Genomic Approaches for Understanding the Characteristics of *Salmonella enterica* subsp. *enterica* Serovar Typhimurium ST1120, Isolated from Swine Feces in Korea

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**Introduction**

The *Salmonella* genus consists of two species: *S. enterica* and *S. bongori*. Species *S. enterica* is divided into six subspecies, of which *S. enterica* subsp. *enterica* can be further subdivided into more than 1,500 serovars [1]. *S. enterica* subsp. *enterica* serovar Typhimurium is one of the leading causes of salmonellosis in humans and mammals, including cattle and rodents [2]. A recent study has revealed that 93.8 million cases of nontyphoidal salmonellosis occur every year, resulting in approximately 155,000 human deaths annually [3].

Transmission routes of *S. enterica* serovars to humans have been reported to be mainly through contaminated animal-derived food products (such as poultry, eggs, pork, beef, and dairy foods) and contaminated produce (such as spinach, peanuts, and tomatoes) [3, 4]. Among them, pork meat is regarded as a major source of human infection in

*Salmonella enterica* subsp. *enterica* serovar Typhimurium, one of the most common foodborne pathogens, is transmitted mainly through contaminated food derived from infected animals. In this study, *S. Typhimurium* ST1120, an isolate from pig feces in Korea, was subjected to whole-genome analysis to understand its genomic features associated with virulence. The genome of ST1120 was found to have a circular chromosome of 4,855,001 bp (GC content 52.2%) and a plasmid of 6,863 bp (GC content 46.0%). This chromosome was predicted to have 4,558 open reading frames (ORFs), 17 pseudogenes, 22 rRNA genes, and 86 tRNA genes. Its plasmid was predicted to have three ORFs. Comparative genome analysis revealed that ST1120 was phylogenetically close to *S. Typhimurium* U288, a critical isolate in piggery farms and food chains in Europe. In silico functional analysis predicted that the ST1120 genome harbored multiple genes associated with virulence and stress resistance, including *Salmonella* pathogenicity islands (SPIs containing SPI-1 to SPI-5, SPI-13, and SPI-14), C63PI locus, ST104 prophage locus, and various antibiotic resistance genes. In accordance with these analysis results, ST1120 showed competence in invasion and survival abilities when it was added to host cells. It also exhibited robust resistance against antibiotics in comparison with other *S. Typhimurium* strains. This is the first report of the complete genome sequence of *S. Typhimurium* isolated from swine in Korea. Comparative genome analysis between ST1120 and other *Salmonella* strains would provide fruitful information toward understanding *Salmonella* host specificity and developing control measures against *S. Typhimurium* infection.

**Keywords:** *Salmonella* Typhimurium ST1120, piggery, genome, comparative analysis, virulence, antibiotic resistance
S. Typhimurium is one of the most frequently isolated serovars in pigs [6]. Its infection in pigs often leads to carriers without exhibiting clinical signs [7]. However, they can excrete Salmonella under stressful conditions, thus increasing the possibility of meat contamination at slaughter [8]. In this context, swine is regarded as a main reservoir of human infection [9]. In addition, the abuse and overuse of antibiotics for disease prevention and growth promotion in livestock facilities have resulted in the emergence of multidrug-resistant bacteria such as S. Typhimurium DT104 [10, 11]. Multiple antibiotic resistance genes at a 43-kb genomic island called Salmonella genomic island I (SGI1) in S. Typhimurium DT104 strain have been reported [11]. This strain has become widespread in livestock and poultry since its first identification from exotic birds in the early 1980s [12], causing several outbreaks worldwide.

S. Typhimurium strain ST1120 (also called HID1120) was first isolated from feces of pigs in Gangwon Province, Korea [13]. Its whole genome was fully sequenced and analyzed in this study. The genome of this strain showed a high sequence homology with that of S. Typhimurium U288, a critical pathogen prevalent in pig production units in the United Kingdom that was also responsible for contaminated pork-mediated deaths in Denmark, 2008 [14]. Furthermore, these two strains possessed an ST104 prophage region common in DT104 strains, suggesting that they might share similar physiological features attributable to ST104 prophage. Given that pigs infected by S. Typhimurium could be potential transmitters responsible for foodborne salmonellosis, genome information of S. Typhimurium isolates might provide insights into various virulence factors and virulence regulation mechanisms in this important serovar.

Materials and Methods

Isolation and Growth Conditions

S. Typhimurium ST1120 was isolated from feces of pigs [13]. This ST1120 strain was cultivated in Luria–Bertani (LB) broth at 37°C for 3 h with constant shaking at 220 rpm until it reached log phase. Morphological features were analyzed using transmission electron microscopy (TEM; JEM1010, Japan) after staining with 2% uranyl acetate.

Genomic DNA Extraction

ST1120 bacterial cells were grown to mid-exponential phase, harvested by centrifugation at 16,000 × g for 1 min, and subjected to genomic DNA extraction using G-spin Genomic DNA Extraction Kit for Bacteria (nIRON Biotechnology, Korea). Bacterial growth was estimated on the basis of optimal density measurements using a SimpliNano spectrophotometer (GE Healthcare, USA).

Genome Sequencing, Annotation, and Comparative Genome Analysis

The genome sequence was determined using PacBio RS II (Pacific Biosciences, USA) at Macrogen Inc. (Korea), which generated 1,517,349,588 bp (200,254 reads, about 150 folds of genome coverage). The sequence reads were assembled de novo using Hierarchical Genome Assembly Process (HGAP) ver. 3.0 [15]. Gene prediction and annotation were conducted using Prokka ver. 1.12 [16], GeneMark [17], and NCBI BLASTP [18]. They were confirmed by manual curation. Genes of rRNA and tRNA were predicted using RNAmmer 1.2 [19] and tRNAscan-SE [20], respectively. The analysis data was deposited in GenBank with accession numbers of CP021909.1 (chromosome) and CP021910.1 (plasmid). The genome was mapped using DNAPlotter [21]. A genome tree was constructed on the basis of average nucleotide identity (ANI) [22] and Roary matrix analysis [23]. Comparative analyses were performed using BLASTN. Results were visualized with Artemis Comparison Tool (ACT) and Easyfig [24, 25]. SPIs and other known virulence factors in Salmonella were analyzed using SPIFinder server ver. 1.0 [26] and the Virulence Factors DataBase (VFDB) [27], respectively. Prediction of genes involved in antibiotic resistance was conducted using the Comprehensive Antibiotic Resistance Database (CARD) [28].

Invasion and Survival Assays

To evaluate bacterial ability to invade mammalian cells and survive inside them, a gentamicin protection assay was performed as described previously [29]. Briefly, epithelial HeLa cells were seeded into 24-well cell culture plates at a density of 1 × 10⁵ cells per well, cultured in DMEM containing 10% fetal bovine serum (Gibco, USA), and infected with Salmonella Typhimurium strains at a multiplicity of infection (MOI) of 100. At 30 min after infection, the medium was removed and the HeLa cells were incubated with fresh DMEM containing gentamicin (100 µg/ml) for 1.5 h. The cells were then lysed with PBS three times and lysed with 1% Triton X-100 (Sigma-Aldrich, USA). The cell lysates were serially diluted and plated onto LB agar to count the number of intracellular bacteria for the invasion assay. For the survival assay, RAW264.7 macrophage-like cells were prepared and infected with Salmonella Typhimurium strains as described in the invasion assay. At 30 min post-infection, the RAW264.7 cells were incubated with fresh DMEM containing gentamicin (100 µg/ml) for 1.5 h to inactivate extracellular Salmonella cells. They were further incubated with fresh DMEM supplemented with gentamicin at 20 µg/ml for an additional 8 h. After three times of washing with PBS, the cells were then lysed with 1% Triton X-100. The cell lysates were serially diluted and plated onto LB agar to enumerate intracellular bacteria.

Lactate Dehydrogenase (LDH) Cytotoxicity Assay

Bacterial cytotoxicity was determined using the CytoTox-ONE Homogeneous Membrane Integrity Assay (Promega, USA) according to the manufacturer’s instruction. Briefly, HeLa cells
were seeded into 96-well cell culture plates at a density of $1 \times 10^3$ cells per well and infected with Salmonella Typhimurium strains at a MOI of 100. At 4 h post-infection, the plates were removed from the incubator and equilibrated at 22°C for 20 min. The cells were treated with CytoTox-ONE reagent (100 µl) containing lactate, NAD$, and resazurin as substrates at 22°C for 10 min. LDH-mediated conversion of resazurin to resorufin was quenched by adding 50 µl of stop solution. Fluorescence was measured immediately at an excitation wavelength of 560 nm and an emission wavelength of 590 nm using a fluorometer (BioTek Synergy HTX Multi-Mode Reader, USA). Host cells not treated with Salmonella were used as negative controls, whereas cells lysed with Triton-X 100 for maximal LDH release were used as positive controls.

### Antimicrobial Susceptibility Test Using Disk Diffusion

Resistance against antibiotics was measured using the disk diffusion method as published previously [30]. Briefly, all bacterial strains were cultured in LB broth overnight. A cell suspension of approximately $1 \times 10^8$ CFU was spread onto Mueller-Hinton agar plates and inoculated with pre-loaded paper disks containing 30 µg of each antibiotic. After incubation at 37°C for 16–24 h, diameters of growth inhibition zones were measured.

### Statistical Analysis

All assays were repeated at least three times. Averaged values with standard deviation are presented. Student’s t-test was used to acquire p-values to determine statistical significance, which was set at $p < 0.05$.

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**Results and Discussion**

### General Genome Properties

S. Typhimurium ST1120 is a rod-shaped bacterium of 0.5–0.8 × 1.0–2.0 µm in size (Fig. S1). Its complete genome is composed of a circular chromosome and a plasmid (Fig. 1). The chromosome is 4,855,001 bp in DNA length with a GC content of 52.2%. It was predicted to have a total of 4,683 ORFs, including 4,558 coding sequences (CDSs), 17 pseudogenes, 22 rRNA genes, and 86 tRNA genes. In addition, its chromosome harbors 10 prophage regions; namely, 4 intact (ST104, Gifsy-1 and -2, and Fels-2) and 6 incomplete regions. The plasmid, designated as pST1120, is 6,863 bp in length with a GC content of 46%, containing three ORFs. In phylogenetic tree analyses based on the ANI value and amino acid sequence matrix (Roary matrix analysis), ST1120 was closely associated with S. Typhimurium LT2 and U288, forming a cluster (Fig. S2).

### Pathogenesis and Virulence Factors

S. Typhimurium can invade intestinal epithelial cells and macrophages and replicate inside these cells during infection of the host. It has been reported that more than 200 virulence genes, approximately 4% of its genome, are required for fatal infection in mice [31]. Many of them are located in SPIs in the chromosome as large cassettes, and

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**Fig. 1.** Genome maps of *Salmonella* Typhimurium ST1120. The genome of ST1120 including the chromosome (A) and plasmid pST1120 (B) was mapped using DNAPlotter [21].
encode determinants responsible for bacterial virulence in the host [32]. In general, SPI-1 is primarily required for bacterial penetration into intestinal epithelial cells. SPI-2 to SPI-4 are necessary for bacterial survival and proliferation inside host cells, and SPI-5 seems to be involved in inflammation and chloride secretion [32]. SPI-13 and SPI-14 are known to be able to promote colonization of \textit{Salmonella} in the host intestine [33, 34]. The presence of SPIs in ST1120 was explored by using the newly developed Web tool SPIFinder 1.0. Results revealed that its genome possessed SPI-1 to SPI-5, SPI-13, SPI-14, and C63PI (Table 1). The \textit{sitABCD} operon (ST1120_03251 to ST1120_03258) in the C63PI genomic region is known to encode protein constituents of manganese(II) and iron(II) uptake system [35]. In view of the role of manganese as a cofactor for superoxide dismutase A in protection against oxidative stress and detoxification of

| Table 1. Predicted virulence factors in \textit{Salmonella} Typhimurium ST1120. |
|-----------------|-----------------|-----------------|
| Virulence factor | Locus tag        | Function        |
| SPI-1           | ST1120_03592 - ST1120_03640 | \textit{Salmonella} pathogenicity island 1 |
| SPI-2           | ST1120_02123 - ST1120_02166 | \textit{Salmonella} pathogenicity island 2 |
| SPI-3           | ST1120_04484 - ST1120_04497 | \textit{Salmonella} pathogenicity island 3 |
| SPI-4           | ST1120_00391 - ST1120_00398 | \textit{Salmonella} pathogenicity island 4 |
| SPI-5           | ST1120_01833 - ST1120_01841 | \textit{Salmonella} pathogenicity island 5 |
| SPI-13          | ST1120_03853 - ST1120_03856 | \textit{Salmonella} pathogenicity island 13 |
| SPI-14          | ST1120_01634 - ST1120_01640 | \textit{Salmonella} pathogenicity island 14 |
| C63PI           | ST1120_03251 - ST1120_03258 | Iron uptake system |
| sseK1           | ST1120_00294     | T3SS effector SseK1 |
| strP            | ST1120_01575     | T3SS effector StrP |
| sopD2           | ST1120_01712     | T3SS effector SopD2 |
| sseI/srfH       | ST1120_01800     | T3SS effector SseI/SrfH |
| sifA            | ST1120_01970     | T3SS effector SifA |
| steA            | ST1120_02332     | T3SS effector SteA |
| sifB            | ST1120_02351     | T3SS effector SifB |
| steB            | ST1120_02378     | T3SS effector SteB |
| steC            | ST1120_02449     | T3SS effector SteC |
| sopE2           | ST1120_02603     | T3SS effector SopE2 |
| sopA            | ST1120_02809     | T3SS effector SopA |
| sseK2           | ST1120_02880     | T3SS effector SseK2 |
| sspH2           | ST1120_02984     | T3SS effector SspH2 |
| sspH1           | ST1120_02984     | T3SS effector SspH1 |
| sseL            | ST1120_03030     | T3SS effector SseL |
| gogB            | ST1120_03322     | T3SS effector GogB |
| pipB2           | ST1120_03515     | T3SS effector PipB2 |
| sopD            | ST1120_03677     | T3SS effector SopD |
| fim operon      | ST1120_01329 - ST1120_01333 | Type 1 fimbriae |
| csg operon      | ST1120_01884 - ST1120_01890 | Curli fimbriae |
| lpf operon      | ST1120_04370 - ST1120_04374 | Long polar fimbriae |
| grvA            | ST1120_01781     | Gifsy-2-related antivirulence factor |
| sedCI           | ST1120_01791     | Gifsy-2 prophage superoxide dismutase |
| ompA            | ST1120_01817     | Outer membrane protein A |
| mig-14          | ST1120_03517     | Antimicrobial peptide resistance protein |

\(^1\)Identified by SPIFinder ver.1.0.

\(^2\)Identified by Virulence Factors DataBase.
reactive oxygen species (ROS) [36], acquisition of this operon may confer competitiveness of Salmonella survival within immune cells generating ROS [37, 38].

In the search against the VFDB, the ST1120 genome revealed a variety of virulence effectors translocated by type 3 secretion systems (T3SS) and additional virulence determinants beneficial for Salmonella pathogenesis (Table 1). For example, ST1120 was predicted to possess at least three different fimbrial structures responsible for bacterial attachment and biofilm formation. Type 1 fimbriae, produced by the fim operon from ST1120_01329 to ST1120_01333 are, important for Salmonella attachment to enterocytes and facilitate intestinal colonization of Salmonella in swine [39]. The curli biogenesis system, a virulence factor for attachment to villi of enterocytes and bacterial auto-aggregation [40], was also predicted to be encoded by seven CDSs (ST1120_01884 to ST1120_01890). Five CDSs from ST1120_04370 to ST1120_04374 were supposed to be required for the biosynthesis of long polar fimbriae (Lpf) that plays a role in the attachment to Peyer’s patches [41]. Besides these, the ST1120 genome contains grvA (ST1120_01781), sodCI (ST1120_01791), ompA (ST1120_01817), and mig-14 (ST1120_03517), whose products are associated with bacterial virulence in many other pathogens. In particular, mig-14 (macrophage-inducible gene-14), encoding an inner membrane-associated protein, enables Salmonella to prevent antimicrobial peptides from penetrating their inner membrane [42].

The presence of multiple virulence factors identified by in silico functional analysis prompted us to examine the virulence of ST1120 strain in comparison with other S. Typhimurium strains such as LT2. Nontyphoidal Salmonellae, including S. Typhymurium, are causative agents of gastroenteritis, which is self-limiting in healthy adults. However, they can become problematic as a result of bacteremic spread and endovascular infection. Dissemination into extra-intestinal sites is largely attributable to the ability of Salmonella to invade epithelial cells and survive inside macrophages [43]. In order to compare the ability to develop systemic diseases among S. Typhimurium strains, ST1120 and the three well-characterized strains LT2, 14028s, and SL1344 were subjected to invasion and survival assays. In the invasion assay using epithelial HeLa cells, ST1120 had invasion ability comparable to SL1344 (Fig. 2A). Both strains showed less invasion of host cells than 14028s. On the other hand, in RAW264.7 macrophage-like cells, ST1120 and SL1344 strains showed higher survival ability than 14028s (Fig. 2B). In both assays, strain LT2 exhibited attenuated virulence compared with the other three strains. In spite of its wide utilization as a representative strain, LT2 is known to be compromised in virulence both in vivo and in vitro [44, 45]. However, these four strains induced comparable cytotoxic activities in epithelial cells (Fig. 2C). Taken together, these results suggest that strain ST1120 has the potential to cause systemic diseases through contaminated food products, in that it could invade into and survive within the host cells.

In the search against the CARD, we identified multiple

![Fig. 2. Salmonella Typhimurium ST1120 virulence toward host cells.](image)

The ability of ST1120 strain to invade and survive inside host cells was compared with those of other S. Typhimurium strains (LT2, 14028s, and SL1344). Invasion (A) and survival (B) assays were conducted using epithelial HeLa cells and macrophage-like RAW264.7 cells, respectively. The cytotoxic activities of these four S. Typhimurium strains were also compared in HeLa cells using the LDH assay (C). The numbers of intracellular bacteria and enzyme activities of host cells infected by ST1120, 14028s, and SL1344 were standardized using values of cells infected with LT2, a well-studied reference strain. Data shown are % mean values of at least three independent experiments. N.S (not significant), p < 0.05; *, p < 0.01; **, p < 0.001.
CDSs associated with antibiotic resistance in the ST1120 genome (Table 2). Antibiotic abuse in livestock has increased the drug resistance risk in zoonotic pathogens. Given that ST1120 was isolated from a piggery farm, which is prone to be exposed to antibiotics, it is worth evaluating its resistance against antibiotics. In order to examine whether the presence of these genes might confer resistance to the ST1120 strain, a disk diffusion assay was performed using four representative antibiotics (streptomycin, chloramphenicol, tetracycline, and ampicillin) (Fig. 3). Interestingly, ST1120 was more tolerant to these four antibiotics tested than the other three S. Typhimurium strains (LT2, 14028s, and SL1344).

### Table 2. Predicted antibiotic resistance-associated coding sequences in Salmonella Typhimurium ST1120.

<table>
<thead>
<tr>
<th>ARO category</th>
<th>No. of genes</th>
<th>Locus_tag</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aminocoumarin resistance protein</td>
<td>2</td>
<td>ST1120_02466, ST1120_03558</td>
</tr>
<tr>
<td>Aminoglycoside resistance protein</td>
<td>2</td>
<td>ST1120_01481, ST1120_02368</td>
</tr>
<tr>
<td>Polymyxin resistance protein</td>
<td>4</td>
<td>ST1120_02823, ST1120_03041, ST1120_03042, ST1120_00428</td>
</tr>
<tr>
<td>Fosfomycin resistance protein</td>
<td>2</td>
<td>ST1120_04521, ST1120_03026</td>
</tr>
<tr>
<td>Fluoroquinolone resistance protein</td>
<td>1</td>
<td>ST1120_01961</td>
</tr>
<tr>
<td>Peptide antibiotic resistance protein</td>
<td>1</td>
<td>ST1120_03945</td>
</tr>
<tr>
<td>Mupirocin resistance protein</td>
<td>1</td>
<td>ST1120_00771</td>
</tr>
<tr>
<td>Isoniazid resistance protein</td>
<td>1</td>
<td>ST1120_00256</td>
</tr>
<tr>
<td>Beta-lactam resistance protein</td>
<td>2</td>
<td>ST1120_02584, ST1120_02215</td>
</tr>
<tr>
<td>Sulfonamide resistance protein</td>
<td>1</td>
<td>ST1120_00839</td>
</tr>
</tbody>
</table>

*ARO (Antibiotic Resistance Ontology) analyzed by Resistance Gene Identifier (RGI) according to CARD (Comprehensive Antibiotic Resistance Database).

**Fig. 3.** Comparison of antibiotic susceptibility between ST1120 and other S. Typhimurium strains.

Antibiotic susceptibility test of S. Typhimurium strains was performed using the agar diffusion assay. Cells of S. Typhimurium strains were spread onto agar plates and incubated with paper disks (8 mm diameter) containing 30 µg of each antibiotic. Images of the plates were taken (A). Diameters of inhibition zones were measured and averaged from three independent tests (B). The following antibiotics were used in the test: STR, streptomycin; CM, chloramphenicol; TET, tetracycline; AMP, ampicillin. CLSI standards in the Enterobacteriaceae family for determining antibiotic resistance are listed in parallel. S, susceptible; I, intermediate; R, resistant.
According to the criteria of Clinical and Laboratory Standards Institute (CLSI) for Enterobacteriaceae, ST1120 has strong resistance to ampicillin and intermediate resistance to streptomycin. It has been reported that substitution of glycine 133 with aspartic acid in the Enterobacter aerogenes Omp36 protein can provide resistance to multiple β-lactam antibiotics by decreasing the permeability of the outer membrane [46]. In this regard, the resistance of strain ST1120 to ampicillin might be attributable to the presence of an equivalent amino acid substitution in the omp36 homolog (ST1120_02215) that shared 67% protein sequence identity with E. aerogenes omp36, at least in part.

Fig. 4. Comparison of amino acid sequences of Omp36 homologs among S. Typhimurium strains.

Multiple alignments of Omp36 amino acid sequences were produced by Clustal Omega (ver. 1.2.4) [47]. Letters in black background indicate identical amino acids present in five Omp36 homologs, and letters in gray background indicate amino acids conserved in at least three Omp36 homologs. The red rectangle indicates differences at amino acid 133 among Omp36 homologs.

Fig. 5. Comparative genome analysis of ST1120 with other representative S. Typhimurium strains.
Pangenome analysis using GView [48] was conducted on ST1120 and three well-studied S. Typhimurium stains (LT2, 14028s, and SL1344) with cutoff value of tblastx at 80% (A). The inner circle indicates COG functional categories of S. Typhimurium ST1120. The red arrow represents an ST1120-specific gene cluster, ST104 prophage. The black curve indicates strain-specific regions among S. Typhimurium strains. The region of ST104 present only in the ST1120 genome was further compared with the LT2 genome counterpart using Easyfig (B). The ST1120-specific region comprised genes from ST1120_01047 to ST1120_01106. It contained the gtrCBA operon.
omp36 homologs annotated as ompN were also detected in the genomes of other S. Typhimurium strains, substitution at amino acid 133 was not found (Fig. 4).

Comparative Genome Analysis

To better understand the unique genetic characteristics of strain ST1120, its genome sequence was compared with those of other closely related strains. In comparison with the publicly available reference genomes of LT2 (Accession No. NC_003197.2), 14028s strain (Accession No. NC_016856.1), and SL1344 (Accession No. NC_016810), a ST1120-unique region (ST1120_01047 to ST1120_01106) composed of mostly whole sequences of the ST104 prophage genome (Fig. 5A) was found through pangenome analysis. This prophage is a P22-like phage (protein sequence identity with P22 > 99%) and known to be conserved in isolates of DT104, a notorious multidrug-resistant Salmonella strain [49]. This prophage region is localized at tRNA loci between ST1120_t025 and ST1120_t026, suggesting that the ST104 prophage might have been acquired through horizontal gene transfer because tRNA loci are generally regarded as hot spots for insertion of transferable elements (Fig. 5B) [50]. Although the role of the ST104 prophage has not been determined yet, it might be involved in S. Typhimurium virulence, in that bacteriophages are able to transfer genetic elements beneficial for bacterial virulence and also change serotypes of host bacteria. Many prevalent prophages such as Gifsy-1, Gifsy-2, Fels-1, and Fels-2 play pivotal roles in bacterial pathogenesis [51, 52]. The ST104 prophage harbors the gtrCBA operon, which encodes serotype-specific glycosyltransferase (GtrC), bactoprenol glucosyltransferase (GtrB), and bactoprenol-linked glucosytranslocase (GtrA) (Fig. 5B). Products of the gtrCBA operon are known to play a role in modifying O-antigen through glucosylation [53]. The O-antigen is a key factor in Salmonella virulence by modulating host immune responses [54, 55]. In this context, the retention of this operon might give ST1120 advantageous competence during host infection.

Considering that ST1120 was isolated from pig fecal samples, it is worthy to compare genomic sequences of piggery farm-related isolates to understand common or unique genome features. To date, two completed genomes are available in public databases for pig-relevant S. Typhimurium isolates: U288 (GenBank Accession No.
and 798 (GenBank Accession No. CP003386.1). Strain U288 is considered to be a significant pathogen to pigs in the United Kingdom. It has been linked to the outbreak of fatal salmonellosis in Denmark [14, 56]. Strain 798 is known to be able to persist long-term in the host, causing asymptomatic and chronic infections in pigs [57]. To compare these genomes, pairwise alignment between ST1120 and U288 or 798 was conducted using ACT. Interestingly, the genome sequences of ST1120 and U288 strains were highly homologous to each other (>99% sequence identity) (Fig. 6A), suggesting a potential outbreak by ST1120 in Korea. Meanwhile, comparison between the ST1120 and 798 genomes revealed strain-specific regions. A prophage region of ST104 conserved in the genomes of ST1120 and U288 was not present in the genome of 798. Instead, strain 798 was found to possess a putative prophage-associated region homologous to the genome sequence of ST64B belonging to the P27-like phage group (Fig. 6B). Since diverse roles of prophage genetic elements are associated with virulence determinants in many bacterial pathogens [58], the beneficial function of these prophage sequences in Salmonella adaptation to various environments, including pigs, merits further study.

In summary, the complete genome sequence of strain ST1120 contains a myriad of genes encoding antibiotic resistance proteins and major virulence factors of Salmonella, including T3SSs and their virulence effectors produced by SPI-1 and SPI-2 or elsewhere in the chromosome, multifaceted virulence determinants produced by SPI-3, SPI-4, SPI5, SPI-13, and SPI-14, and C63PI, Lpf, and curli biogenesis system, and others. In accordance with the in silico analysis results, strain ST1120 showed substantial resistance to several antibiotics and also had considerable virulence after having contact with host cells compared with other reported Salmonella enterica serovars (Fig. 6A), suggesting a potential outbreak by ST1120 in Korea. Meanwhile, comparison between the ST1120 and 798 genomes revealed strain-specific regions. A prophage region of ST104 conserved in the genomes of ST1120 and U288 was not present in the genome of 798. Instead, strain 798 was found to possess a putative prophage-associated region homologous to the genome sequence of ST64B belonging to the P27-like phage group (Fig. 6B). Since diverse roles of prophage genetic elements are associated with virulence determinants in many bacterial pathogens [58], the beneficial function of these prophage sequences in Salmonella adaptation to various environments, including pigs, merits further study.

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### Conflict of Interest

The authors have no financial conflicts of interest to declare.

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