

Complete genome sequence of *Staphylococcus* phage vB_SauM-HM01, a novel member of the genus *Silviavirus*[§]

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Staphylococcus aureus is a clinically important pathogen with increasing antibiotic resistance. Here, we report the complete genome sequence of the bacteriophage vB_SauM-HM01, a linear dsDNA (138,868 bp; 29.8% G + C) encoding 196 proteins without tRNA, lysogeny, virulence, or antibiotic resistance genes. Phylogenetic and comparative genome analyses classified it as a member of the genus *Silviavirus* (subfamily *Twortvirinae*), closely related to phage Stau2. Mosaic regions near homing endonuclease genes suggest recombination events contributing to genetic diversity. These genomic features, together with specific broad host range against *S. aureus*, support its potential as a safe and effective biocontrol agent and improve our understanding of phage diversity and classification.

Keywords: *Staphylococcus* phages, biocontrol agent, phage diversity, phage Stau2

Staphylococcus aureus is a major opportunistic pathogen and a leading cause of nosocomial infections ranging from mild skin infections to life-threatening diseases, such as fatal pneumonia and sepsis (Cheung et al., 2021). With the increasing threat of antibiotic resistance, bacteriophages

(phages) have emerged as promising alternatives, offering host specificity, minimal disruption of normal flora, and self-replication at infection sites (Loc-Carrillo and Abedon, 2011). Members of the subfamily *Twortvirinae* are distinguished by their broad host range, strong bacteriolytic activity, and efficacy in both in vivo and food-related applications (Kitamura et al., 2020; Lertsittikul et al., 2024; Lu et al., 2023; Pelyuntha et al., 2025). Among the available genera in the *Twortvirinae*, *Silviavirus* is a relatively recently established genus in the International Committee on Taxonomy of Viruses (ICTV) classification, and research on this group remains limited compared to other well-studied *Twortvirinae* phages (Lavigne et al., 2015). Moreover, for therapeutic use, comprehensive genomic characterization is essential to confirm the exclusion of undesirable genes, such as virulence or antibiotic resistance determinants (Cheung et al., 2021). Herein, we present the complete genome sequence of vB_SauM-HM01, a new member of *Silviavirus* (subfamily *Twortvirinae*) phage isolated in Korea, and propose it as a potential alternative antimicrobial for *S. aureus* biocontrol.

Staphylococcus phage vB_SauM-HM01 was isolated from a sewage sample collected in Daejeon, Korea, using *S. aureus* ATCC 12598 as the host. The morphological feature of the phage was analyzed as previously described (Lim et al., 2024), and the host range of the phage was determined

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using various available reference strains of *Staphylococcus* spp. of clinical and food origins (Table S1). The gDNA of phage vB_SauM-HM01 was extracted using the conventional phenol-chloroform extraction method with minor modifications (Lim et al., 2024). A 100 bp paired-end library was prepared using the TruSeq Nano DNA library preparation kit (Illumina, USA), sequenced on an Illumina HiSeq X-10 platform (Illumina, USA), yielding 19,981,860 reads (3,017,260,860 bases; G + C content, 29.8%; Q30, 92.6%), and de novo assembled using SPAdes v.3.13.0 (Bankevich et al., 2012). Genome annotation was performed with RAST (<https://rast.nmpdr.org/>), and predicted open reading frames (ORFs) were subsequently characterized via BLASTp (<https://blast.ncbi.nlm.nih.gov>) against non-redundant protein databases (*E*-value < 1e-5). Protein features were assessed using InterProScan (<https://www.ebi.ac.uk/interpro/>; Pfam v.36), TMHMM v.2.0 (<https://services.healthtech.dtu.dk/services/TMHMM-2.0/>), SignalP v.6.0 (<https://services.healthtech.dtu.dk/services/SignalP-6.0/>), and DePolymerase Predictor v1.0.0 (Magill and Skvortsov, 2023), all run with their default settings. Additionally, tRNAs were identified using tRNAscan-SE v.2.0 (<http://gttnadb.ucsc.edu/faq.html>) with default parameters; antibiotic resistance genes were screened with CARD Resistance Gene Identifier v.6.0.5 (<https://card.mcmaster.ca/>) applying 'Perfect and Strict hits only' criteria; and virulence factors were detected by VFDB VFAnalyzer 2022 (<http://www.mgc.ac.cn/cgi-bin/VFs/v5/main.cgi>) with thresholds of *E*-value < 1e-5 and sequence identity > 60%. Biophysical properties (Mw/pI) were estimated using the ExPASy Compute pI/Mw tool (Wilkins et al., 1999), and the circular genome map was visualized using the Proksee server (<https://proksee.ca/>). For comparative genomic analyses, closely related phage genomes (based on ICTV classification; <https://ictv.global/taxonomy/>) were retrieved from the GenBank database and aligned using MUSCLE. A phylogenetic tree was then constructed in MEGA X (Kumar et al., 2018) via the Neighbor-Joining method with 1,000 bootstrap replicates. Pairwise genome comparisons were visualized with EasyFig (Sullivan et al., 2011), which employed the BLASTn algorithm (*E*-value < 1e-5) to display nucleotide identity as a grey-scale gradient.

Phage vB_SauM-HM01, with an icosahedral head and long

contractile tail typical of Myovirus (Fig. 1A), lysed all the tested *S. aureus* strains from both clinical and food sources, thus demonstrating a broader applicability than previously reported *Silviavirus* phages (Hsieh et al., 2011; Lertsittikul et al., 2024). The isolated phage did not exhibit lytic activity against coagulase-negative *Staphylococcus* spp., and this high specificity against *S. aureus* minimizes the risk of off-target disruption of commensal flora, supporting its potential as a targeted approach for *S. aureus* control in diverse settings.

The linear dsDNA genome of phage vB_SauM-HM01 was 138,868 bp in length (209.7× coverage, 29.8% G + C content) and contained 196 predicted ORFs and no tRNA genes (Table 1). Functional analysis results showed that proteins could be grouped into five categories: 121 hypothetical proteins; 27 nucleotide metabolism proteins, including DNA polymerase (ORF152) and RNA polymerase (ORF107); 24 structural and packaging proteins, including five terminase large subunits (ORF71, 73, 76, 77, and 79), and the major capsid protein (ORF91); seven lysis-related proteins including endolysin-like proteins (ORF59, 60, and 111), and holin (ORF 61); and 17 proteins with additional functions, such as homing endonuclease (ORF50 and 100), transposase (ORF94, 137, 155, and 156), and Group I intron protein (ORF78) (Fig. 1B, Table S2). Notably, no antimicrobial resistance-, virulence-, or lysogeny-associated genes were detected. Genome termini analysis predicted a T5-like packaging mechanism with a 10,902-bp terminal repeat spanning positions 118,037–128,938. Comparative genomic analysis showed > 98% BLASTn identity with *Staphylococcus* phages Stau2 (KP 881332), Remus (MW546076), and Romulus (MW546075), all of which belong to the subfamily *Twortvirinae*. Phylogenetic analysis further classified vB_SauM-HM01 as belonging to the genus *Silviavirus* (Fig. 1C). Although the overall genome organization was largely conserved with phage Stau2, reduced nucleotide similarities were observed near putative recombination-associated elements, including homing endonuclease genes (HEG) (Fig. 1D). This pattern suggests a mosaic genome architecture shaped by recombination events, a common feature of *Staphylococcus* phages (Deghorain and Van Melderren, 2012; Vandersteegen et al., 2013). HEGs in phages are generally recognized as mobile genetic elements that promote their own propagation by

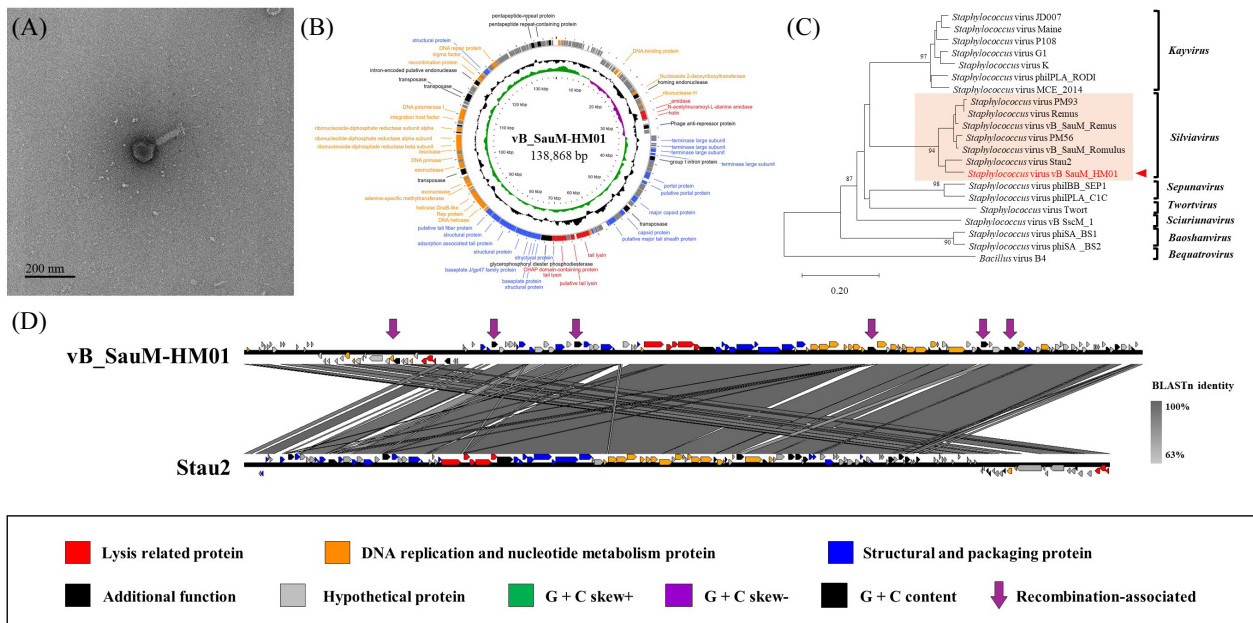


Fig. 1. Morphological and genomic profile of *Staphylococcus* phage vB_SauM-HM01. (A) Transmission electron micrograph of the isolated phage (scale bar = 200nm). (B) Circular genome map of phage vB_SauM-HM01. (C) Genome-based phylogeny of phage vB_SauM-HM01 generated with 19 *Twortvirinae* phages (including six representative members of the genus *Silviavirus*) with MEGA X. (D) Comparative genome analysis of phage vB_SauM-HM01 with *Staphylococcus* phage Stau2 (KP881332) using EasyFig with BLASTn (E -value < $1e-5$). Arrows indicate the orientation of the predicted open reading frames (ORFs), color-coded by function category: Lysis-related (red), DNA replication and nucleotide metabolism protein (orange), Structural and packaging protein (blue), Additional function (black), and Hypothetical protein (grey). Putative recombination-associated elements, such as homing endonucleases, transposases, and Group 1 introns, are highlighted by colored arrows. Grey shading between genomes reflects nucleotide identity (63%-100%), with darker tones indicating higher similarity.

Table 1. General genomic features of *Staphylococcus* phage vB_SauM-HM01

Features	Value
Genome size (bp)	138,868
G + C content (%)	29.8
Coverage	209.7 ×
Contigs	1
Total genes	196
Protein coding genes (CDS)	196
tRNA	0

inducing site-specific DNA cleavage in alleles lacking the HEG, thereby driving recombination and gene conversion (Edgell et al., 2010). Additionally, the HEGs in phages have been adapted for the targeted DNA modifications due to their long recognition sequences and high specificity, thus proving their potential for biotechnological applications (Stoddard, 2011). The presence of HEGs in the genome of vB_SauM-HM01 reflects its genomic plasticity and evolutionary adaptability, while offering insights into the genetic diversity

within the genus *Silviavirus*.

Here, we report the complete genome sequence of *Staphylococcus* phage vB_SauM-HM01, a newly identified member of the genus *Silviavirus*. Its genomic profile, along with specific lytic activity, supports its potential as a phage-based alternative against *Staphylococcus* infection in the post-antibiotic era.

Nucleotide sequence accession number

The complete genome sequence of *Staphylococcus* phage vB_SauM-HM01 has been deposited in the GenBank database under the accession number OL436246, and the phage has been deposited in the Korean Collection for Type Cultures (KCTC) as KCTC 4840.

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Conflict of Interest

The authors declare no conflicts of interest.

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