was identified to be an $epf^{-}/mrp^{+}/sly^{-}$ 54 S. suis, which is the most prevalent genotype in Korea [1]. Additionally, several virulence-associated 55 factors of the genus *Streptococcus*, including choline-binding protein D (*cbpD*), fibronectin/fibrinogen-56 binding proteins (*fbp54/pavA*), glyceraldehyde-3-phosphate dehydrogenase (*plr/gapA*), extracellular 57 hyaluronidase (*hylA*), periplasmic serine endoprotease (*htrA/degP*), trigger factor (*tig/ropA*), and zinc 58 metalloproteinase (*zmpC*) were detected. However, no genetic determinants associated with antibiotic 59 resistance were detected. These results suggest that other unknown resistance determinants are present in 60 INT-01, warranting further investigation. To our knowledge, this is the first study to report the genome of 61 S. suis from Korea. The present results potentially provide important insights into the development of 62 control strategies against S. suis infections by providing the genomic information of the most prevalent 63 type of the pig pathogen in Korea. 64 Streptococcus suis INT-01 has been deposited in the Culture Collection of Antimicrobial Resistant

Microbes (CCARM) as CCARM4634, and its genome has been deposited in the DDBJ/ENA/GenBank
database under accession number CP041994.1.

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Tables and Figures

Table 1. Characteristics of the *Streptococcus suis* strain INT-01 genome

Features	Value
Genome Size (bp)	2,092,054
G+C content (%)	41.3
Contigs	1
Total genes	2,073
tRNAs	58
rRNAs (5S, 16S, 23S)	4, 4, 4
ncRNAs	4
Protein-coding genes	1,929
Pseudogenes	70

Tables and Figures



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Figure 1. Phylogenetic trees constructed on the basis of OrthoANI values determined with the available complete genomes of *S. suis* INT-01 and other species in the *S. suis* strains. The result of each two-strain comparison is provided, where the diagonals departing from each strain meet, e.g., the OrthoANI value between *S. suis* INT-01 and *S. suis* ST3 is 99.95%. (2-column fitting image).

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