Article Type: Research article

Article Title (within 20 words without abbreviations): Complete genome sequence of serotype 3 *Streptococcus suis* INT-01, isolated from a domestic pig in South Korea

Running Title (within 10 words): Genomic sequence of *Streptococcus suis* INT-01

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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.

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Formal analysis: Park SY, Kim IH. 
Methodology: Park SY, Kim IH, Yu HJ, Paik HR. 
Software: Park SY, Kim IH. 
Validation: Park SY, Kim IH, Paik HR. 
Investigation: Park SY, Kim IH, Kim JH. 
Writing - original draft: Park SY, Kim IH, Kim JH. 
Writing - review & editing: Kim JH, Son JS.

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

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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 G + 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 epf⁺/mrp⁺/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 (3 to 6): *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 S735T (AY585196), thus suggesting that strain INT-01 is a member of S. suis. The antimicrobial susceptibility of strain INT-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, CA, USA) with P6-C4 chemistry by constructing a 20 kb SMRTbellTM template library, using the DNA/polymerase binding kit P6 (Pacific Biosciences, CA, USA) in accordance with the manufacturer’s instructions. Genome assembly of the filtered reads (1,245,264,540 bp, 153,790 reads, N₅₀, and 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% G + 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 S735T (NC_018526, serotype 2); furthermore, this strain is most similar to S. suis ST3 (NC_015433, serotype 3) [6,7] (Figure 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 \textit{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 \textit{epf}/\textit{mrp}^+/\textit{sly}^- \textit{S. suis}, which is the most prevalent genotype in Korea [1]. Additionally, several virulence-associated factors of the genus \textit{Streptococcus}, including choline-binding protein D (\textit{cbpD}), fibronectin/fibrinogen-binding proteins (\textit{fbp54/pavA}), glyceraldehyde-3-phosphate dehydrogenase (\textit{plr/gapA}), extracellular hyaluronidase (\textit{hylA}), periplasmic serine endoprotease (\textit{htrA/degP}), trigger factor (\textit{tig/ropA}), and zinc metalloproteinase (\textit{zmpC}) were detected. However, no genetic determinants associated with antibiotic resistance were detected. These results suggest that other unknown resistance determinants are present in INT-01, warranting further investigation. To our knowledge, this is the first study to report the genome of \textit{S. suis} from Korea. The present results potentially provide important insights into the development of control strategies against \textit{S. suis} infections by providing the genomic information of the most prevalent type of the pig pathogen in Korea.

\textit{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.

\textbf{Acknowledgments}

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References


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**Tables and Figures**

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

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