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Complete genome sequence of serotype 3 *Streptococcus suis* INT-01, isolated from a domestic pig in Korea

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Abstract

Streptococcus suis is a major pig pathogen causing severe economic losses to the swine industry. This study aimed to analyze the genome of *S. suis* strain INT-01 isolated from a domestic pig in Korea. We found that the genome of strain INT-01 contains 2,092,054 bp, with a guanine (G) + cytosine (C) content of 41.3%, and the capsular polysaccharide synthesis locus of this strain is almost identical to that of serotype 3 *S. suis* strain 4961 isolated from China, suggesting that these isolates can be classified as serotype 3. Genomic analyses revealed that strain INT-01 is an extracellular protein factor (*epf*)$^-$/ muraminidase-released protein (*mrp*)$^+$/ suilysin (*sly*)$^-$ *S. suis*, which is the most prevalent genotype in Korea, and several virulence-related genes associated with the pathogenicity of *S. suis* were also detected. The genomic information of strain INT-01 may provide important insights into the development of control strategies against *S. suis* infections in Korea.

Keywords: *Streptococcus suis*, Pathogen, Genotype, Control strategy, Swine industry

Streptococcus suis is a major pig pathogen causing severe economic losses to the swine industry and is considered a prominent zoonotic agent [1]. Thus far, 35 capsular serotypes have been reported for this species, and its distribution among pigs geographically varies [2]. Several virulence factors have been reported for *S. suis*, and extracellular protein factor (*epf*), muraminidase-released protein (*mrp*), and suilysin (*sly*) are considered to be the major virulence-associated genes underlying the pathogenesis of this bacterium [3]. Although a recent study has reported that serotype 3, with the *epf*$^-$/*mrp*$^+$/*sly*$^-$ genotype, is the most prevalent serotype among Korean isolates [1], its genomic characteristics remain unknown.

In this study, strain INT-01 was isolated in 2018 from the tonsillar swab of a growing pig with respiratory clinical signs that had been reared at a private farm in Yesan (Chungcheongnam-do, Korea). The α-hemolytic isolate displayed 99.7% 16S rRNA identity to *S. suis* S735^T (AY585196), thus suggesting that strain INT-01 is a member of *S. suis*. The antimicrobial susceptibility of strain INT-

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Competing interests
No potential conflict of interest relevant to this article was reported.

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Not applicable.

Availability of data and material
Upon reasonable request, the datasets of this study can be available from the corresponding author. *Streptococcus suis* INT-01 has been deposited in the Culture Collection of Antimicrobial Resistant Microbes (CCARM) as CCARM4634.

Authors' contributions
Conceptualization: Son JS, Kim JH.
Data curation: Park SY, Kim IH, Yu HJ.
Formal analysis: Park SY, Kim IH.
Methodology: Park SY, Kim IH, Yu HJ, Paik HR.
Software: Park SY, Kim IH.
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Investigation: Park SY, Kim IH, Kim JH.
Writing - original draft: Park SY, Kim IH, Kim JH.
Writing - review & editing: Son JS, Kim JH.

Ethics approval and consent to participate
This article does not require IRB/IACUC approval because there are no human or animal participants.

01 was evaluated according to the testing guidelines and interpretive breakpoints in the M100S document of the Clinical and Laboratory Standards Institute [4]. The isolate was resistant to tetracycline, erythromycin, gentamicin, lincomycin, and levofloxacin. Its genome was sequenced using the PacBio RS II system (Pacific Biosciences, Menlo Park, CA, USA) with P6-C4 chemistry by constructing a 20 kb SMRTbellTM template library, using the DNA/polymerase binding kit P6 (Pacific Biosciences) in accordance with the manufacturer's instructions. Genome assembly of the filtered reads (1,245,264,540 bp, 153,790 reads, N_{50}, 10,696 bp) was performed using the PacBio HGAP (v.2.3) pipeline with default settings, using the *de novo* assembly protocol, and the reads (2,113,334 bp, 409×) were polished using Quiver. Annotation was performed using the Prokaryotic Genome Annotation Pipeline (National Center for Biotechnology Information, http://www.ncbi.nlm.nih.gov/books/NBK174280/).

The genome of INT-01 was found to comprise 2,092,054 bp (41.3% guanine [G] + cytosine [C] content), encoding 2,054 coding sequences, 12 rRNAs, 57 tRNAs, and 4 non-coding RNAs (Table 1). Genome similarities among 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 *S. suis* ST3 (NC_015433, serotype 3) [6,7] (Fig. 1). Moreover, the capsular polysaccharide synthesis (*cps*) locus of INT-01 is almost identical (> 99.9%) to that of strain 4961 (JF273646, serotype 3), thus suggesting that INT-01 can be classified as serotype 3 [8].

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

Streptococcus suis INT-01 has been deposited in the Culture Collection of Antimicrobial

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

G, guanine; C, cytosine.

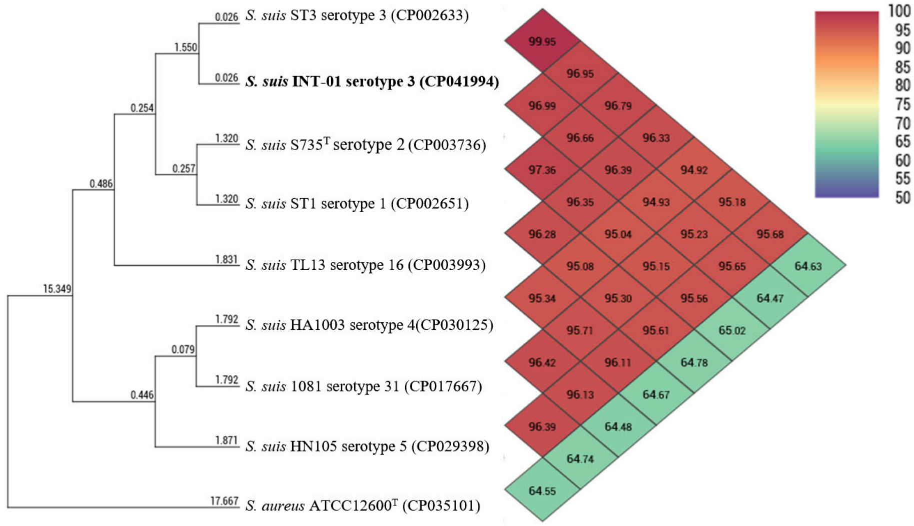

Fig. 1. Overall genome relatedness and heatmaps on the basis of OrthoANI values determined with the available complete genomes of *Streptococcus suis* INT-01 and other species in the *S. suis* strains. The result of each two-strain comparison is provided, where the diagonals departing from each strain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

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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