Archives of Virology (2024) 169:4
https://doi.org/10.1007/s00705-023-05940-1

BRIEF REPORT

Genomic and biological characteristics of a novel lytic phage, vB_MscM-PMS3, infecting the opportunistic zoonotic pathogen *Mammaliicoccus sciuri*

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Received: 11 August 2023 / Accepted: 14 November 2023

Abstract

Mammaliicoccus sciuri is an opportunistic zoonotic pathogen in humans and animals. We isolated the *Mammaliicoccus* phage vB_MscM-PMS3, which was also able to infect and lyse *M. sciuri* and *M. lentus*. The phage genome is a linear dsDNA that is 147,811 bp in length and contains 206 ORFs and three tRNA genes. It showed low genome coverage (< 17%) and sequence identity (< 91.3%) to other phage genomes. Phylogenetic analysis based on the whole genome and major capsid protein revealed that this phage clustered with members of the subfamily *Twortvirinae* of the family *Herelleviridae*, but it was distinctly separated from the other members, indicating its uniqueness.

Coagulase-negative staphylococci (CoNS) are ubiquitous worldwide. Previously considered non-pathogenic commensal bacteria, they are now recognized as important opportunistic pathogens of humans and animals [1, 2]. In 2020, the genus *Mammaliicoccus* was established to include five species from the CoNS group (*M. sciuri*, *M. lentus*, *M. fleurettii*, *M. stepanovicii*, and *M. vitulinus*) [3]. *M. sciuri* is an opportunistic zoonotic pathogen that causes severe clinical infections in humans and other animals [4–6]. Moreover, it is a natural reservoir of the antibiotic resistance gene *mecA* for *S. aureus*, and the emergence of multidrug-resistant strains poses a serious threat to public health worldwide [7, 8]. Bacteriophages (phages) are promising alternatives to control staphylococcal infections in humans and animals. Several phages infecting *Staphylococcus aureus* and some species of CoNS have been reported to date [9–11]. However, reports on phages infecting *M. sciuri* are relatively scarce compared to those on phages infecting *Staphylococcus* spp. despite their potential clinical relevance. In this study, we report the genomic and biological characteristics of a novel lytic phage (isolate designated vB_MscM-PMS3) infecting *M. sciuri*.

We previously reported the emergence of multidrug-resistant *Mammaliicoccus* species in Korean ducks [12]. Among the duck tremor-associated isolates, *M. sciuri*

Handling Editor: Johannes Wittmann.

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Published online: 11 December 2023

SNUDS-18 (= KCCM 43253 = KCTC 43031) [13] was selected as the host strain for phage isolation. Phage vB_MscM-PMS3 was isolated from wastewater from a sewage treatment plant (Daejeon, Korea). Phage isolation and propagation were conducted as described previously [14], using the double-layer agar method on tryptic soy agar (TSA; Difco, USA) at 37 °C, the optimal culture condition for bacterial growth.

The host range of phage vB_MscM-PMS3 was determined using *Mammaliicoccus* spp. (*M. sciuri* and *M. lentus*; n = 8), several species of CoNS (n = 8), and *S. aureus* (n = 8) (Table 1). Ten µl of phage suspension (~ 10^8 PFU/mL) was dropped onto each bacterial lawn in TSA and incubated overnight at 37 °C. The isolated phage infected and formed clear lytic plaques on several strains of *M. sciuri* and *M. lentus*; however, no plaques were formed on the other staphylococcal strains used in this study (Table 1). For morphological analysis, a drop of the phage suspension (~ 10^8 PFU/mL) was deposited onto a glow-discharged carbon-coated copper grid and negatively stained with 2% (w/v) uranyl acetate (Electron Microscopy Sciences, Inc., USA). The stained phage particles were observed using a JEM-1400 transmission electron microscope (JEOL Ltd., Japan) at 120 kV. As shown in Fig. 1A, phage vB_MscM-PMS3 has an icosahedral head and a long contractile tail with a sheath wrapped around the tail tube. To evaluate its lytic activity against the planktonic state of *M. sciuri*, we treated exponentially growing host bacteria (~ 10^7 CFU/mL) with various concentrations of phages as described previously [14]. The phage-treated group was treated at a multiplicity of infection (MOI) of 0.001, 0.01, 0.1, or 1, and the non-phage-treated group was used as a control. Each group was cultured in tryptic soy broth (TSB; Difco, USA) at 37 °C with shaking at 130 rpm, and bacterial growth was measured at 600 nm using a UV/Vis spectrophotometer (K-Lab Co., Ltd., Korea). All phage-treated groups effectively inhibited the growth of host bacteria for 24 h compared to the control group (Fig. 1B). Next, we estimated the adsorption rate of phage vB_MscM-PMS3 against host bacteria at an MOI of 0.001 and performed a one-step growth experiment to estimate the burst size of

Table 1 Detailed information on the bacterial strains used in this study and their susceptibility to *Mammaliicoccus* phage vB_MscM-PMS3

Bacterial species			Phage sensitivity	Origin	Source
Mammaliicoccus	*Mammaliicoccus sciuri*	SNUDS-1	–	South Korean ducks exhibiting tremor	[12]
		SNUDS-9	–		
		SNUDS-17	++		
		SNUDS-18	++		
		KCCM 41468	++	Unknown	KCCM
	Mammaliicoccus lentus	SNUDS-5	++	South Korean ducks exhibiting tremor	[12]
		SNUDS-12	–		
		KCTC 3577	++	Udder of a goat	KCTC
Coagulase-negative	*Staphylococcus ureilyticus*	ATCC 49330	–	Human skin	ATCC
	Staphylococcus warneri	ATCC 27836	–	Human skin	ATCC
	Staphylococcus epidermidis	ATCC 12228	–	Unknown	ATCC
		CCARM 3A794	–	Human skin	CCARM
	Staphylococcus haemolyticus	ATCC 29970	–	Human skin	ATCC
		CCARM 3A638	–	Human skin	CCARM
	Staphylococcus xylosus	ATCC 29971	–	Human skin	ATCC
	Staphylococcus saprophyticus	ATCC 15305	–	Urine	ATCC
Coagulase-positive	*Staphylococcus aureus*	ATCC 12598	–	Human with septic arthritis	ATCC
		ATCC 29213	–	Human wound	ATCC
		ATCC 6538	–	Human lesion	ATCC
		CCARM 3A170	–	Human skin	CCARM
		CCARM 3A200	–	Human skin	CCARM
		CCARM 3798	–	Human skin	CCARM
		CCARM 3832	–	Human skin	CCARM
		CCARM 3915	–	Human skin	CCARM

++, complete lysis; +, turbid lysis; -, no lysis

KCCM, Korean Culture Center of Microorganisms; KCTC, Korean Collection for Type Cultures; ATCC, American Type Culture Collection; CCARM, Culture Collection of Antimicrobial Resistant Microbes

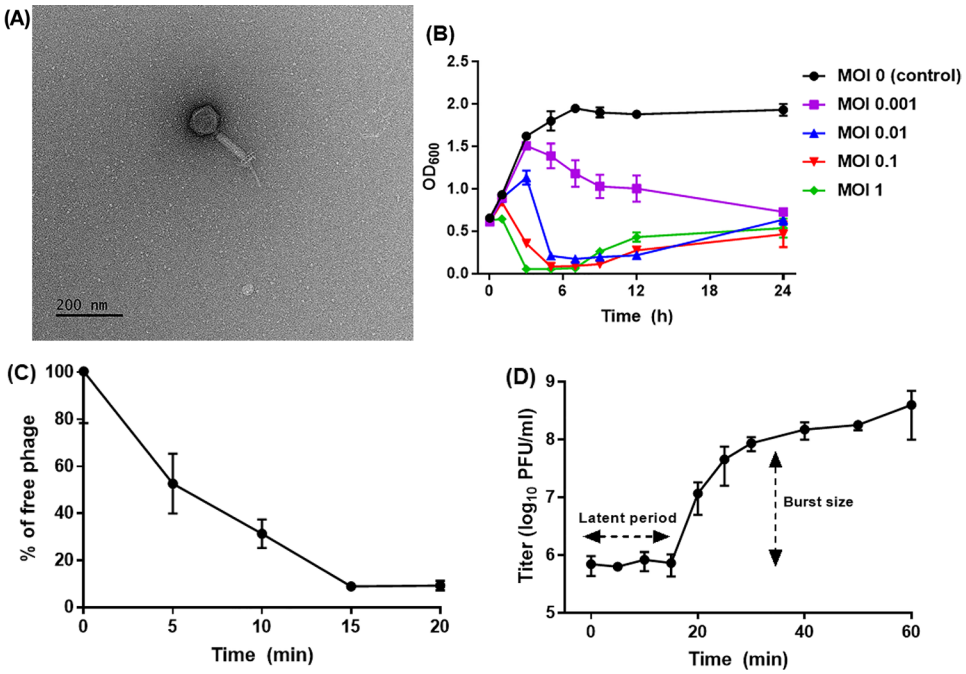

Fig. 1 Biological characteristics of *Mammaliicoccus* phage vB_MscM-PMS3. (A) Transmission electron micrograph of a negatively stained phage virion. Scale bar, 200 nm. (B) The bacteriolytic activity of the phage against the host bacterium, *M. sciuri* SNUDS-18, at various multiplicities of infection (MOIs). (C) Adsorption rate of the phage to *M. sciuri* SNUDS-18 at an MOI of 0.001. (D) One-step growth curve of the phage in *M. sciuri* SNUDS-18. The data represent the mean ± standard deviation (SD) of three independent experiments

the phage as described previously [14]. Adsorption analysis revealed that < 10% of the free phage particles remained in the culture medium after 15 min (Fig. 1C). Therefore, the growth curve was measured after 15 min. The latent period of phage vB_MscM-PMS3 was approximately 20 min, and its burst size was approximately 125 PFU/mL (Fig. 1D). Moreover, phage stability was evaluated after exposure to various organic solvents, temperatures, and pH conditions using a previously described method [14], with slight modifications. Briefly, the phage suspension (~ 10^7 PFU/mL) was incubated with different organic solvents (phosphate-buffered saline, dimethyl ether, chloroform, dimethyl sulfoxide [DMSO], and ethanol [EtOH]) at different temperatures (4, 15, 25, 30, 37, 50, and 60 °C) and pH conditions (pH 3, 5, 7, 9, and 11) for 3 h, and the phage titer was determined using the double-layer agar method. Phage vB_MscM-PMS3 was not affected by organic solvents, such as dimethyl ether and chloroform; however, it did not survive treatment with DMSO and EtOH (Supplementary Fig. S1A). In addition, we tested the phage titers at various temperatures and pH conditions, and the phage maintained stable infectivity under the wide range of these conditions (Supplementary Fig. S1B and C).

Next, the genomic DNA of vB_MscM-PMS3 was extracted and purified as described previously [11]. The whole genome was sequenced via a hybrid approach, using a PacBio RSII System (Pacific Biosciences, Inc., USA) by constructing a 20-kb SMRTbell™ template library (Pacific Biosciences, Inc.) and using a HiSeq X-10 platform (Illumina, Inc., USA) by preparing a DNA library using a TruSeq Nano DNA Library Prep Kit (Illumina, Inc.). Initial assembly of the PacBio reads was performed using HGAP3 [15], and Illumina reads were used to improve the accuracy of the genome sequence using Pilon [16]. Phage termini were analyzed using the PhageTerm online tool [17], and the results indicated that the isolated phage belonged to the direct terminal repeat (DTR) class with 5,334-bp terminal repeats (Supplementary Fig. S2). Open reading frames (ORFs) were identified using the RAST server [18], and putative functions were annotated using the BLASTp and HHpred servers [19, 20]. The presence of tRNA was determined using tRNAscan-SE (v.2.0) [21].

The genome of phage vB_MscM-PMS3 is a 147,811-bp linear dsDNA with 31.25% G+C content. Genome annotation revealed the presence of 206 ORFs and three tRNA genes in the genome (Fig. 2A). The molecular weight and isoelectric point (pI) of the proteins encoded by the ORFs were calculated using ExPASy [22], and transmembrane regions were identified using TMHMM v.2.0 [23]. The results are presented in Supplementary Table S1.

To verify the novelty of phage vB_MscM-PMS3, BLASTn was used to compare its genome sequence with those of other known phages in the GenBank database. This revealed that the *Staphylococcus* phage ZCSS1 (MW430345), belonging to the genus *Sciuriunavirus*, was most similar to our isolated phage, with a sequence identity value of 91.3%; however, the query coverage between the two phages was only 17%. Then, the Virus Intergenomic Distance Calculator (VIRIDIC) [24] was used to calculate the intergenomic similarity between phage vB_MscM-PMS3 and other similar phages, and the results also indicated that the newly isolated phage showed less than 35% nucleotide

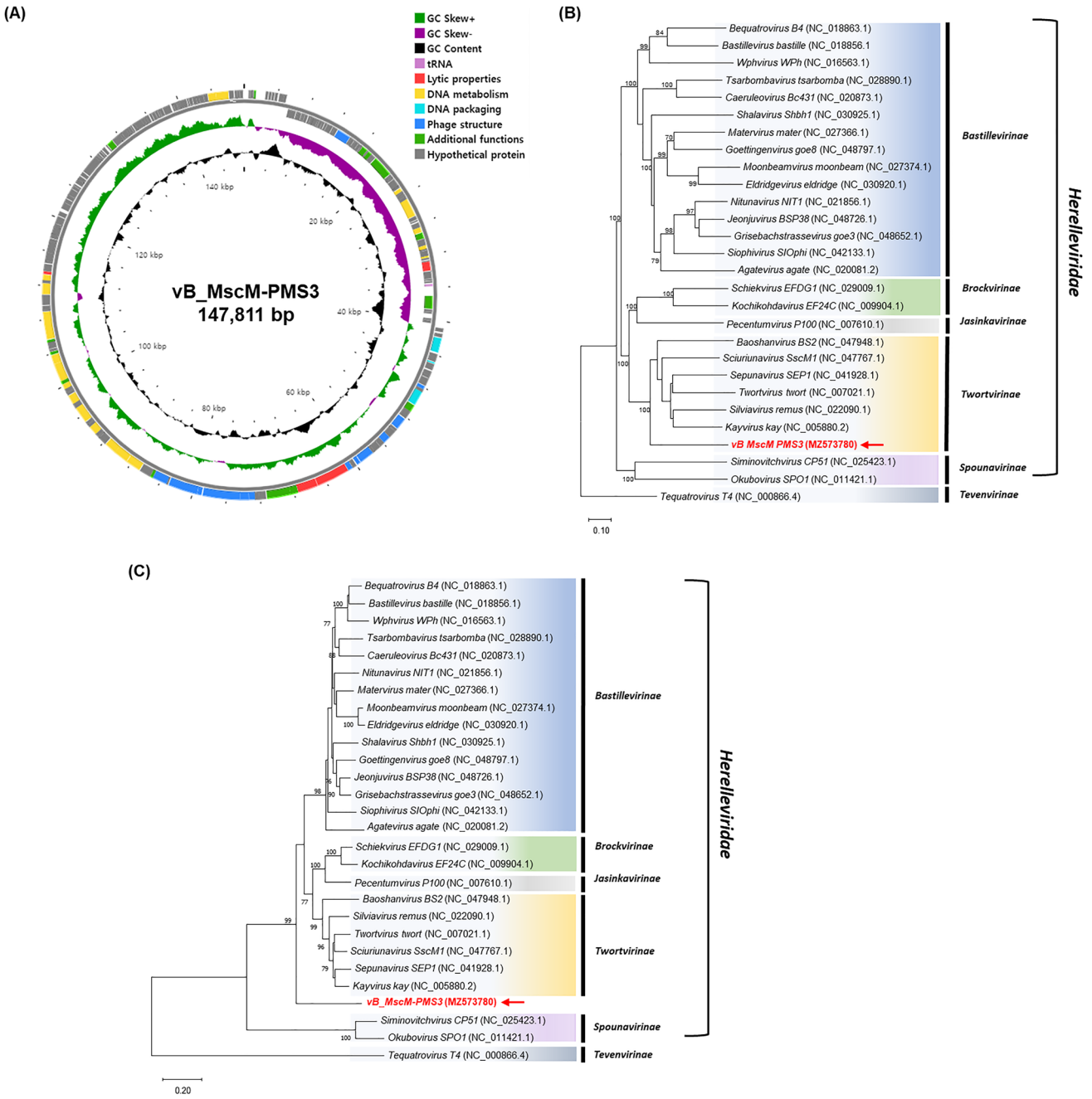

Fig. 2 Genomic characteristics of the *Mammaliicoccus* phage vB_MscM-PMS3. (A) Whole genome map of the phage. Each open reading frame (ORF) is colored according to its putative function. (A and B) Phylogenetic trees based on whole-genome (B) and major capsid protein (C) sequences of the phage vB_MscM-PMS3 and its relatives in the family *Herelleviridae*. The genome of tequatrovirus T4 (NC_000866.4) was used as an outgroup

similarity to other available phage genomes (Supplementary Fig. S3). The genomes of phage vB_MscM-PMS3 and phage vB_SscM-1 (NC_047767), representative of the genus *Sciuriunavirus* [25], were compared and visualized using Easyfig [26] (Supplementary Fig. S4).

To determine the taxonomic position of the isolated *Mammaliicoccus* phage, we generated phylogenetic trees at the genus level by comparing whole-genome and major capsid protein (MCP) sequences of our phage with those of other representative phages in the International Committee on Taxonomy of Viruses database version MSL38. V1 [25]. The Virus Classification and Tree Building Online Resource [27] and MEGA X [28] were used to reconstruct phylogenetic trees by the neighbor-joining method with

1000 bootstrap replicates. In the whole-genome-based phylogeny, phage vB_MscM-PMS3 was most closely related to members of the genus *Sciuriunavirus* of the subfamily *Twortvirinae*; however, this branch was separated from the other members of this subfamily (Fig. 2B). The MCP-based phylogeny revealed that the newly isolated phage could be classified as a member of one subfamily of the family *Herelleviridae*, but it was distinctly separated from the other subfamilies (Fig. 2C).

In conclusion, we isolated phage vB_MscM-PMS3, which exerts a strong lytic effect on *M. sciuri* under various environmental stresses. Analysis of its genome confirmed that phage vB_MscM-PMS3 is distinct from the other members of the subfamily *Twortvirinae* in the family *Herelleviridae*. Therefore, the *Mammaliicoccus* phage vB_MscM-PMS3 represents a new evolutionary lineage and may be classified as a new member of the subfamily *Twortvirinae* in the family *Herelleviridae.*

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00705-023-05940-1.

Author contributions JHK and JEH conceived the idea and designed the experiments. Material preparation and data collection were performed by HK, SYP, SL, and YBK. SGK and SCP helped with the experimental design. The first draft of the manuscript was written by HK, SYP, and SL, and all authors commented on previous versions of the manuscript and read and approved the final manuscript.

Funding This work was supported by the 'Development of Technology for the Biomaterialization of Marine Fishery Byproducts' of the Korea Institute of Marine Science and Technology Promotion [KIMST], funded by the Ministry of Oceans and Fisheries [KIMST-20220128], and also supported by a research grant from the National Institute of Biological Resources [NIBR202322101], funded by the Ministry of Environment of the Republic of Korea.

Data availability The complete genome sequence of phage vB_MscM-PMS3 has been deposited in the NCBI GenBank database under accession number MZ573780.2.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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