

Research Note

식품에서 분리된 살모넬라(*Salmonella enterica* Enteritidis MFDS1022168)의 유전체 서열 분석

김예은 · 이우정 · 안은숙 · 김승환 · 주인선*
식품의약품안전처 식품의약품안전평가원 미생물과

Complete Genome Sequence of *Salmonella enterica* Enteritidis MFDS1022168 Isolated from Food

Yeeun Kim, Woojung Lee, Eun Sook An, Seung Hwan Kim, Insun Joo*
Food Microbiology Division, Ministry of Food and Drug Safety, Cheongju, Korea

(Received September 23, 2024/Revised October 22, 2024/Accepted October 23, 2024)

ABSTRACT - *Salmonella enterica* is an infectious foodborne pathogen causing major public health problems worldwide. We present here the complete genome sequence of *Salmonella enterica* subsp. *enterica* serovar Enteritidis isolate MFDS1022168, that was isolated from a gimhap in Gyeonggi-do, South Korea, in 2022. The complete genome sequence of MFDS1022168 consists of a circular chromosome of 4,771,958 bp with a G + C content of 52.0%, and a plasmid of 59,372 bp with a G + C content of 51.9%. The chromosome is characterized by the presence of *Salmonella* pathogenicity islands containing virulence factor-encoding genes such as *invA*. Gene prediction analysis showed that the chromosome possesses 4,776 coding sequence (CDS) as well as 84 *tRNA* and 22 *rRNA* genes.

Key words: *Salmonella enterica* subsp. *enterica* serovar Enteritidis, Complete genome, Gimhap

Body

Nontyphoidal salmonellae are among the most prevalent foodborne pathogens causing gastrointestinal infections worldwide. Cardoso *et al.*¹⁾ reported a high number of cases and outbreaks of salmonellosis are associated with the consumption of eggs and egg products.

The pathogenicity of *Salmonella* spp. is mediated by several of their genes, such as invasion gene A (*invA*), fimbrial gene A (*fimA*), *Salmonella* enterotoxin gene (*stn*), *Salmonella* plasmid virulence gene R (*spvR*), *Salmonella* plasmid virulence gene C (*spvC*), *Salmonella* pathogenicity island 2 gene C (*spiC*), and pathogenicity island-encoded proteins gene D (*pipD*). The invasion gene (*invA*), located

on the pathogenicity island 1 (SPI-1), has been widely studied for its ability to promote virulence and as a biomarker for the detection of *Salmonella* spp. Nikiema *et al.*²⁾ reported the *invA* gene of *Salmonella* spp. is also involved in the invasion of host epithelial cells. In this study, we present the complete genome sequence of *Salmonella enterica* subsp. *enterica* serovar Enteritidis isolate MFDS1022168, the causative agent of a food poisoning incident in Gyeonggi-do, South Korea, in 2022. The virulent strain, isolated from gimhap and detected using real-time PCR, was identified during a food poisoning investigation managed by the Ministry of Food and Drug Safety (MFDS, Seoul, Korea). Following isolation, the MFDS1022168 isolate was incubated on tryptic soy agar plates overnight at 37°C. The total genomic DNA of MFDS1022168 was extracted using a Blood and Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. The genomic DNA was qualitatively and quantitatively assessed using a NanoDrop 2000 UV-visible spectrophotometer (Thermo Fisher Scientific, Waltham, MA,

*Correspondence to: Insun Joo, Food Microbiology Division, Ministry of Food and Drug Safety, Cheongju, Korea
Tel: +82-43-719-4301, Fax: +82-43-719-4300
E-mail: jis901@korea.kr

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USA) and a Qubit 3.0 fluorometer (Invitrogen, Waltham, MA, USA), respectively. The qualified genomic DNA was

then used to construct a PacBio library utilizing the single-molecule real-time (SMRT) bell® express template prep kit 2.0 (Pacific Biosciences, Menlo Park, CA, USA). The library was sequenced using a Sequel binding kit 3.0 (Pacific Biosciences) and Sequel sequencing kit 3.0 (Pacific Biosciences) on a single-molecule real-time cell (SMRT Cell 1M v3, Pacific Biosciences). The raw sequence reads were *de novo* assembled using the PacBio SMRT analysis system with the hierarchical genome assembly process (HGAP) 2 assembler (version 8; Pacific Biosciences). The total number of generated reads was 4,831,330 bp. The final assembly had a coverage of 664×. The genome was annotated using the Bacterial and Viral Bioinformatics Resource Center (Bacterial and Viral

Table 1. Genetic characteristics of *Salmonella enterica* Enteritidis MFDS1022168

Features	Characteristics	
	Chromosome	plasmid
Genome size	4,771,958 bp	59,372 bp
GC content	52.0%	51.9%
No. of CDS	4776	100
No. of tRNAs	84	-
No. of rRNAs	22	-

CDS: coding sequence

Table 2. Virulence genes associated with pathogenicity islands in *Salmonella enterica* Enteritidis MFDS1022168

Classification	Gene (s)	Function	Reference
SPI-1 (<i>Salmonella</i> pathogenicity island-1)	<i>invA</i>	Gene encoding an effector protein related to delivery of type III secretion system.	Marcus <i>et al.</i> ⁶⁾
	<i>hilA</i>	Gene encoding a transcriptional activator of SPI genes	Marcus <i>et al.</i> ⁶⁾
	<i>sipB</i>	Assists in the delivery of effector proteins during invasion	Lou <i>et al.</i> ⁹⁾
	<i>sipC</i>	Facilitates T3SS formation and membrane ruffle creation for invasion	Lou <i>et al.</i> ⁹⁾
	<i>sopE</i>	Activates host GTPases to enhance membrane ruffling and invasion	Lou <i>et al.</i> ⁹⁾
SPI-2 (<i>Salmonella</i> pathogenicity island-2)	<i>ssrA</i>	Gene encoding a membrane-located sensor kinase that is involved in the two-component system with SsrB. The system activates the type III secretion system and effector proteins.	Marcus <i>et al.</i> ⁶⁾ Tomljenovic-Berube <i>et al.</i> ¹⁰⁾
	<i>ssaR</i>	Encoding a protein related to the translocation of effectors to the host cell via the type III secretion system. These effectors are involved in the processes of invasion, internalization, intracellular proliferation, and survival.	Sever and Akan ¹¹⁾

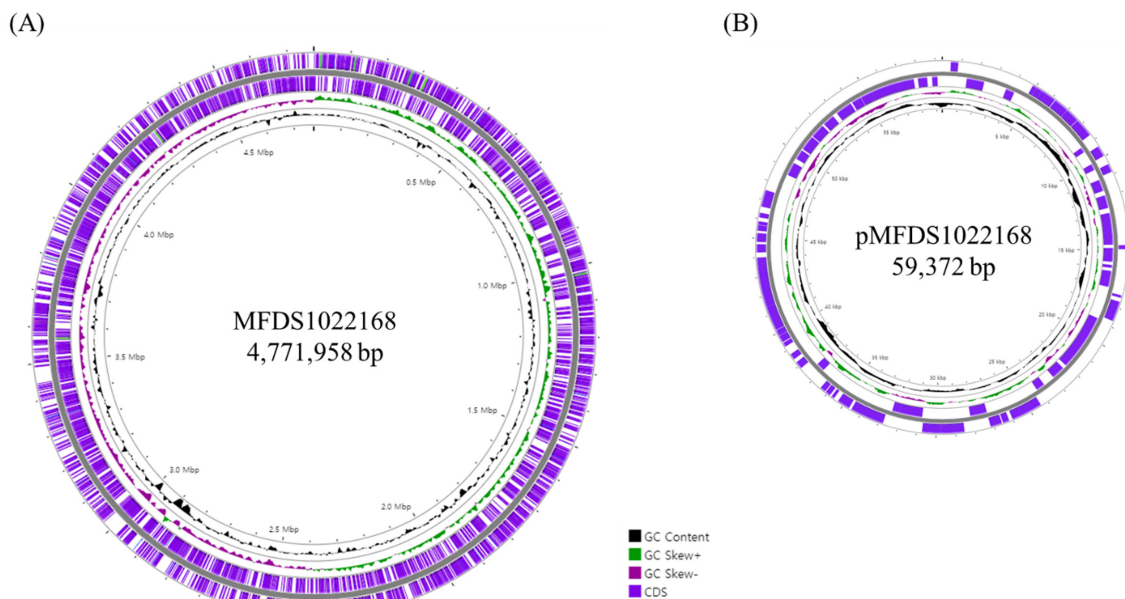


Fig. 1. Complete genome map of *Salmonella enterica* Enteritidis MFDS1022168. (A) Chromosome. (B) Plasmid sequences were visualized using the Proksee tool (<https://proksee.ca>). The outer ring data represent CDS forward strands, CDS reverse strands, GC skew, and GC content.

Bioinformatics Resource Center, BV-BRC, v3.32.13.a) web server³). Virulence-associated genes were predicted using the Virulence Factor Database (VFDB)⁴ and Pathosystems Resource Integration Center_Virulence Factor (PATRIC_VF)⁵). The complete genome sequence of MFDS1022168 consists of two contigs of 4,771,958 bp and 59,372 bp, with G+C contents of 52.0% and 51.9%, and containing 4,776 and 100 coding sequences, respectively. The chromosome encodes 84 tRNAs and 22 rRNAs (Table 1). Marcus *et al.* reported *Salmonella* pathogenicity islands (SPI) are known to confer virulence traits⁶. For instance, Raffatelli *et al.* reported SPI-1 promotes bacterial invasion into epithelial cells⁷. Hensel reported *Salmonella* pathogenicity island 2 (SPI-2) is central for the ability of *S. enterica* to cause systemic infections and for intracellular pathogenesis⁸. Accordingly, SPI-1 (*invA*, *hilA*, *Salmonella* invasion protein B (*sipB*), *Salmonella* invasion protein C (*sipC*), and *sopE*) and SPI-2 (*ssrA*, *ssaR*), which are located on the chromosome of MFDS1022168, may play an important role in the strong pathogenicity of this isolate (Table 2). The complete genomic information of *Salmonella enterica* Enteritidis MFDS1022168 provides a genetic basis for a more detailed analysis of its virulence factors, thus facilitating the further investigation of this food-borne pathogen.

Nucleotide sequence accession number(s)

The complete genome sequence of *Salmonella enterica* serovar Typhimurium MFDS1022168 has been deposited in NCBI GenBank under accession numbers CP168659 (chromosome, MFDS1022168) and CP168660 (plasmid, pMFDS1022168). The strain has been deposited in the Korean Culture Collection for Foodborne Pathogens under strain number MFDS1022168.

Acknowledgements

This research was supported by a grant from the Ministry of Food and Drug Safety, Republic of Korea (23194MFDS017).

국문요약

*Salmonella enterica*는 세계적으로 가장 널리 알려진 식 중독균이다. *Salmonella enterica* Enteritidis MFDS1022168 은 인천보건환경연구원에서 계란에서 분리된 균주로, 이 균주가 지니고 있는 병원성에 대한 genome 수준의 메커니

즘을 이해하기 위하여 whole genome sequencing을 진행하여 유전체를 분석하였다. PacBio Sequel 장비를 이용하여 유전체염기서열 분석을 진행한 결과 *invA*를 비롯한 여러 종류의 병원성유전자를 갖고 있는 것으로 확인됐다. 이에 따라 해당 균주의 독성을 파악하기 위해 유전체분석을 진행하였다. 유전자 총 수 예측 결과, 4,776개의 CDSs, 84 개의 tRNA, 22개의 rRNA를 보유한 것으로 나타났다.

Conflict of interests

The authors declare no potential conflict of interest.

ORCID

Woojung Lee <https://orcid.org/0000-0002-3174-8033>

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