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Complete genome sequence of *Zindervirus* phage vB_SenAt-pSL2 infecting *Salmonella enterica* subsp. *enterica* serovar Enteritidis from Korean broiler chickens

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국내 육계에서 분리한 *Salmonella enterica* subsp. *enterica* serovar Enteritidis를 특이적으로 감염하는 *Zindervirus* 파지 vB_SenAt-pSL2의 전장 유전체 분석

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Salmonella enterica subsp. *enterica* serovar Enteritidis is a major food-borne pathogen responsible for severe economic loss in the poultry industry. We report the complete genome sequence of the novel bacteriophage (phage) vB_SenAt-pSL2 infecting *Salmonella enterica* subsp. *enterica* serovar Enteritidis KCTC 82777 isolated from Korean broiler chickens. The genome comprised a linear double-stranded DNA of 44,304 bp with 47.4% G + C content and 54 ORFs. Genome-based comparison revealed that the phage was closely related to *Zindervirus* and distinguished by having the largest genome in the genus. The high nucleotide similarity of the predicted tail fiber and spike proteins in vB_SenAt-pSL2 with other broad-host-range phages in *Zindervirus* suggests its potential to infect diverse *Salmonella* serotypes. Based on these results, the newly isolated *Salmonella* phage vB_SenAt-pSL2 is a new member of the genus *Zindervirus* and its genomic information provides insights into the biodiversity and taxonomy of *Autographiviridae*.

Keywords: *Salmonella* phage, *Zindervirus*, biodiversity, tail protein

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Salmonella enterica subsp. *enterica* serovar Enteritidis (*Salmonella* Enteritidis) is a major food-borne pathogen. Its cells tightly attach to food contact surfaces, forming biofilms on poultry products, which leads to severe food-borne illnesses (Myszka and Czaczyk, 2011). *Salmonella* bacteriophages (phages) have attracted global interest as potential biocontrol agents because of their ability to rapidly lyse a broad range of host bacteria (Pelyuntha *et al*., 2021). *Zindervirus* (synonyms SP6-like viruses, Sp6likevirus, and Sp6virus) is a genus in the family *Autographiviridae*, subfamily *Molineuxvirinae* (Adriaenssens *et al*., 2020). The genus is characterized by an isometric head

and a non-contractile tail consisting of host-recognizing six prominent tail spike proteins (TSPs) (Knecht *et al*., 2020), and includes phages infecting *Escherichia coli*, *Salmonella* spp., and *Vibrio* spp. (Cao *et al*., 2021). Here, we report the complete genome sequence of phage vB_SenAt-pSL2, a new member of the genus *Zindervirus*, infecting *Salmonella* Enteritidis from Korean broiler chickens.

The phage vB_SenAt-pSL2 was isolated from the water sample collected from the sewage treatment plant in Deajeon using the host strains *Salmonella* Enteritidis KCTC 82777 obtained from Korean broiler chickens. Phage propagation and amplification were carried out as described previously (Kim *et al*., 2012), and total genomic DNA was extracted by Macrogen and sequenced on an Illumina HiSeq X-10 platform of coverage 100x by preparing a DNA library using a TruSeq Nano DNA kit (Illumina). The *de novo* assembly of the filtered reads (1,972,589,259 bp; 13,126,724 reads; N50, 44,304 bp) with 100× coverage was performed using SPAdes (v3.13.0). Annotation of the putative open reading frames (ORFs) was performed with RAST (https://rast.nmpdr.org/rast.cgi), and conserved domain functions were determined using BLASTP (https://blast.ncbi.nlm.nih.gov/Blast.cgi), Pfam (http://pfam.xfam.org/), and HHpred (https://toolkit.tuebingen.mpg.de/tools/hhpred). Phage termini and packaging mechanism were determined using PhageTerm through the Galaxy server (https://cpt.tamu.edu/galaxy-pub). Transmembrane domains were predicted by using TMHMM (http://www.cbs.dtu.dk/services/TMHMM-2.0/), and signal peptides were predicted using SignalP (http://www.cbs.dtu.dk/services/SignalP/). Phylogenetic analysis based on genome sequences was performed using VICTOR (https://ggdc.dsmz.de/victor.php).

Transmission electron microscopy of phage vB_SenAt-pSL2 revealed morphological characteristics similar to those of other members of *Autographiviridae*, featuring an isometric head and a putative non-contractile tail (Fig. 1A). The vB_SenAt-pSL2 genome was a linear double-stranded DNA 44,304 bp in length with 47.4% G + C content, 54 predicted protein-coding genes, but no tRNA (Table 1), and including a short terminal repeat region (177 bp). The genome was similar to the *Salmonella* phages UAB_Phi78 (NC_020414.2, 98.4%) and BP12B (KM 366097.1, 96.5%). Phylogenetic analysis using 20 genome sequences belonging to the subfamily *Molineuxvirinae* (*Autographiviridae*) clustered vB_SenAt-pSL2 together with other members of *Zindervirus*, from which is it distinguished by possessing the largest genome in the genus (Fig. 1B).

Of the putative 54 open reading frames (ORFs), 11 ORFs

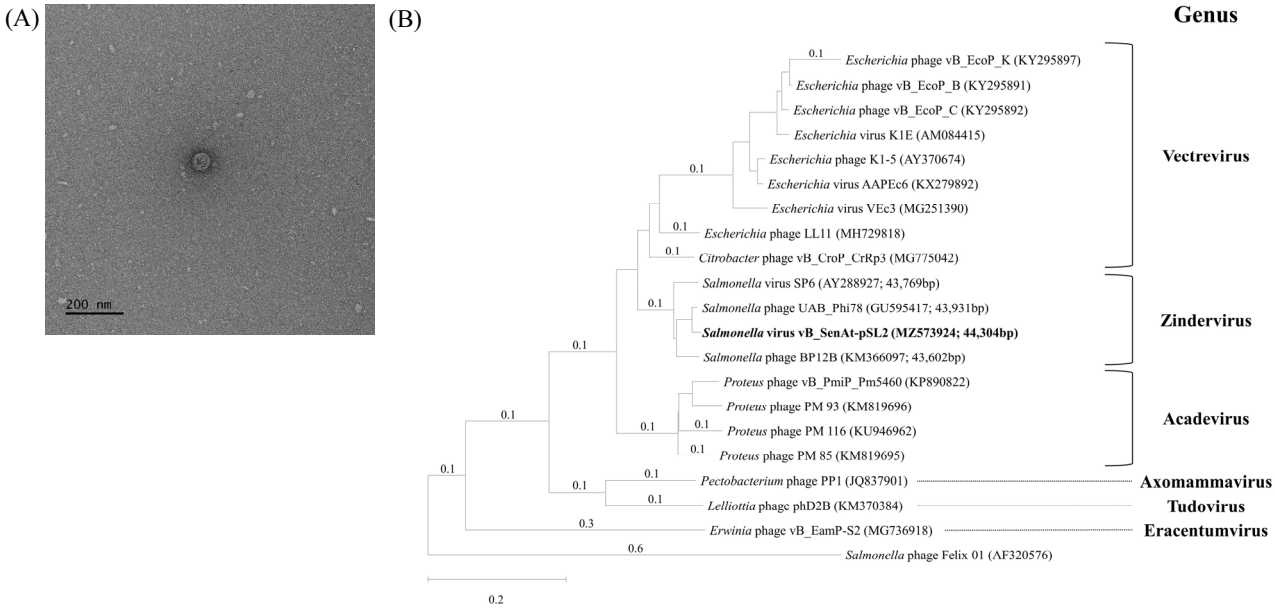

Fig. 1. (A) Transmission electron micrograph (TEM) of *Salmonella* phage vB_SenAt-pSL2. The scale bar is 200 nm. (B) Genome-BLAST Distance Phylogeny (GBDP) tree based on genome sequences of the strain vB_SenAt-pSL2 and phages of six genera belonging to the subfamily *Molineuxvirinae*. The tree was created in VICTOR, with branch support determined in terms of formula D0 and inferred from 100 pseudo-bootstrap replicates with an average support of 18%.

Table 1. The features of *Salmonella* phage vB_SenAt-pSL2 genome

Features	Value
Genome Size (bp)	44,304
G + C content (%)	47.4
Conitg	1
Total genes	54
Protein-coding genes (CDS)	54

were functionally annotated as nucleotide metabolism-related proteins: DNA-directed RNA polymerase, HNH homing endonucleases, DNA primase/helicase, DNA polymerase, exonuclease, DNA ligase, lysozyme, holin and peptidase. The presence of virion-encoded RNA polymerase is the defining characteristic of *Autographiviridae* (Adriaenssens *et al*., 2020), and its presence in vB_SenAt-pSL2 strongly supported the genome-based phylogeny. 13 ORFs were identified as packaging- and structure-related proteins such as collar head-to-tail connector protein, capsid assembly scaffolding protein, major capsid protein, tail tubular proteins, tail fiber proteins, terminase subunits, and TSPs. Tail fiber proteins are critical components for host recognition and phage DNA ejection (Scholl *et al*., 2002; Gebhart *et al*., 2017). TSP, also known as depolymerase, specifically degrades cellular polysaccharides involved in biofilm formation by the host bacterial strain (Harper *et al*., 2014; Knecht *et al*., 2020). The high nucleotide similarities of the predicted tail fiber proteins (ORF 37, 38, 39, 41, 42, and 53) TSPs (ORF 51 and 52) between vB_SenAt-pSL2 and other broad-host-range *Salmonella* phages in the genus *Zindervirus* (Bardina *et al*., 2012) suggests a wide host range of the phage against *Salmonella* serotypes. Moreover, a putative lysozyme (ORF 40) and holin (ORF 43) which showed high sequence similarities to other members in the genus *Zindervirus* were detected in the genome. The remaining ORFs were assigned to hypothetical proteins, four of which were screened for conserved domains by Pfam search, and antibiotic resistance or bacterial virulence genes were not found in the genome of the phage (Supplementary data Table S1).

Based on the genome comparison and its distinct genome size, the newly isolated *Salmonella* phage vB_SenAt-pSL2 is a new member of the genus *Zindervirus*. Its genomic information will provide insights into the biodiversity of phages in *Autographiviridae*.

Nucleotide sequence accession number

Salmonella phage vB_SenAt-pSL2 was deposited in the Korean Collection for Type Cultures (KCTC 4823). The genome of vB_SenAt-pSL2 was deposited in GenBank (accession number MZ573924.3).

적 요

Salmonella enterica subsp. *enterica* serovar Enteritidis는 전 세계적으로 발생되는 주된 식중독 유발 세균으로 양계 산업 전반에 심각한 피해를 초래한다. 본 연구에서는 국내 도계장에서 분리된 *Salmonella enterica* subsp. *enterica* serovar Enteritidis를 특이적으로 감염하는 파지 vB_SenAt-pSL2의 전장 유전체를 분석하였다. 분석 결과, vB_SenAt-pSL2 파지는 총 44,304 bp, G + C 함량 47.4%의 유전체를 지니며 54개의 단백질 코딩 유전자로 구성됨을 확인하였다, 또한 전장 유전체 기반 계통수 분석 결과 vB_SenAt-pSL2 파지는 *Zindervirus*로 분류되었으며, 동일 속 중 가장 큰 유전체를 지니는 것으로 확인되었다. 또한 *Zindervirus* 내 광숙주역 파지들과의 tail fiber 및 spike 단백질의 높은 상동성에 근거하여, vB_SenAt-pSL2 파지 역시 광숙주역을 지닐 것으로 추정된다. 본 연구 결과는 *Autographiviridae* 파지의 분류학적 특성 및 다양성 연구의 기반 자료로 활용될 것이다.

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Conflict of Interest

The authors declare that they have no conflict of interest.

References

Adriaenssens EM, Sullivan MB, Knezevic P, van Zyl LJ, Sarkar BL, Dutilh BE, Alfenas-Zerbini P, Łobocka M, Tong Y, Brister JR, *et al.* 2020. Taxonomy of prokaryotic viruses: 2018–2019 update from the ICTV Bacterial and Archaeal Viruses Subcommittee. *Arch. Virol.* **165**, 1253–1260.

Bardina C, Spricigo DA, Cortés P, and Llagostera M. 2012. Significance of the bacteriophage treatment schedule in reducing *Salmonella* colonization of poultry. *Appl. Environ. Microbiol.* **78**, 6600–6607.

Cao Y, Zhang Y, Lan W, and Sun X. 2021. Characterization of vB_VpaP_MGD2, a newly isolated bacteriophage with biocontrol potential against multidrug-resistant *Vibrio parahaemolyticus*. *Arch. Virol.* **166**, 413–426.

Gebhart D, Williams SR, and Scholl D. 2017. Bacteriophage SP6 encodes a second tailspike protein that recognizes *Salmonella* enterica serogroups C_2 and C_3. *Virology* **507**, 263–266.

Harper DR, Parracho HMRT, Walker J, Sharp R, Hughes G, Werthén M, Lehman S, and Morales S. 2014. Bacteriophages and biofilms. *Antibiotics* **3**, 270–284.

Kim JH, Son JS, Choi YJ, Choresca CH, Shin SP, Han JE, Jun JW, Kang DH, Oh C, Heo SJ, *et al.* 2012. Isolation and characterization of a lytic *Myoviridae* bacteriophage PAS-1 with broad infectivity in *Aeromonas salmonicida*. *Curr. Microbiol.* **64**, 418–426.

Knecht LE, Veljkovic M, and Fieseler L. 2020. Diversity and function of phage encoded depolymerases. *Front. Microbiol.* **10**, 2949.

Myszka K and Czaczyk K. 2011. Bacterial biofilms on food contact surfaces – a review. *Pol. J. Food Nutr. Sci.* **61**, 173–180.

Pelyuntha W, Ngasaman R, Yingkajorn M, Chukiatsiri K, Benjakul S, and Vongkamjan K. 2021. Isolation and characterization of potential *Salmonella* phages targeting multidrug-resistant and major serovars of *Salmonella* derived from broiler production chain in Thailand. *Front. Microbiol.* **12**, 662461.

Scholl D, Adhya S, and Merril CR. 2002. Bacteriophage SP6 is closely related to phages K1-5, K5, and K1E but encodes a tail protein very similar to that of the distantly related P22. *J. Bacteriol*. **184**, 2833–2836.