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Research Note

Whole-Genome Analysis of *Salmonella Enterica* subsp. *Enterica* serovar Gallinarum biovar Gallinarum Strain IJES3-1 Isolated from a Retail Chicken Shell Egg in Korea

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ABSTRACT - *Salmonella enterica* subsp. enterica serovar Gallinarum biovar Gallinarum causes fowl typhoid in poultry. In this study, we isolated *Salmonella* from a Korean retail chicken shell egg and performed whole-genome sequencing, from which we identified one chromosome (4,659,977-bp) and two plasmids (plasmid_1: 87,506 bp and plasmid_2: 2,331 bp). The isolate serotype was confirmed to be Gallinarum, with a biovar type of Gallinarum, which was finally identified as *Salmonella enterica* subsp. *enterica* serovar Gallinarum biovar Gallinarum. Multilocus sequence typing confirmed that the isolate was that of sequence type 78. The antimicrobial resistance gene, *aac(6')-laa*, was identified on the chromosome, and 166 virulence genes were detected on the chromosome and plasmid_1.

Key words: *Salmonella enterica* subsp. *enterica* serovar Gallinarum *biovar* Gallinarum, Whole-genome sequence, Chicken egg

Salmonella is a rod-shaped, gram-negative, facultative anaerobic bacterium belonging to the *Enterobacteriaceae* family[1]. It can be observed in various environments and hosts[2]. *Salmonella enterica* subsp. *enterica* serovar Gallinarum biovar Gallinarum (*S*. Gallinarum) is adapted to captive birds and primarily affects chickens and turkeys[2]. Fowl typhoid is a septicemic disease of poultry caused by *S.* Gallinarum[2]. Although fowl typhoid is controlled or eliminated in numerous developed countries, it remains prevalent in developing countries, including South Africa[3]. Additionally, the disease causes significant economic losses owing to reduced production and high mortality rates in captive poultry[4].

S. Gallinarum strain IJES3-1 was isolated from a retail chicken shell egg purchased from a domestic supermarket. Whole-genome sequencing (WGS) was performed using Illumina MiSeq and Nanopore sequencing techniques. For MiSeq sequencing, libraries were prepared using the TruSeq Nano DNA Prep kit (Illumina, San Diego, CA, USA), following the manufacturer's protocol. Libraries were sequenced using the NextSeq P1 600 cycles in a NextSeq 2000 system using 2×300 bp pair-ends. Following sequencing, the individual sequence reads were analyzed using FastQC-v.0.11.8. For nanopore sequencing, libraries were prepared using a Ligation Sequencing kit (Oxford Nanopore Technologies, Oxford, UK). Nanopore sequencing data were based on the Guppy v4.2.2. Illumina and nanopore sequencing data were processed and de novo assembled using Unicycler v0.5.0. The genome was annotated using the National Center for Biotechnology Information (NCBI) Prokaryotic Genome Annotation Pipeline[5]. The complete genome sequence of IJES3-1 has been deposited in the NCBI GenBank database under the accession numbers CP157387 (chromosome), CP157388 (plasmid_1), and CP157389 (plasmid_2).

The genome of the *S.* Gallinarum strain IJES3-1 consists of a single chromosome (4,659,977 bp, with 52.20 % GC content), plasmid_1 (87,506 bp, with 53.55 % GC content), and plasmid_2 (2,331 bp, with 47.32 % GC content) (Table 1 and Fig. 1).

Multilocus sequence typing (MLST) analysis using MLST 2.0 (version 2.0.9) confirmed this strain as sequence type (ST) 78[6]. This bacterium was identified as *Salmonella enterica* subsp. *enterica* serovar Gallinarum using SeqSero 1.2 and Kmerfinder 3.2[7,8]. The biovar type was confirmed as *Salmonella enterica* subsp. *enterica* serovar Gallinarum biovar Gallinarum using in silico polymerase chain reaction (PCR) on Unipro UGENE 50.0, using the primer sequences listed in Table 2[9]. AMRFinderPlus and ResFinder were used to identify antimicrobial resistance (AMR) genes[10,11]. No antimicrobial

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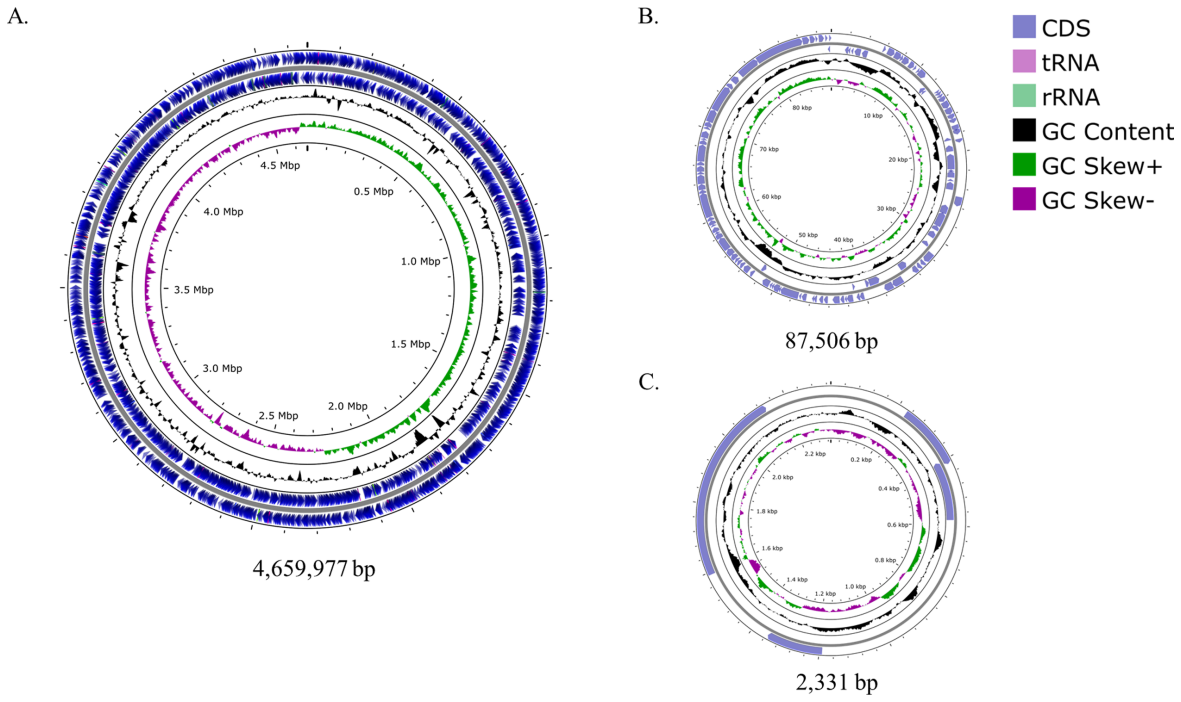

Fig. 1. Complete genome map of *S. gallinarum* strain IJES3-1. (A) Chromosome, (B) plasmid_1, and (C) plasmid_2 sequences are visualized using the Proksee (https://proksee.ca). The outer ring data represents: CDS forward strands, CDS reverse strands, GC content, and GC skew. tRNA (light pink) and rRNA (light green) are located in the same ring as that of the CDS.

Table 1. Genetic characteristics of *S.* Gallinarum strain IJES3-1

Features	Characteristics		
	Chromosome	Plasmid_1	Plasmid_2
Genome size	4,659,977 bp	87,506 bp	2,331 bp
GC content	52.20%	53.55%	47.32%
No. of CDS	4,400	109	4
No. of rRNA	22	-	-
No. of tRNA	77	-	-
MLST	ST78	-	-
AMR gene	*aac(6')-laa*	N.D[1)]	N.D[1)]
No. of virulence gene	160	6	0

[1)] N.D.: not detected.

Table 2. Confirmation of biovar type using in scilco PCR

Gene		Primer sequence (5' → 3')	Size (bp)	Salmonella		IJES3-1 location (Amplicon size)	Reference
				SP[1)]	SG[2)]		
stn	Forward:	TATTTTGCACCACAGCCAGC	131	+	+	4,476,169-4,476,299 (131bp)	14)
	Reverse:	CGACCGCGTTATCATCACTG					
I137_08605	Forward:	CACTGGAGACTCTGAGGACA	290	+	+	2,773,759-2,774,048 (290bp)	
	Reverse:	GGGCAGGGAGTCTTGAGATT					
ratA	Forward:	ATTGCTCTCGTCCTGGGTAC	571	-	+	1,278,381-1,278,951 (571bp)	
	Reverse:	TACCGATACGCCCAACTACC					

[1)] SP: *Salmonella enterica* subsp. *enterica* serovar Gallinarum biovars Pullorum.
[2)] SG: *Salmonella enterica* subsp. *enterica* serovar Gallinarum biovars Gallinarum.

resistance genes were identified in AMRFinderPlus, but *aac(6')-laa* gene responsible for amikacin, kanamycin, and tobramycin resistance was identified in ResFinder (Table 1)[12]. However, this gene appears to have no clinical significance or evolutionary advantage[12]. Virulence genes were identified using the Virulence Factor Database (VFDB), and 166 genes (160 on the chromosome and 6 on the plasmid_1) were identified (Table 1)[13].

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Conflict of interests

The authors declare no potential conflict of interest.

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