BMC Microbiology

RESEARCH

Open Access

The change of long tail fibers expanded the host range of a T5-like *Salmonella* phage and its application in milk

Xiaofeng Zheng[1,2,3,4], Xin Wang[1,3,4], Pei Li[1,3,4], Yu Zhou[1,3,4], Xihui Zhu[1,3,4], Zimeng Hu[1,3,4], Hui Wang[1,3,4], Mianmian Chen[5], Xiang Huo[6,7,8], Yingyu Liu[2*] and Wei Zhang[1,2,3,4*]

Abstract

We engineered novel T5-like bacteriophage (phage) with extended host ranges by editing the long-tail fibers (PB3 and PB4) to combat *Salmonella* Enteritidis. By replacing the long-tail fibers PB3 and PB4 regions of phage PH204 with those from phage SP76, we created phages RPA_{1-3} and RPB_{1-3}, which exhibited expanded host ranges, lysing 54 strains compared to the original 30 strains. These phages retained the biological characteristics of PH204, including temperature, pH stability and adsorption rate. In milk, RPA_{1-3} and RPB_{1-3} inhibited *Salmonella* ZWSA605 growth, reducing bacterial counts to 1.51 log10 CFU/mL and 2.18 log10 CFU/mL after 8 h, respectively. Although the bacteriolytic activity of recombinant phages is lower than that of the parent phage, our findings suggest that these phages hold promise as candidates for future phage biocontrol applications in food.

Keywords *Salmonella*, Homologous recombination, T5-like phages, Host range expansion

Introduction

Milk quality and safety are highly valued by the dairy industry. Specifically, microbial contamination has become a serious problem for raw milk and dairy products, such as cheese and yogurt. *Salmonella* is one of the most common foodborne pathogens found in milk [1]. *Salmonella*, a genus of facultative intracellular pathogens, poses a significant threat to both human and animal health [2]. With over 2600 serotypes, *Salmonella* is a leading cause of gastroenteritis, particularly in vulnerable populations, such as the elderly, infants, and immunocompromised individuals [3]. The majority of disease-causing serotypes belong to *Salmonella enterica* ssp. *enterica*, which encompasses at least 1450 serotypes, including *S.* Enteritidis and *S.* Typhimurium [4, 5]. Contaminated food is a primary source of human infection [6], and increasing antimicrobial use has raised concerns about resistance and multidrug resistance [7].

*Correspondence:
Yingyu Liu
xjlyy1028@163.com
Wei Zhang
vszw@njau.edu.cn
[1]College of Veterinary Medicine, Nanjing Agricultural University, Nanjing 210095, China
[2]College of Veterinary Medicine, Xinjiang Agricultural University, Urumqi 830052, China
[3]The Sanya Institute of Nanjing Agricultural University, Sanya 572024, China
[4]Key Lab of Animal Bacteriology, Ministry of Agriculture, Nanjing 210095, China
[5]College of Animal Science and Technology, College of Veterinary Medicine of Zhejiang A&F University, 666 Wusu Street, Lin'an District, Hangzhou, Zhejiang Province 311300, China
[6]Jiangsu Provincial Center for Disease Control and Prevention, Nanjing 210009, China
[7]Jiangsu Provincial Medical Key Laboratory of Pathogenic Microbiology in Emerging Major Infectious Diseases, Nanjing 210009, China
[8]Jiangsu Province Engineering Research Center of Health Emergency, Nanjing 210009, China

Both typhoid and non-typhoid *Salmonella* pose significant public health threats, ranking among the top 15 drug-resistant bacterial threats in the United States [8]. The rise in multidrug-resistant *Salmonella* isolates has raised concerns about the effectiveness of traditional treatments, specifically the decreased susceptibility to ampicillin, streptomycin, sulfonamide, tetracycline, and chloramphenicol [9]. With the development of new antimicrobial agents, such as phage and probiotics, there is an urgent need to explore alternative treatments for *Salmonella* infections.

Phages are the most abundant biological entities on earth [10], outnumbering bacteria by nearly 10 times, and can be found in diverse environments, including the gastrointestinal tracts of animals and the ocean floor [11]. These viruses have shown promise as low-cost, safe, effective, and rapidly self-replicating antimicrobials for treating various bacterial infections [12]. The successful use of phages in controlling *Salmonella* contamination of milk has been widely reported [13–16]. However, there have been relatively few studies examining the effects of engineered phages on the growth of *Salmonella* in milk.

Phages are utilized as antimicrobial agents because of their specificity and precise targeting of hosts. However, these methods cannot target all species of the same bacteria, presenting a significant limitation against various pathogens. Consequently, major clinical challenges remain associated with the use of phages in treating diverse bacterial infections. To address this, a combination of various phages, or phage cocktails, is necessary to target a wide range of bacterial pathogens. Nonetheless, the diversity in the physical structure, genomic composition, and life cycle of phage cocktails poses regulatory approval challenges [12]. Recently, phage engineering has emerged as a strategy to expand the host range of phages and enhance their ability to kill resistant bacteria. For instance, clinicians successfully treated a patient infected with drug-resistant *Mycobacterium abscessus* using engineered phages. Another transgenic *Staphylococcus aureus* phage was used to treat tibial infections, osteomyelitis and soft tissue infections in mice. In addition, modified phages can be used as antigen delivery carriers to prevent animal diseases [17]. Therefore, it is urgent to expand the host range of phages through genetic engineering for antimicrobial therapy [18]. Phage tails have been reported to contain receptor-binding proteins (RBPs) that determine host range [19]. Modifying these parts could potentially generate phages with an extended host range. Nonetheless, it remains undetermined whether the PB3 and PB4 parts outside the pb1 part are host-range-determining regions of T5-like phages. Efforts have been made to alter the sequence of the long-tail fiber gene to change the host range of specific phages. For instance, in T2 family phages, the gene 38 product (gp38) located at the tip of the long tail fiber determines the host range. Replacing the gene products (gp37 and gp38) in the T2 genome with the gene product from the *E. coli* O157:H7 specific phage PP01 resulted in the recombinant T2 phage T2ppD1, which exhibited the same host range as the donor phage PP01. However, T2ppD1 showed weaker binding affinity to *E. coli* O157:H7 compared to PP01 and produced turbid plaques on a lawn of *E. coli* O157:H7 cells [20]. Similarly, in T4-like phages, the C-terminal region of gp37 in the T4 family may be crucial for receptor specificity. Exchanging gene 37 fragments between the T4-like phage WG01 and another T4-like phage QL01 expanded the host range of the recombinant phage WQD for infecting *Escherichia coli* through homologous recombination [21, 22]. Jing Zhang et al. demonstrated that editing the long tail fiber (pb1) of T5-like phages could produce engineered phages with an expanded host range, indicating that the pb1 component may be a major determinant of host specificity for some T5-like phages [23]. Furthermore, studies have identified the outer membrane transport protein *FhuA* as the receptor for T5 phages, with the receptor-binding protein located at the tip of the phage's long tail, consisting of the PB3 and PB4 parts [24]. In the T5 family of phages, the PB3 and PB4 genes form the distal tail tip complex and play a crucial role in the flexible tail assembly process. Specifically, the lower parts of PB4 and PB5 constitute the tail tip straight fiber, while the upper part of PB4 connects to the tail tip cone, which is composed of PB3.

The host range of the T5-like phage PH204 isolated in this study differs significantly from that of another phage, SP76 isolated in a previous study. While SP76 can lyse 64 strains of *Salmonella*, PH204 can lyse only 30 strains. Despite these differences, the two phages share high genomic homology, making them suitable models for studying the factors influencing host range. We modified and expanded the host range of PH204 through homologous recombination and studied the effect of the long tail fiber on the host range by measuring the host range, biological characteristics, gene passage stability of the recombinant phage, and their efficacy in milk. The findings of this study could enhance engineered phage diversity and provide guidance for selecting engineered phage candidates for future phage preparations, as well as evaluating their effectiveness in controlling *Salmonella* contamination in milk.

Materials and methods

Bacteria strains, phages, and medium

Bacterial strains

Salmonella Enteritidis ZWSA337, isolated from a poultry farm in Hubei Province, was used as the host bacterium to isolate phage SP76. ZWSA337 had been previously

isolated and preserved. Similarly, *Salmonella* Enteritidis ZWSA605, isolated from a poultry farm in Hainan Province, was used to isolate phage PH204. ZWSA605 had also been previously isolated and preserved. All strains were cultured overnight in LB broth (Beijing Biotek Biotechnology Co., LTD, China) at 37 °C.

Phage isolation, purification and propagation

Phages SP76 and PH204 were isolated from sewage collected from livestock and poultry farms in Jiangsu Province, China. A mixture of 200 µL of filtrated sewage and phage was combined with 100 µL of logarithmic phase *Salmonella* ZWSA337 and ZWSA605 cultures (OD600 = 0.4, 2-2.9 × 10^8 CFU /mL), then mixed with semi-solid LB agar (Beijing Biotek Biotechnology Co., LTD, China) and plated on LB agar. The plates were incubated at 37℃ for 8 h [25]. Plaques with clear, large spots and well-defined boundaries were selected for phage isolation. Using a sterilized 1µL pipette, a single plaque was isolated and transferred into 1 mL of SM buffer (Guangzhou Luofake Tong Enterprise Management Co., LTD, China). The mixture was vortexed thoroughly. Then, 100 µL of the mixture was taken for a tenfold serial dilution from 10^{-1} to 10^{-6}, with each dilution spread onto double agar plates to obtain single plaques, thereby purifying the phage. This process was repeated five times. Finally, the phage suspension was filtered and sterilized using a 0.22 µm microporous membrane (Merck Millipore, Germany). The purified phage suspensions were stored in 25% glycerol at -20℃ [26]. After mixing the phage and host bacteria in a 1:1 ratio, the mixture was spread on double agar plates and incubated for 8 h. Subsequently, 5 mL of SM buffer was added to each plate, which was then shaken at 4 °C for approximately 2 h at a rotating speed of 50–60 rpm. The liquid from the plates was drawn into a sterile EP tube, centrifuged at 5,000 × g for 20 min, and the supernatant was transferred to a new sterile EP tube. The bacteria were filtered out using a 0.22 µm filter, and the filtrate was collected.

Electron microscopy

To photograph phages with electron microscopy more clearly, purified and filtered phages were evenly smeared on the surface of the copper grid and stained negatively with 2% phosphotungstic acid [27]. Phage electron micrographs were observed and visualized using an HT-7700 transmission electron microscope (Hitachi, Japan) with an accelerating voltage of 80 kV.

Genome sequencing, assembly, and annotation

We extracted genomic DNA from phages PH204, RPA_{1-3}, and RPB_{1-3} using the Fast Pure phage DNA Separation Mini-Kit (ZOMANBIO). The genome of phage SP76 had been previously annotated [28]. We performed genome sequencing (300 bp reads, PE150) using the Illumina HiSeq system (Illumina, San Diego, CA) [29]. The sequencing reads were de novo assembled using Unicycler [30]. The entire genome of phages SP76 and PH204 were annotated with Prokka 1.14 [31] and RAST server (https://rast.nmpdr.org/).

Phylogenetic tree and comparative genomics analysis

To further analyze the relationship between phages PH204 and SP76, we downloaded 46 highly homologous phage genomes from NCBI (https://blast.ncbi.nlm.nih.gov/). We constructed a phylogenetic tree using MEGA (version 11) and visualized it with iTOL (https://itol.embl.de/itol.cgi). Additionally, we employed Easyfig (version 2.2.3) [32] to compare the sequence arrangements of PH204 and SP76 genomes.

Prediction of the 3D structures of fiber genes Peg158, Peg159, Peg162, and Peg163

Using the SWISS - MODEL server (https://swissmodel.expasy.org/), we predicted the tertiary structure of the Peg158, Peg159, Peg162, and Peg163 genes. To do this, we accessed the SWISS-MODEL website, created a new modeling project, and uploaded the amino acid sequences of Peg158, Peg159, Peg162, and Peg163. After the template search was completed, the templates were ranked based on the expected quality of the final model, estimated by a GMQE score close to 1 and the QMEAN scoring function [32–34].

Comparative genomic analysis of long-tail fiber genes

We conducted a comparative genomic analysis of the caudal fibers of two T5-like phages, PH204 and SP76, focusing on the corresponding genes Peg158, Peg159, Peg162, and Peg163. The amino acid sequences of Peg158, Peg159, Peg162, and Peg163 were uploaded to the Clustal Omega online services website (https://www.ebi.ac.uk/Tools/msa/clustalo/). Using the default parameter settings, we submitted the sequences for alignment. The result indicated common sequences represented by "*" below, while non-homologous regions were denoted by "–". The multi-sequence alignment results were then visualized using Jalview software.

Construction of recombinant plasmid

We replaced the TFP fragment in PH204 (Peg158 and Peg159) with the corresponding fragment from SP76 (Peg162 and Peg163) (Fig. 2) using a modified protocol [21]. We amplified the SP76 Peg162 and Peg163 fragments, as well as upstream and downstream homologous arm fragments, and pUC118-AMP plasmid fragments using PCR with primers listed in Table 1. The PCR reaction procedure involved an initial denaturation at 94℃ for 5 min, followed by 30 cycles of amplification with the

Table 1 Primers used for constructing the plasmids for alteration of phages

Target	Primer	Primer sequence (5′–3′)
RPA	RPA-1 F	caggtcgactctagaggatccGTTAACGAAGTTAAGG-TATCTGCTCAA
	RPA-1R	tttcttcatAAACTTTCCCGTAAATTTAATGAGATG
	RPA-2 F	cgggaaagtttATGAAGAAAATTCTTGACAGTGCAA
	RPA-2R	caTTAAATAGATGCCCAATCCACAGA
	RPA-3 F	gattgggcatctatttaaTGATTTCGAATAATGCACCAGC
	RPA-3R	tatgaccatgattacgaattcAAATTTAATAG-TAGAACCTAAAGCACCAA
RPB	RPB-1 F	caggtcgactctagaggatccCCTTATATTGTGCAG-CAACTAGAAACA
	RPB-1R	TAAATGGATGCCCACTCCACA
	RPB-2 F	gtggagtgggcatccatttaATGATTTCGAATAATG-CACCAGC
	RPB-2R	cagtactcattatTTACCTTATATGGATATACCCAC-TATTTAGG
	RPB-3 F	aaggtaaATAATGAGTACTGAAAATAGAGT-TATAGACTTAGTAA
	RPB-3R	tatgaccatgattacgaattcAAACTTACTAGCAG-TAGCTTTAGTATCATCA
M13	F	CGCCAGGGTTTTCCCAGTCACGAC
	R	AGCGGATAACAATTTCACACAGGA

following steps: 94℃ for 30 s, 55℃ for 30 s, and 72℃ for 90 s. The process concluded with a final extension at 72℃ for 10 min. The fragments were then spliced together using fusion PCR, resulting in two recombinant plasmids, pUC-RPA$_{1-3}$ and pUC-RPB$_{1-3}$. These plasmids were generated through double digestion of the pUC118 plasmid with BamHI and EcoRI restriction endonuclease enzymes.

Construction and isolation of recombinant phage

We performed homologous recombination and modification of the phage using previous methods [21]. Plasmids pUC118-RPA$_{1-3}$ and pUC118-RPB$_{1-3}$ were transformed into *Salmonella* ZWSA605 and cultured in LB medium with 50 μg/mL ampicillin at 37℃ until an OD_{600} of 0.4 was reached. Phage PH204 was then added and incubated for 8 h. The phage filtrate was filtered, mixed with *Salmonella* ZWSA605 and 0.5% agar, poured into an agar plate, dried naturally, and incubated at 37℃ for 8 h to obtain plaques. A single plaque was picked using a sterilized 1 μL pipette tip and placed into 1 mL of SM buffer, vortexed to mix well. Then, 100 μL of this mixture was serially diluted from 10^{-1} to 10^{-6}, spread on double-layer agar plates, and incubated to grow single plaques, thus purifying the phage. The target fragment size was detected using the corresponding primers listed in Table 1.

Host range analysis

To evaluate the phage host range, 97 *Salmonella* strains were used. The host profiles of recombinant phages RPA$_{1-3}$ and RPB$_{1-3,}$ and their parental phages SP76 and PH204, were determined using the drop method [35]. Logarithmic phase *Salmonella* cultures were mixed with 0.5% LB agar and spread onto plates. Phage suspensions (5 μL, approximately 10^9 PFU /mL) were then dropped onto each plate, dried, and incubated at 28 °C for 12 h. Plaques formed on 64 host strains by parental phage SP76 were observed, while plaques formed on 30 host strains by parental phage PH204 were observed. Additionally, recombinant phages RPA$_{1-3}$ and RPB$_{1-3}$ formed plaques on 24 host strains that were not lysed by PH204. These 24 host strains served as markers for successful recombinant phage screening.

Multiplicity of infection

We determined the optimal MOI for phages SP76, PH204, RPA$_{1-3}$, and RPB$_{1-3}$ using a modified standard protocol [36]. Phage dilutions ranged from 10^4 to 10^9 PFU /mL. For each phage, we mixed 100 μL of logarithmic phase host bacteria culture (ZWSA337 for SP76, ZWSA605 for PH204, RPA$_{1-3}$, and RPB$_{1-3}$) with 4800 μL of LB medium and incubated overnight at 37℃ and 180 rpm. The culture was centrifuged at 5,000 × g, centrifuged for 10 min, the supernatant was collected and diluted, and the phage titer was determined using the double-layer plate method. The sample with the highest phage titer was considered to have the optimal MOI, calculated using the formula: PFU/mL = number of plaques × dilution factor × 10.

Thermal and pH stability tests

We assessed the thermal and pH stability of the phages using a modified protocol [37, 38]. For thermal stability, 1 mL of phages (1×10^8 PFU /mL) was incubated at various temperatures (4 °C to 80 °C) for 1 h. For pH stability, 100 μL of phage solution (1×10^8 PFU /mL) was added to 900 μL of SM solution (pH 1–12) and incubated at 37℃ for 1 h [39]. After each incubation period, the phage titers were determined using the double-layer plate method.

One-step growth curve

We determined the latent period and burst size of phages PH204, RPA$_{1-3}$, RPB$_{1-3}$, and SP76 using the one-step growth curve method [40]. Logarithmic *Salmonella* cultures (1×10^8 CFU /mL, OD = 0.4) were mixed with 1×10^7 PFU /mL phage suspensions at 37℃. After 10 min adsorption, centrifugation, and resuspension in PBS, the samples were incubated at 37℃ and 180 rpm in 10 mL LB medium. Phage titers were determined every 10 min (up to 180 min) using the double-layer agar plate method [41].

Phage adsorption assay

We mixed 500 µL of fresh host bacteria ZWSA026 (OD = 0.4, 1×10^8 CFU /mL) with 500 µL of diluted phage (1×10^6 PFU /mL) for the experimental group, and with 500 µL of PBS for the control group. After incubating at 37℃ for 10 min and centrifugation at 5000 g for 1 min, we calculated the phage titer in the supernatant using the double-layer plate method. The adsorption rate was calculated as follows [38]:

Adsorption rate (%)=(Initial phage titer-Tested phage titer)/ Initial phage titer ×100.

Propagating stability of recombinant phage RPA_{1-3} and RPB_{1-3}

To evaluate the propagating stability of recombinant phages RPA_{1-3} and RPB_{1-3}, we collected samples from the 1th, 10th, and 20th generations of both phages. PCR amplification of Peg162 and Peg163 was performed using M13F /M13R primers, and the amplified PCR fragments were sent to Nanjing Jinsirui Co., LTD for sequencing. By comparing the sequencing results, we evaluated the genetic stability of the recombinant phages across different generations.

Efficacy of phages on inhibiting *Salmonella* in the planktonic state

The lytic capacities of phages RPA_{1-3}, RPB_{1-3}, PH204, and SP76 were evaluated by measuring the OD_{600} values of planktonic *Salmonella* ZWSA605 and ZWSA337 at MOIs of 0.1, 0.01, and 0.001. We mixed 20 mL of host bacteria (2×10^8 CFU /mL) with 200 µL of diluted phage lysate ($2 \times 10^7 - 2 \times 10^5$ PFU /mL) and incubated the mixture at 180 rpm at 37℃. The control group consisted of host bacteria and fresh LB medium. Record OD_{600} values of different MOIs at an interval of one hour. All experiments were performed in triplicate.

Inhibitory effect of phage on milk model

Pasteurized milk from Nanjing Weigang Dairy Co., LTD was purchased locally and stored at room temperature. To simulate bacterial contamination, 8 mL of milk containing phages RPA_{1-3}, RPB_{1-3}, and PH204 (10^8 PFU / mL) or SP76 (10^8 PFU /mL) was mixed with 1 mL of *Salmonella* ZWSA605 (10^6 CFU /mL) or ZWSA337 (10^6 CFU /mL), resulting in a final concentration of 10^5 CFU / mL. The mixtures were incubated at 20℃ or 4℃ for 2, 4, 6, and 8 h [15, 42]. At each time point, 100 µL of the liquid was sampled and serially diluted with PBS for counting of viable bacteria. A blank control was used, where PBS buffer replaced phage.

Statistical analysis

Graphs were produced using GraphPad Prism 8. For the phage RPA_{1-3} and RPB_{1-3} experiments in milk models, the Student's t-test was used to compare the blank control group with the phage treatment group. Differences with $P \leq 0.05$ were considered significant.

Results

Morphology of phages

Transmission electron microscopy (TEM) images of the two phages are shown in Fig. 1. Both phages exhibited

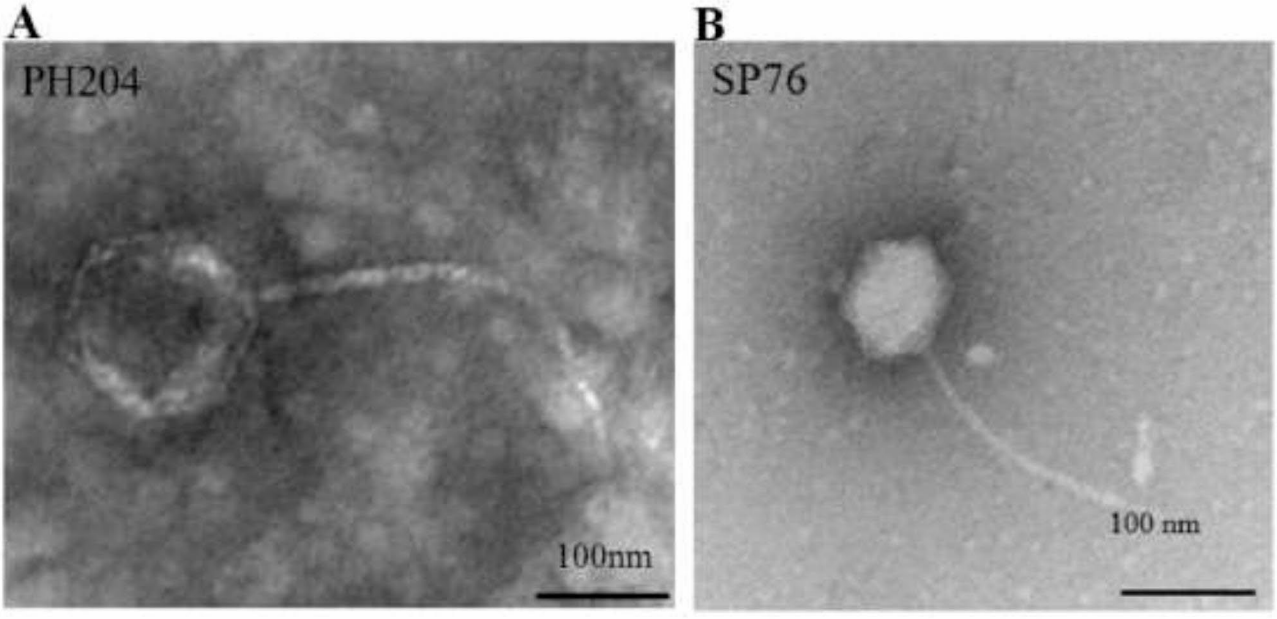

Fig. 1 TEM images of phage PH204 (**A**) and SP76 (**B**). Phage PH204 and SP76 had a polyhedral head, with a head of approximately 80 ± 2 nm and a tail of approximately 230 ± 2 nm

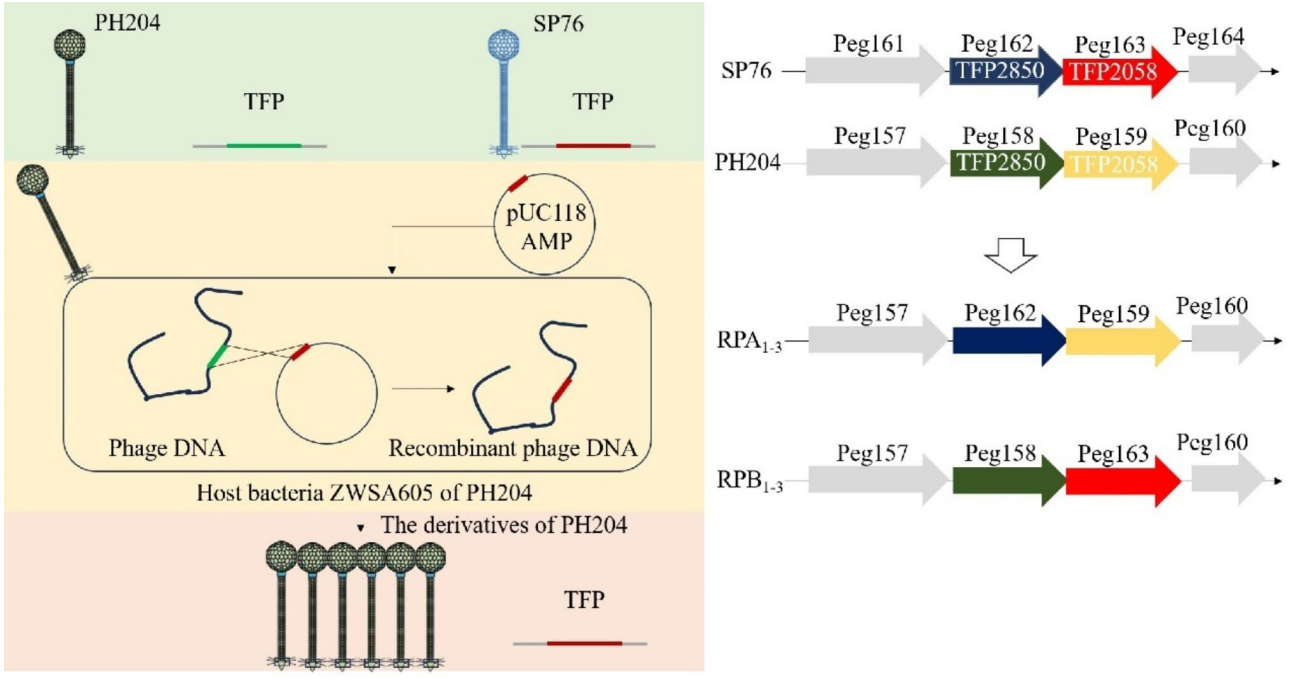

Fig. 2 Basic principle of homologous recombination of PH204 and a draft of cauda protein gene

characteristic features of the *Demerecviridae* family, including polyhedral heads approximately 80 ± 2 nm in diameter and long, non-shrinkable tails around 230 ± 2 nm in length.

Genomic and phylogenetic tree analysis of phage PH204 and SP76

The complete genomes of phages PH204 and SP76 were sequenced and submitted to GenBank under accession numbers OR492254.1 and MW206381.1, respectively. Genome sizes were 110, 316 bp for PH204 and 111, 639 bp for SP76, with average GC contents of 39% and 39.9%, respectively.

Phylogenetic analysis of PH204 and SP76, along with 46 other T5-like phages, revealed that they belong to the T5-like phage group (Fig. 3). Phage PH204 is closely related to phages SE11, siur09, and BB1, which belong to the *Caudoviricetes*, *Demerecviridae*, and *Tequintavirus* species, respectively. Conversely, phage SP76 is closely related to phages 8sent1748 and vB Eco mar003J3, which are also members of the *Caudoviricetes*, *Demerecviridae*, and *Epseptimavirus* species.

Comparison of long-tail fibers from phages PH204 and SP76

The PB3 and PB4 sequences of phages SP76 and PH204 were compared to those of *Salmonella* phage vB_Sen-E22, another member of the *Tequintavirus* genus. Our analysis revealed high homology among the three phages, with consistent amino acid sequences in the PB3 and PB4 regions (Supplementary materials FIG. S1). Easyfig comparison analysis showed that over 78% of the protein sequences in the genomes of PH204 and SP76 were similar (Fig. 4A). BLASTn comparison results showed a genome homology of 94.58% and a query coverage rate of 72%. These findings suggest a high level of genomic similarity between phages PH204 and SP76.

The long-tail protein sequences of phages PH204 and SP76 each contain two open reading frames (ORFs) (Fig. 4B). Comparison of the amino acid sequence of Peg158, Peg159, Peg162, and Peg163 revealed sequence homologies of 89.67% and 95.62%. These variations may contribute to the distinct host ranges of phages PH204 and SP76.

Three-dimensional structural modeling of Peg158, Peg159, Peg162, and Peg163

The three-dimensional structures of the PB3 (Peg158 and Peg162) and PB4 (Peg159 and Peg163) proteins of phages PH204 and SP76 were predicted through homology modeling. Following template selection based on the Global Model Quality Estimate (GMQE), the top-ranked templates were employed for predictive protein modeling. As depicted in Fig. 5A and B, the GMQE values for the Peg158 gene of PH204 and the Peg162 gene of SP76 were 0.83 and 0.84, respectively. Similarly, the GMQE values for the Peg159 gene of phage PH204 and the Peg163 gene of SP76 were 0.85 and 0.86, respectively (Fig. 5C and D).

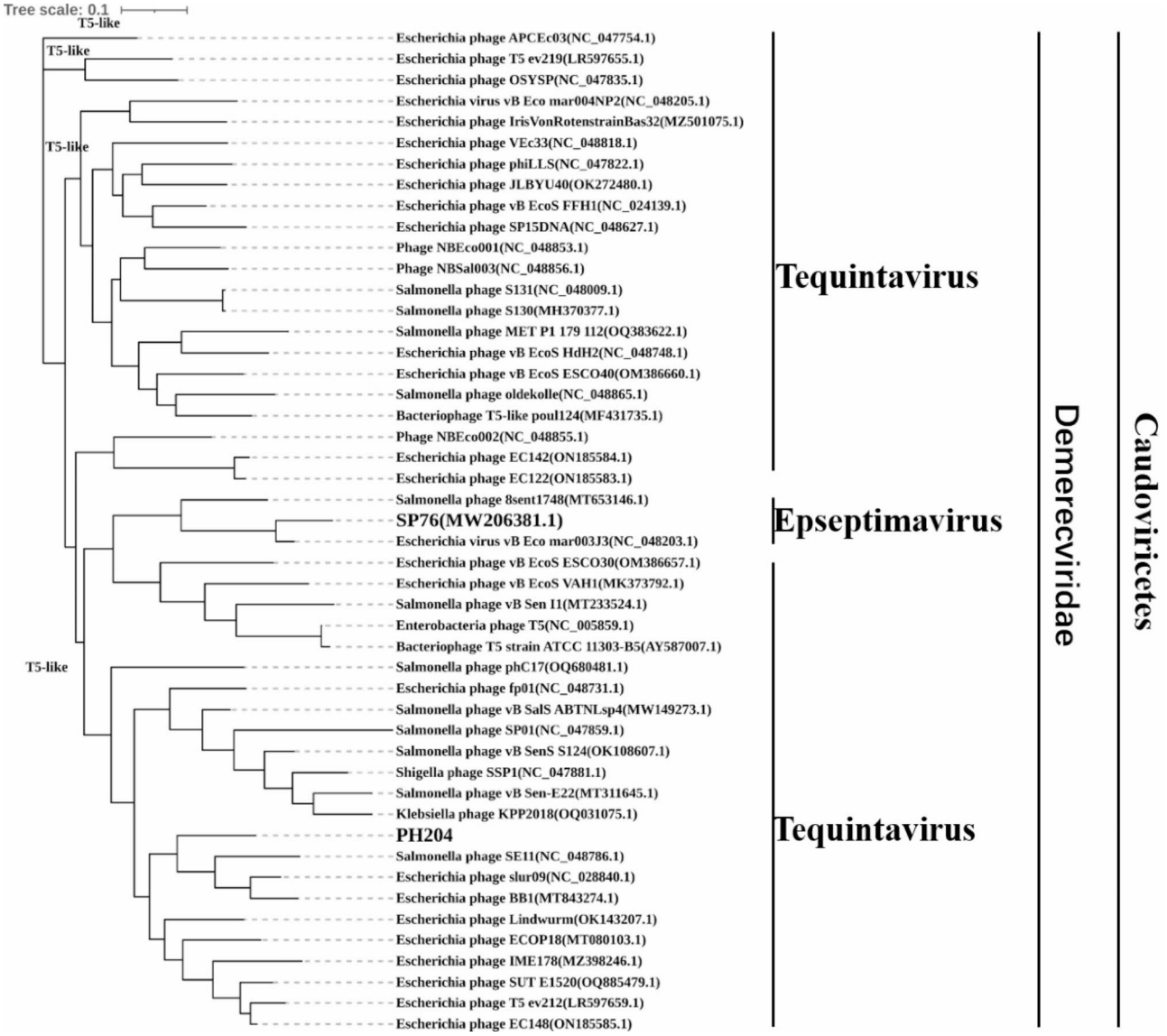

Fig. 3 Systematic genomic analysis of phages SP76 and PH204 at the nucleotide level. The outer layers of the tree (from right to left) represent class, family, genus, and species. The scale represents the approximate number of gene clusters determined by genetic distance

Isolation of recombinant phages

The Peg162 and Peg163 genes encoding TFP in phage SP76 were selected to replace the corresponding DNA segments of the Peg158 and Peg159 genes encoding TFP in PH204. Two recombinant phages, RPA_{1-3} and RPB_{1-3}, were isolated from *Salmonella* ZWSA337 by plaque purification and PCR identification. After 12 h incubation at 28℃, the recombinant phages formed circular lytic areas (0.1 cm) on *Salmonella* ZWSA605 and ZWSA337 without visible halos (Fig. 6A and D). However, plaques on *Salmonella* ZWSA605 were more transparent (Fig. 6E and F). Whole genome sequencing confirmed that RPA_{1-3} and RPB_{1-3} originated from PH204, with Peg162 and Peg163 gene segments from SP76.

Host range analysis of phages PH204, SP76, RPA_{1-3} and RPB_{1-3}

The host range of phages PH204, SP76, and their two derivatives was investigated using 97 *Salmonella* strains (Supplemental Table 1). The results are presented in Fig. 7. Among the 97 strains, phages PH204 and SP76 were able to lyse 30 and 64 strains, respectively. Notably, the chimeric phages RPA_{1-3} and RPB_{1-3} exhibited a broader host range compared to phage PH204, each capable of lysing 54 strains.

Multiplicity of infection

MOI of phages serves as a crucial bioindicator for quantifying phage input and output. Lowering the MOI can decrease the costs of phage production, thereby

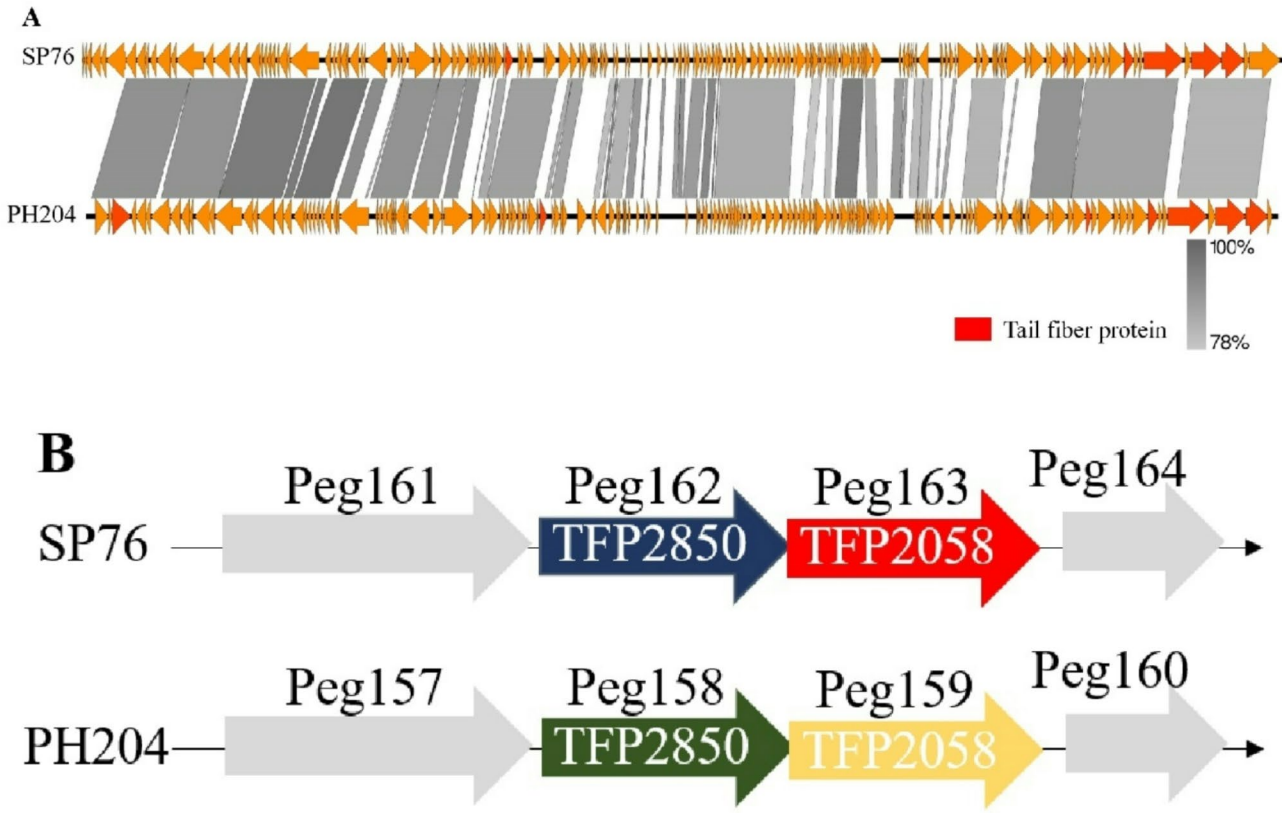

Fig. 4 Comparison of T5 phages SP76 and PH204 tail fibers using Easyfig. (**A**) Whole-genome collinear comparison of SP76 and PH204, with predictive tail fiber genes heightened in red. (**B**) PB3 and PB4 regions of SP76 and PH204 each consist of two ORFs

facilitating the large-scale production and application of phage-based products. When the MOI was 0.0001, the titer of SP76 was the highest, indicating that the optimal infection multiplicity for SP76 was 0.0001. Phages PH204 and RPA_{1-3} had the highest titer at an MOI of 0.1, making their optimal infection multiplicity 0.1. The titer of bacteriophage RPB_{1-3} was highest at an MOI of 0.001, thus its optimal infection multiplicity was 0.001 (Fig. 8A).

Thermal and pH stability

The thermal stability test results (Fig. 8B) show that RPA_{1-3} and RPB_{1-3} remained stable across various temperatures, with active particles ranging from 76.6% at 4℃ to 50℃ for 1 h, similar to parent phages PH204 (90.8%) and SP76 (68.9%). Notably, at 60℃, PH204, RPA_{1-3}, and RPB_{1-3} particles remained active(>21.6%), whereas SP76 particles were less than 1% active.

The pH stability results (Fig. 8C) demonstrate that RPA_{1-3} and RPB_{1-3} have similar stability to PH204 and SP76, with a survival rate exceeding 76.7% within a pH range of 4–11. However, all four phages failed to survive under extremely acidic (pH = 1,2) or highly alkaline (pH = 12) conditions.

One-step growth curve

The one-step growth curves of the chimeric phages RPA_{1-3} and RPB_{1-3}, along with their parent phages PH204 and SP76, are shown in Fig. 8D. The latent period for all phages was approximately 30 min. The burst times for phages RPA_{1-3}, PH204, RPB_{1-3}, and SP76 were 20 min, 30 min, 40 min, and 20 min, respectively. Following an infection period of 50 min, 60 min, 70 min, and 90 min, phages RPA_{1-3}, PH204, RPB_{1-3}, and SP76 entered the rest phase successively, with burst sizes of 20 PFU, 20 PFU, 37 PFU, and 31 PFU per cell, respectively.

Adsorption rates of phages PH204, SP76, and recombinant phages

To evaluate the adsorption properties of phages PH204, SP76, RPA_{1-3}, and RPB_{1-3}, we selected a *Salmonella* strain ZWSA026 based on its susceptibility to these phages and measured their adsorption rates, as shown in Fig. 9. The adsorption rate of parental phages PH204 and SP76 were 31.47% and 46.47%, respectively. In contrast, RPB_{1-3} had a significantly slower ($P<0.05$) adsorption rate (25.3%) compared to PH204 (31.47%). RPA_{1-3} had an adsorption rate of 23.8%.

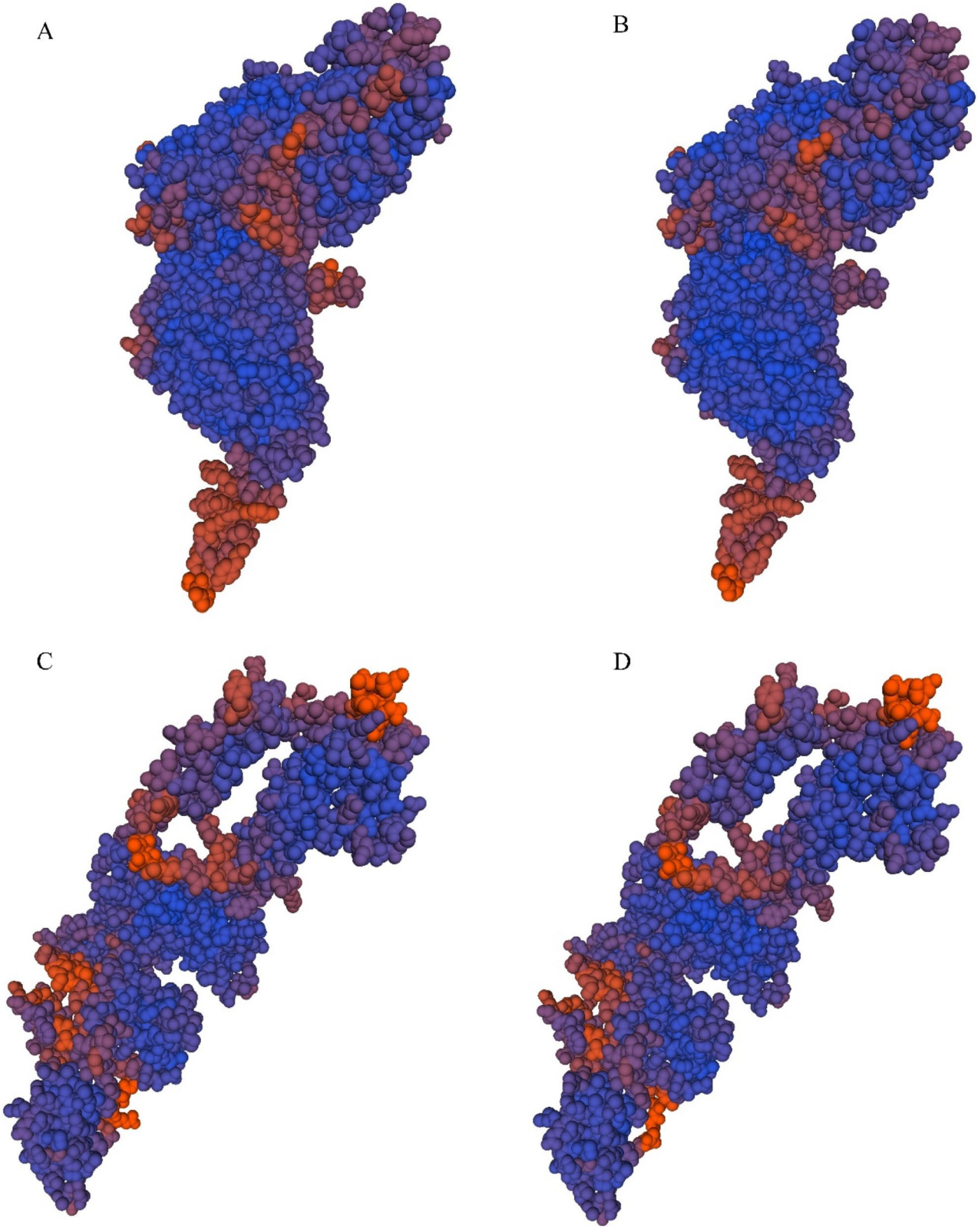

Fig. 5 SWISS-MODEL was used to predict the amino acid three-dimensional structure model corresponding to Peg158, Peg162, Peg159, and Peg163 genes. (**A**-**D**) Target protein structure of PH204 and SP76 genes, with quality indicated by color coding (blue: high, red: low)

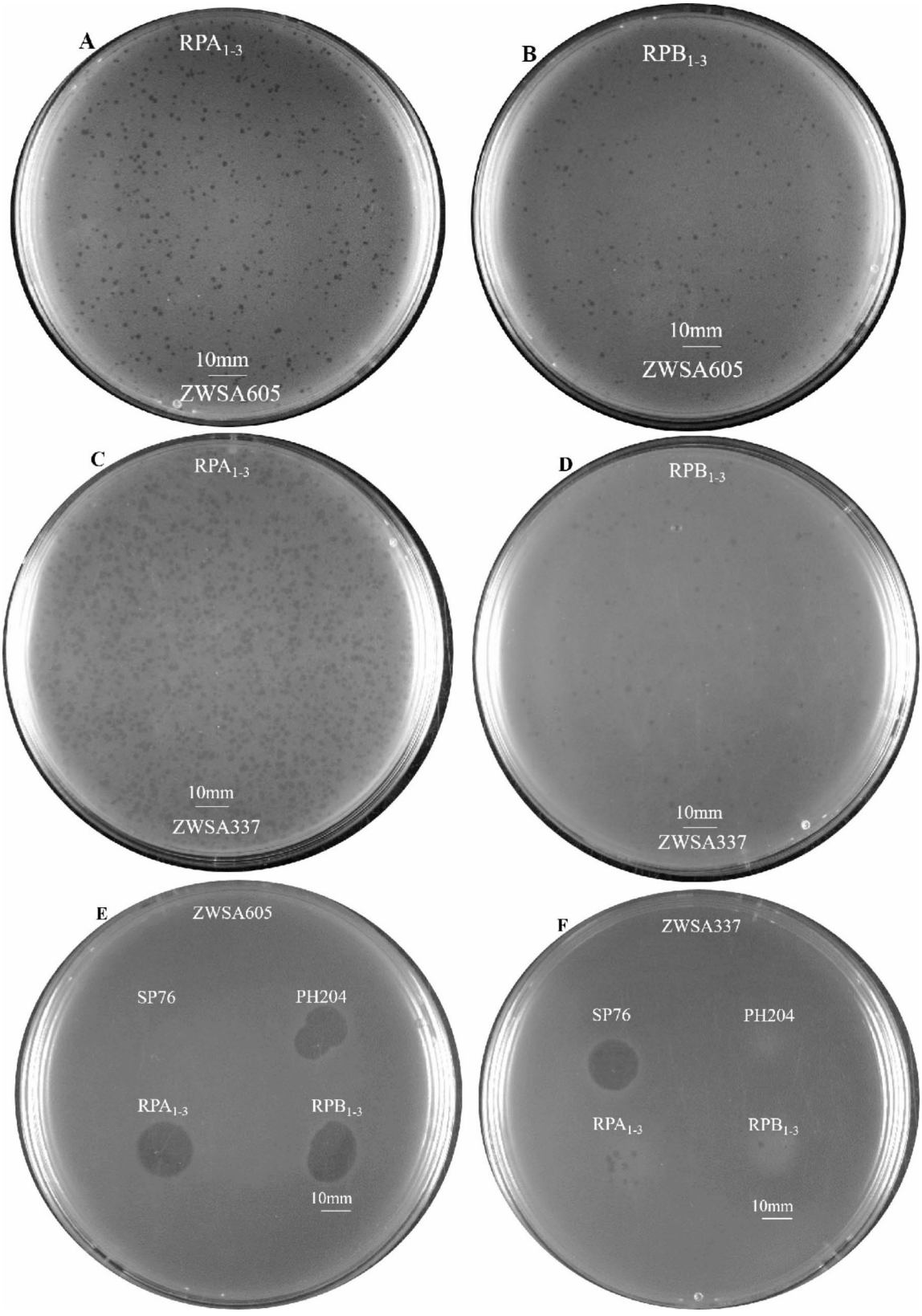

Fig. 6 Characteristics of recombinant phages. (**A**-**B**) Morphology of phages RPA_{1-3} and RPB_{1-3} on host bacteria ZWSA605 on LB double-layer plate. (**C**-**D**) Morphology of phages RPA_{1-3} and RPB_{1-3} on host bacteria ZWSA337 on LB double-layer plate. (**E**-**F**) Lytic ability of phages SP76, PH204, RPA_{1-3}, and RPB_{1-3} on strains ZWSA605 and ZWSA337

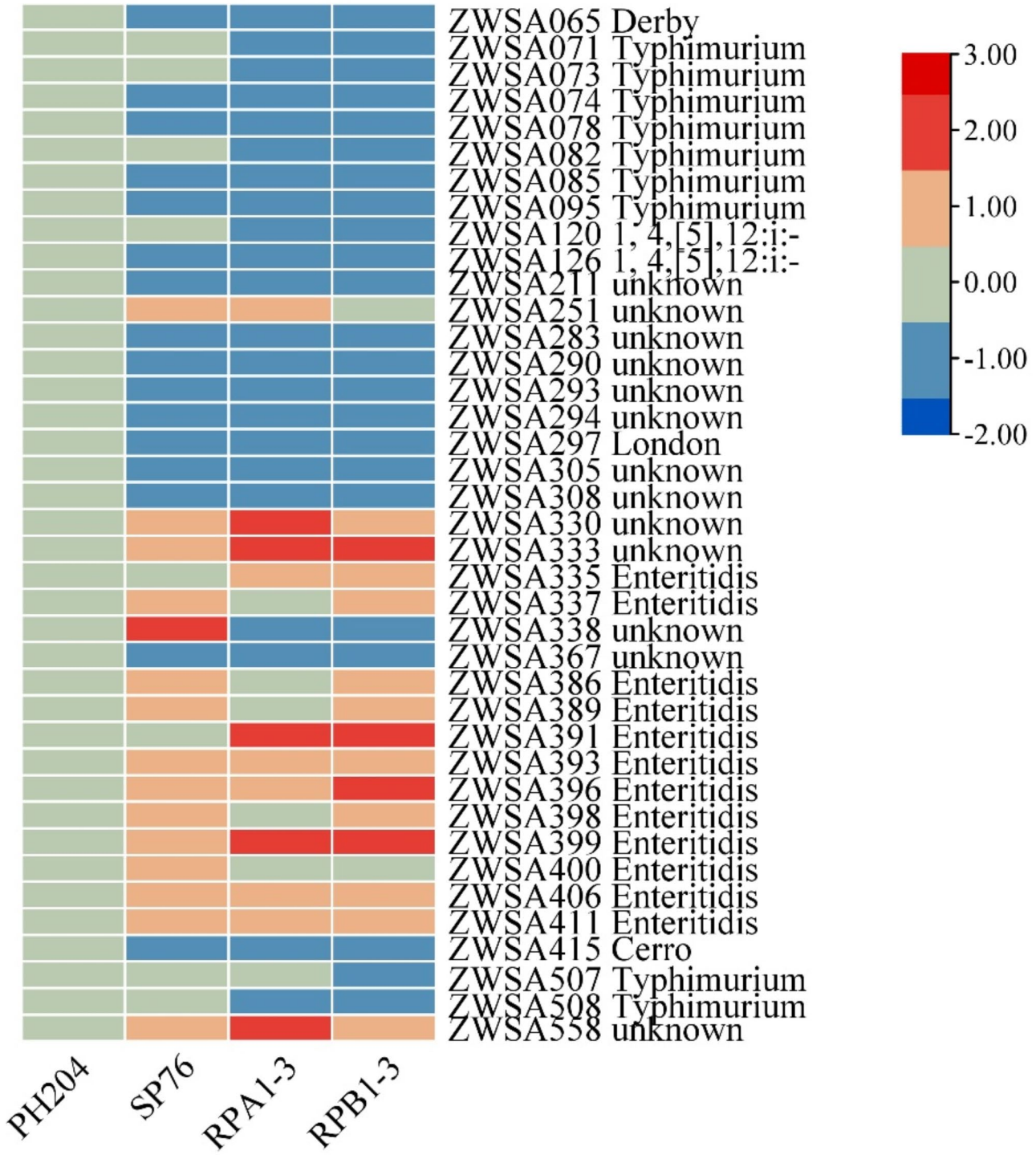

Fig. 7 Comparison of the host range of phages PH204, SP76, RPA_{1-3}, and RPB_{1-3} using the EOP values. Only 39 strains are shown in the image because the other 58 strains are sensitive to these phages. The gray colour (EOP<0) indicates that the phage has no EOP value, meaning it has no activity to lyse *Salmonella*

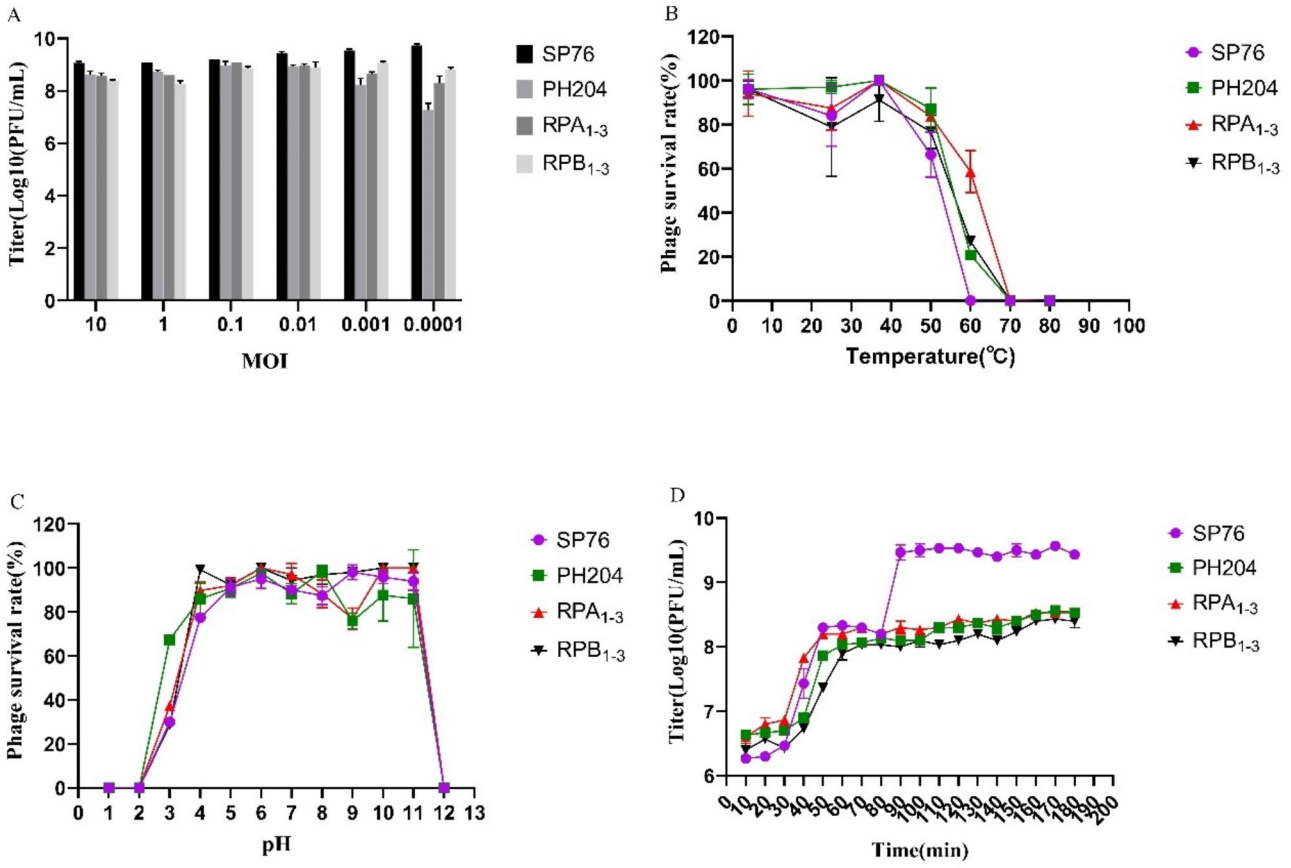

Fig. 8 Biological characteristics of recombinant phages RPA_{1-3}, RPB_{1-3} and their parent phages SP76 and PH204. (**A**) Optimal multiplicity of infection of recombinant phages RPA_{1-3}, RPB_{1-3}, and their parent phages SP76 and PH204. (**B**) Temperature stability of recombinant phages RPA_{1-3}, RPB_{1-3}, and their parent phages SP76 and PH204. (**C**) pH stability of recombinant phages RPA_{1-3}, RPB_{1-3} and their parent phages SP76 and PH204. (**D**) One-step growth curves of recombinant phages RPA_{1-3}, RPB_{1-3}, and their parent phages SP76 and PH204

Propagating stability of recombinant phages RPA_{1-3} and RPB_{1-3}

The Peg162 and Peg163 genes of RPA_{1-3} and RPB_{1-3} remained unmutated in the 1th, 10th, and 20th generations, indicating that phages RPA_{1-3} and RPB_{1-3} are genetically stable.

Lysing capacity of recombinant phages against *Salmonella* in planktonic form

Recombinant phages RPA_{1-3}, RPB_{1-3} and parent phage PH204 effectively inhibited the proliferation of *Salmonella* ZWSA605. The OD_{600} value of the host bacteria in the untreated group continued to increase within the first 12 h, while the OD_{600} values of the phage treatment groups (MOI 0.1, 0.01, and 0.001) initially increased and then decreased (Fig. 10A-C). Compared to the untreated group, phage RPA_{1-3} treatment groups (MOI of 0.1, 0.01, and 0.001) reduced OD_{600} values by 3.58, 3.55, and 3.35, respectively, at 12 h (Fig. 10A). Similarly, phage RPB_{1-3} treatment groups (MOI of 0.1, 0.01, and 0.001) decreased the OD_{600} value by 1.95, 1.87, and 1.99, respectively, at 12 h (Fig. 10B). The parent phage PH204 treatment groups (MOI of 0.1, 0.01, and 0.001) decreased OD_{600} values by 1.79, 1.71, and 1.72, respectively, at 12 h (Fig. 10C). This suggests that the phages effectively inhibited the growth of *Salmonella* ZWSA605.The smallest MOI (0.001) resulted in the most pronounced increase within the first 3 h, indicating that higher doses of phages led to greater inhibition of bacterial growth. Between 4 h and 12 h, the recombinant phages effectively inhibited the growth of *Salmonella* ZWSA605.

The donor phage SP76 and recombinant phages RPA_{1-3} and RPB_{1-3} had lower inhibitory ability against *Salmonella* ZWSA337. The OD_{600} values of the phage-treated first decreased and then increased (Fig. 10D, E, and F), indicating that the phages inhibited the growth of *Salmonella* ZWSA337, but only for a short period (2–5 h). The phage-treated groups had lower OD_{600} values than the phage-untreated groups, but the inhibitory effect was not sustained after 6 h. Compared to the untreated groups, the phage SP76 treatment groups (MOI of 0.1, 0.01, and 0.001) decreased OD_{600} values by 0.71, 0.8, and 0.87, respectively, at 12 h (Fig. 10D). Phage RPA_{1-3} treatment groups (MOI of 0.1, 0.01, and 0.001) reduced

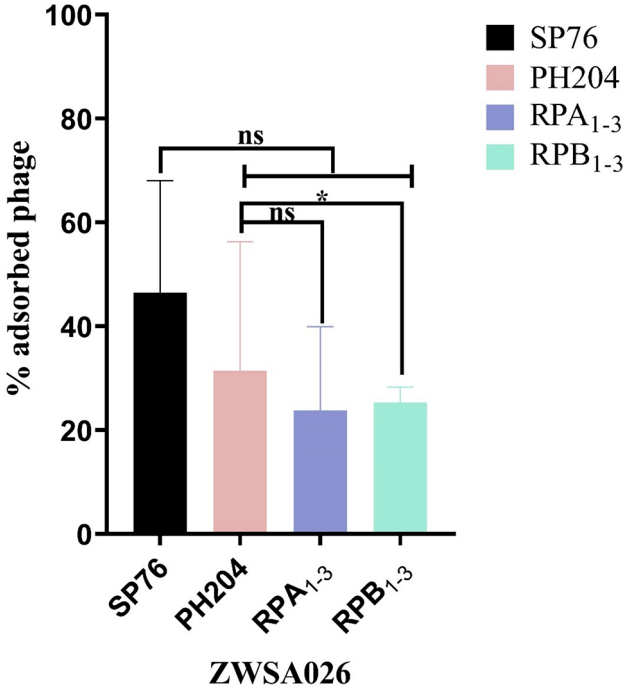

Fig. 9 Adsorption of ZWSA026 by phages SP76, PH204, RPA_{1-3} and RPB_{1-3}. Columns marked with * indicate significant differences at the $0.01 < P < 0.05$ level. Error bars represent the mean ± SEM

OD_{600} values by 0.37, 0.17, and 0.26, respectively, at 12 h (Fig. 10E). Phage RPB_{1-3} treatment groups (MOI of 0.1, 0.01, and 0.001) reduced OD_{600} values by 0, 0.12, and 0.2, respectively, at 12 h (Fig. 10F).

Evaluation of the application of phage in milk

The milk biocontrol assay showed that phages PH204 and SP76 exhibited statistically significant inhibition of ZWSA605 and ZWSA337, respectively, at 4℃ and 20℃ (Fig. 11). PH204 reduced ZWSA605 to 0.72 log10 CFU/mL at 4℃ for 8 h, while SP76 reduced ZWSA337 to 0.26 log10 CFU/mL. At 20℃, PH204 reduced ZWSA605 to 3.11 log10 CFU/mL after 8 h, and SP76 reduced ZWSA337 to 0.33 log10 CFU/mL.

Phages RPA_{1-3} and RPB_{1-3} also showed statistically significant inhibition of ZWSA605 at 20℃, with RPA_{1-3} reducing ZWSA605 to 1.51 log10 CFU/mL and RPB_{1-3} reducing it to 2.18 log10 CFU/mL after 8 h. However, no inhibitory effects were observed on ZWSA337 at 4℃ and 20℃.

Notably, the number of viable bacteria in the 20℃ phage treatment group was significantly lower than that in the 4℃ phage treatment group (RPA_{1-3}, $P < 0.001$; RPB_{1-3}, $P < 0.0001$, PH204 < 0.0001) after 8 h. Additionally, phages RPA_{1-3} and RPB_{1-3} were significantly less effective than PH204 at both temperatures.

Discussion

In this study, we engineered two chimeric phages, RPA_{1-3} and RPB_{1-3}, by modifying the long tail fiber gene of a T5-like phage. These chimeras were based on the backbone of phage PH204, with partial replacement of the Peg162 and Peg163 genes from phage SP76. This modification expanded the host range of both RPA_{1-3} and RPB_{1-3}. Notably, these chimeric phages retained the ability to infect 30 strains of the parent phage PH204 and additionally acquired the ability to infect 24 strains of the parent phage SP76.

To investigate the genomic features of phages PH204 and SP76, we conducted phylogenetic and sequence analyses. Phylogenetic analysis revealed that phages PH204 and SP76 belong to *Demerecviridae*-like phages within the order *Caudoviricetes*, specifically classified under *Tequintavirus* and *Epseptimavirus*. Despite their high genomic homology, the plaque host ranges of PH204 and SP76 differ significantly. A detailed sequence comparison of the PB3 and PB4 regions of phages PH204 and SP76 with another broad-spectrum T5-like phage, vB_Sen-E22, showed notable differences in the PB3 and PB4 regions of phage SP76. These differences may contribute to the varying host ranges of phages PH204 and SP76 [43]. Further investigation into the differences in phage-binding regions is required to demonstrate that variations in these regions may lead to changes in the phage host range.

Meanwhile, to investigate the relationship between the PB3 and PB4 regions of T5-like phages and their host range, we used homologous recombination to replace the PB3 and PB4 regions of SP76 with those from phage PH204 [44]. Previous studies have indicated that the successful reconstruction of long fibers in T2-, T4-, and T5-like phages depends heavily on the high homology of the edited sequences [23, 45–48].

Furthermore, phage adsorption rates were analyzed to evaluate the ability of phages to bind to host bacteria. The adsorption rates of phages RPA_{1-3} and RPB_{1-3} were lower than those of PH204 and SP76. Our findings suggest that PB3 and PB4 may serve as primary host-range determinants for certain T5-like phages, with minimal effects on phage uptake. As early as 1982, Heller et al. showed that the long tail fibers of T5 phage were closely related to the adsorption rate of the phage, and no in-depth studies were conducted on the influence of the host range [49].

Generally, the biological characteristics of RPA_{1-3} and RPB_{1-3}, such as MOI, thermal stability, pH stability, and one-step growth curve, are essential for assessing their environmental stability against pathogenic bacteria [50, 51]. Our study demonstrated that the thermal and pH stability of RPA_{1-3} and RPB_{1-3} were comparable to those of PH204, and the modifications in PB3 and PB4 did not

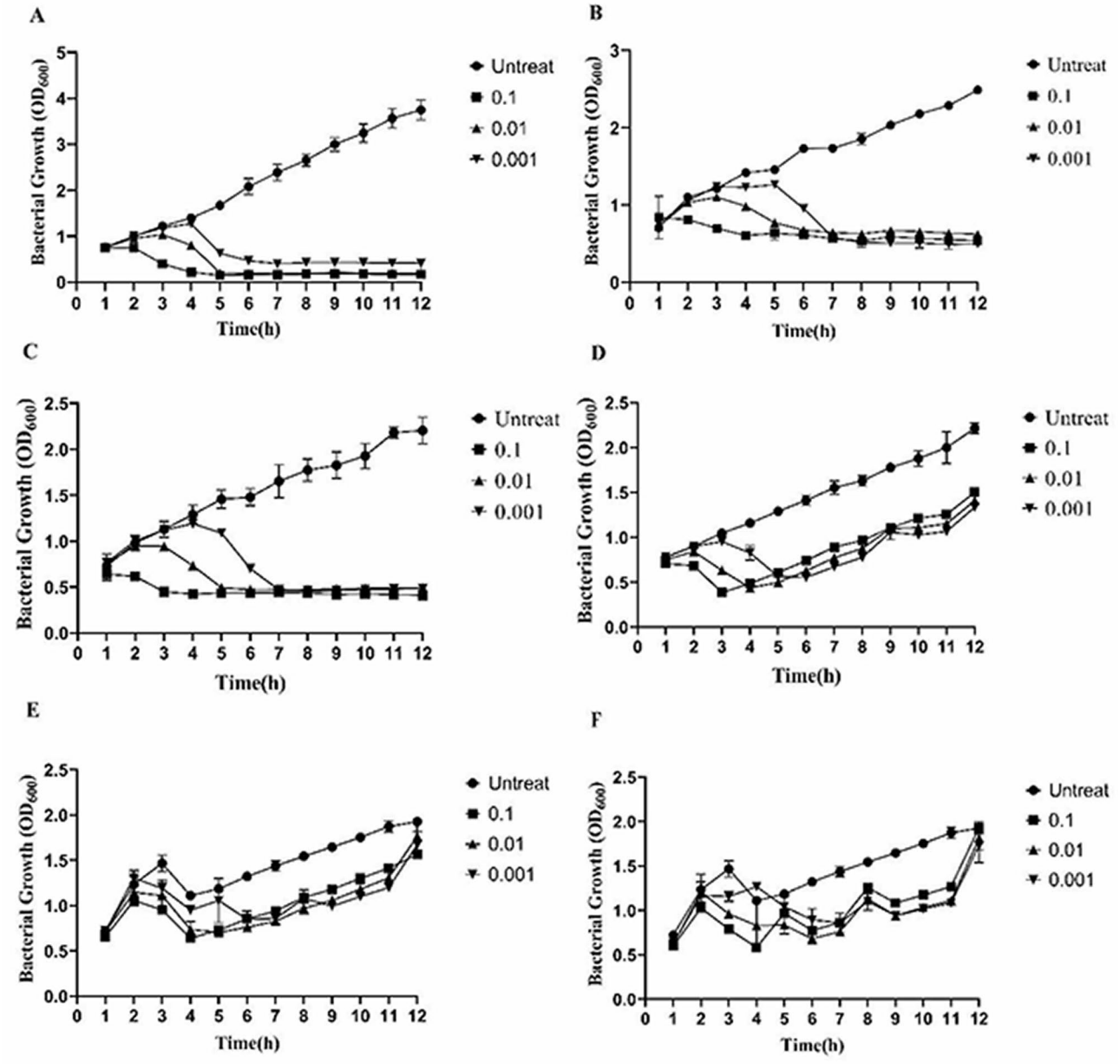

Fig. 10 Inhibition curves of phages RPA_{1-3}, RPB_{1-3}, PH204 and SP76 against *Salmonella*. (**A**-**C**) Inhibition curve of RPA_{1-3}, RPB_{1-3}, and PH204 against ZWSA605. (**D**-**F**) Inhibition curve of phage SP76, RPA_{1-3}, and RPB_{1-3} against ZWSA337

significantly impact their growth characteristics. According to the one-step growth curve, the latency periods of RPA1-3 and RPB1-3 were both 30 min and, burst times were 20 min and 40 min, respectively, and burst sizes were 20 particles and 37 particles, respectively. Compared with PH204 (its latency time was 30 min, the burst time was 30 min, and the burst was 20 particles), it indicated that the one-step growth curves of RPA_{1-3} and RPB_{1-3} closely those of PH204, indicating that their growth cycles were not notably altered. Compared to previously reported genetically manipulated phages, recombinant phages RPA_{1-3}, and RPB_{1-3} exhibited higher tolerance to environmental conditions, including pH = 3 and temperatures of 60℃ [52].

Additionally, the engineered phages RPA_{1-3} and RPB_{1-3} did not effectively inhibit *Salmonella* ZWSA337 in a planktonic state, and their inhibitory capacity was lower than that of phage SP76. However, our results demonstrated that these engineered phages can inhibit *Salmonella* ZWSA605 in a planktonic state, with an inhibitory effect comparable to that of phage PH204. These findings indicate that the modifications of PB3 and PB4 did not significantly alter the bacteriostatic activity of RPA_{1-3} and RPB_{1-3}. Moreover, these results suggest

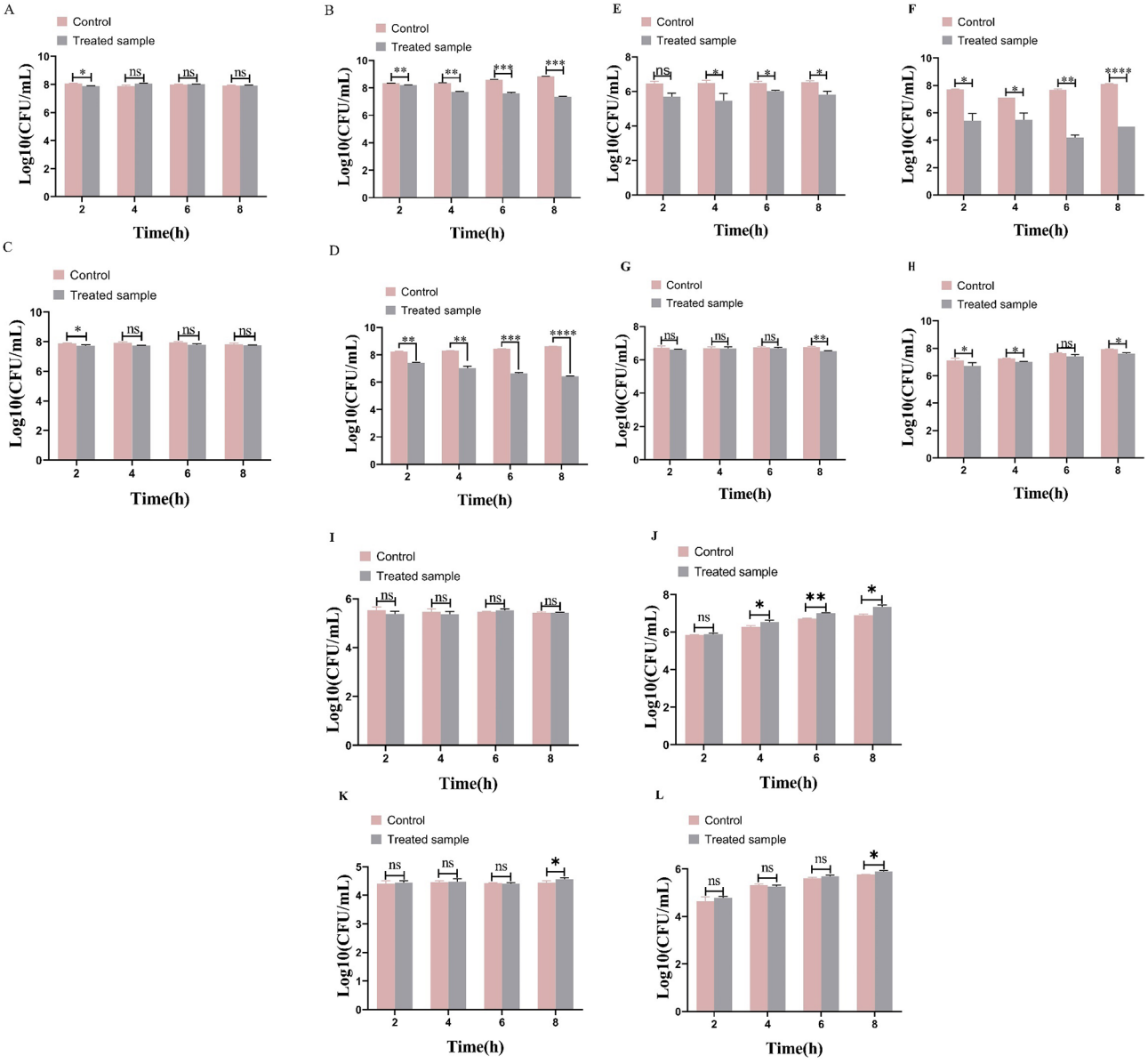

Fig. 11 Application of phages RPA_{1-3}, RPB_{1-3}, PH204, and SP76 in milk. (A-B) RPA_{1-3} inhibited the growth of *Salmonella* ZWSA605 in milk at both 4℃ and 20℃ using an MOI of 100. (C-D) RPB_{1-3} inhibited the growth of *Salmonella* ZWSA605 in milk at both 4℃ and 20℃ using an MOI of 100. (E-F) PH204 inhibited the growth of *Salmonella* ZWSA605 in milk at both 4℃ and 20℃ using an MOI of 100. (G-H) SP76 inhibited the growth of *Salmonella* ZWSA337 in milk at both 4℃ and 20℃ using an MOI of 100. (I-J) RPA_{1-3} inhibited the growth of *Salmonella* ZWSA337 in milk at both 4℃ and 20℃ with MOI of 100. (K-L) RPB_{1-3} inhibited the growth of *Salmonella* ZWSA337 in milk at both 4℃ and 20℃ with MOI of 100. **** Highly significant P-value < 0.001 (Tukey's B)

that recombinant phages RPA_{1-3} and RPB_{1-3} have been successfully isolated, and that long-tail fibers PB3 and PB4 may be essential for host recognition and absorption of certain T5-like phages. The basic biological characteristics and functions of other regions of long-tail fibers and their relationship with bacteriolytic activity of phages require further investigation. Several studies have confirmed the potential of phages to inhibit bacteria growth. Yoichi M et al. found that a recombinant T2 phage, T2ppD1, could inhibit the growth of *Escherichia coli* O157:H7 for 7 h at MOI of 0.1 [20]. Mahichi F et al. identified a site-specific recombination phage, IP008, which effectively inhibited the growth of *E. coli* within 11 h [53]. Additionally, Li Min et al. modified a T4-like phage that inhibited the growth of pathogenic *Escherichia coli* within 14 h. These studies suggest that both naturally isolated and engineered phages can effectively inhibit the growth of planktonic *Salmonella* within a specific range.

Since phages are natural predators of bacteria and replicate only on target bacteria, they may enhance the biosafety of animal-derived foods [54]. Milk is a staple

food consumed daily in many countries [55], and due to its high nutrient content, various bacteria can easily grow in it [56]. Contaminated milk is reported to be a major source of *Salmonella* infections [57]. Phages RPA_{1-3} and RPB_{1-3} demonstrated significant antibacterial effects against *Salmonella* ZWSA605 in milk, comparable to phage PH204, but showed no antibacterial activity against *Salmonella* ZWSA337. These findings indicate that the modifications of PB3 and PB4 did not substantially alter the antibacterial activity of RPA_{1-3} and RPB_{1-3} in milk. When incubated at 4℃ and a MOI of 100 for 8 h, PH204 effectively inhibits *Salmonella* in milk, reducing the number of bacteria by 0.72 log10 CFU /mL compared to the control group. However, bacteriophages RPA_{1-3} and RPB_{1-3} did not inhibit *Salmonella* in milk. This is similar to the result reported by Marcia Braz et al., which stated that phages have a low inhibitory effect on *Salmonella* in milk at 4℃ because temperature can affect bacteria activity, and phages replicate depending on the growth of their bacterial host [58]. At 20℃ for 8 h, phages RPA_{1-3}, RPB_{1-3} and PH204 effectively inhibited *Salmonella* in milk, with a decrease in the bacterial count of 1.51 $\log_{10}$ CFU /mL, 2.18 $\log_{10}$ CFU /mL and 3.11 $\log_{10}$ CFU /mL with MOI of 100, respectively. Similar results were observed at room temperatures in various dairy products contaminated with *Salmonella* [59]. Junjie Li et al. found that phage T156 reduced the number of *Salmonella* in milk to 4.15 $\log_{10}$ CFU /mL at MOI of 1000 [60].

Conclusion

In this study, the PB3 and PB4 regions of two highly homologous T5 phages, SP76 and PH204, belonging to different genera, were modified to broaden their host range. Our results indicate that PB3 and PB4 may determine the host range of certain T5-like bacteriophages, with PB4 being partially necessary for the T5 bacteriophage *Tequintavirus* genus to perform its adsorption function. Notably, the engineered phages RPA_{1-3} and RPB_{1-3} were able to control the growth and survival of *Salmonella* ZWSA605 in milk, although their bacteriostatic effect was lower than that of the parental phage PH204. This study provides a reference for exploring the functions of PB3 and PB4 in *Tequintavirus* and other T5-like phages, as well as for modifying these phages to alter or expand their host range.

Abbreviations

RPA_{1-3}	Recombinant phage A
RPB1 – 3	Recombinant phage B; tail protein
PB3	Tail protein
PB4	tail protein
phage	bacteriophage
TFP	Tail fiber protein

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12866-025-03895-8.

Supplementary Material 1

Supplementary Material 2

Acknowledgements

The author wishes to express their gratitude to the College of Veterinary Medicine, Nanjing Agricultural University and the Department of College of Veterinary Medicine, Xinjiang Agricultural University for the support during this study.

Author contributions

Xiaofeng Zheng: Conceptualization, Writing– original draft, Formal analysis, Investigation. Xin Wang, Pei Li: Data curation, Software, Methodology. Yu Zhou, Xihui Zhu, Zimeng Hu: Software, Validation. Hui Wang, Mianmian Chen: Validation. Xiang Huo: Investigation. Yingyu Liu: Supervision. Wei Zhang: Project administration, Writing– review & editing. All authors reviewed the manuscript.

Funding

This work was supported by the NHC Key Laboratory of Enteric Pathogenic Microbiology (Jiangsu Provincial Center for Disease Control and Prevention);The Natural Science Foundation Project of Xinjiang Uygur Autonomous Region (No. 2024D01A128); The Major Science and Technology Projects in the Autonomous Region (No. 2023A02007); The Ecological Prevention and Control of hemorrhagic septicemia in yaks in Qinghai Province (No. 2024-ZJ-753); The Shandong Province science and technology smes innovation ability improvement project (No. 2022TSGC2305); The Guizhou Province Science and Technology Plan Project (No. QKH [2023]008); The Key Research and Development of Hainan Province: (No. ZDYF2022XDNY236).;

Data availability

The raw sequence data reported in this paper have been deposited in the Genome Sequence Archive (Genomics, Proteomics & Bioinformatics 2021) in National Genomics Data Center (Nucleic Acids Res 2022), China National Center for Bioinformation / Beijing Institute of Genomics, Chinese Academy of Sciences (GSA: CRA020973) that are publicly accessible at https://ngdc.cncb.ac.cn/gsa.

Declarations

Ethical approval

Not required, because no animal object was involved.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Conflict of interest

The authors declare no competing financial or commercial interests.

Received: 18 November 2024 / Accepted: 14 March 2025
Published online: 26 March 2025

References

1. Phongtang W, Choi GP, Chukeatirote E, Ahn J. Bacteriophage control of Salmonella typhimurium in milk. FOOD SCI BIOTECHNOL. 2019;28(1):297–301.
2. Göser V, Sander N, Schulte M, Scharte F, Franzkoch R, Liss V, Psathaki OE, Hensel M. Single molecule analyses reveal dynamics of Salmonella translocated effector proteins in host cell endomembranes. NAT COMMUN 2023, 14(1).

3. Yuan Q, Gu B, Liu W, Wen XR, Wang JL, Tang JW, Usman M, Liu SL, Tang YR, Wang L. Rapid discrimination of four Salmonella enterica serovars: A performance comparison between benchtop and handheld Raman spectrometers. J CELL MOL MED. 2024;28(8):e18292.
4. Sun R, Fang L, Dai J, Chen K, Ke B, Sun J, Ke C, Wai CCE, Liu Y, Chen S et al. Antimicrobial resistance and population genomics of emerging multidrug-resistant Salmonella 4,[5],12:i:- in Guangdong. China MSYSTEMS 2024:e0116423.
5. Lu Z, Xing T, Zhao Z, Li Z, Hou D, Ma Z, Chen S, Yang Y, Li S, Zhang H. Induction of Salmonella enteritidis into a viable but nonculturable state by cinnamaldehyde and its resuscitation. FOODBORNE PATHOG DIS; 2024.
6. Tian Y, Gu D, Wang F, Liu B, Li J, Kang X, Meng C, Jiao X, Pan Z. Prevalence and characteristics OfSalmonella spp. From a pig farm in Shanghai, China. FOODBORNE PATHOG DIS. 2021;18(7):477–88.
7. Michael GB, Schwarz S. Antimicrobial resistance in zoonotic nontyphoidal Salmonella: an alarming trend? CLIN MICROBIOL INFEC. 2016;22(12):968–74.
8. Teng J, Chan E, Dai A, Ng G, Li TT, Lai C, Wu A, Lau S, Woo P. In vitro susceptibility of typhoidal, nontyphoidal, and Extended-Spectrum-beta-Lactamase-Producing Salmonella to Ceftolozane/Tazobactam. ANTIMICROB AGENTS CH. 2022;66(1):e0122421.
9. Mohakud NK, Panda RK, Singh D, Patra SD, Simnani FZ, Sinha A, Nandi A, Jha E, Singh S, Kaushik NK, et al. Intrinsic insights to antimicrobial effects of Nitrofurantoin to multi drug resistant Salmonella enterica serovar typhimurium ms202. BIOMED PHARMACOTHER. 2023;165:115180.
10. Salmond GPC, Fineran PC. A century of the phage: past, present and future. NAT REV MICROBIOL. 2015;13(12):777–86.
11. Dion MB, Oechslin F, Moineau S. Phage diversity, genomics and phylogeny. Nat Rev Microbiol. 2020;18(3):125–38.
12. Hussain W, Yang X, Ullah M, Wang H, Aziz A, Xu F, Asif M, Ullah MW, Wang S. Genetic engineering of bacteriophages: key concepts, strategies, and applications. BIOTECHNOL ADV. 2023;64:108116.
13. Phothaworn P, Supokaivanich R, Lim J, Klumpp J, Imam M, Kutter E, Galyov EE, Dunne M, Korbsrisate S. Development of a broad-spectrum Salmonella phage cocktail containing viunalike and jerseylike viruses isolated from Thailand. FOOD MICROBIOL. 2020;92:103586.
14. Islam MS, Hu Y, Mizan M, Yan T, Nime I, Zhou Y, Li J. Characterization of Salmonella Phage LPST153 That Effectively Targets Most Prevalent Salmonella Serovars. MICROORGANISMS. 2020;8(7).
15. Huang C, Virk SM, Shi J, Zhou Y, Willias SP, Morsy MK, Abdelnabby HE, Liu J, Wang X, Li J. Isolation, characterization, and application of bacteriophage LPSE1 against Salmonella enterica in ready to eat (RTE) foods. FRONT MICROBIOL. 2018;9:1046.
16. Braz M, Pereira C, Freire C, Almeida A. Evaluation of the potential of phage phSE-5 to fight Salmonella typhimurium in milk, liquid whole egg and eggshell. J APPL MICROBIOL 2025.
17. Song J, Liu Z, Zhang Q, Liu Y, Chen Y. Phage engineering for targeted Multidrug-Resistant Escherichia coli. INT J MOL SCI 2023, 24(3).
18. Lobocka M, Dabrowska K, Gorski A. Engineered bacteriophage therapeutics: rationale, challenges and future. BIODRUGS. 2021;35(3):255–80.
19. Yuan X, Zhu Z, Huang Z, Yu S, Jin H, Chen B, Yu S, Xue L, Chen M, Zhang J, et al. Engineered lytic phage of Bacillus cereus and its application in milk. INT J FOOD MICROBIOL. 2023;405:110339.
20. Yoichi M, Abe M, Miyanaga K, Unno H, Tanji Y. Alteration of tail fiber protein gp38 enables T2 phage to infect Escherichia coli O157:H7. J BIOTECHNOL. 2005;115(1):101–7.
21. Chen M, Zhang L, Abdelgader SA, Yu L, Xu J, Yao H, Lu C, Zhang W. Alterations in gp37 expand the host range of a T4-Like phage. APPL ENVIRON MICROB 2017, 83(23).
22. Li M, Shi D, Li Y, Xiao Y, Chen M, Chen L, Du H, Zhang W. Recombination of T4-like phages and its activity against pathogenic Escherichia coli in planktonic and biofilm forms. VIROL SIN. 2020;35(5):651–61.
23. Zhang J, Ning H, Lin H, She J, Wang L, Jing Y, Wang J. Expansion of the plaquing host range and improvement of the absorption rate of a T5-like Salmonella phage by altering the long tail fibers. APPL ENVIRON MICROB 2022, 88(17).
24. Golomidova A, Kulikov E, Prokhorov N, Guerrero-Ferreira R, Knirel Y, Kostryukova E, Tarasyan K, Letarov A. Branched lateral tail fiber organization in T5-Like bacteriophages DT57C and DT571/2 is revealed by genetic and functional analysis. VIRUSES-BASEL. 2016;8(1):26.
25. Li P, Wang H, Li M, Qi W, Qi Z, Chen W, Dong Y, Xu Z, Zhang W. Characterization and genome analysis of a broad lytic spectrum bacteriophage P479 against multidrug-resistant Escherichia coli. VIRUS RES. 2022;308:198628.
26. Gu J, Xu W, Lei L, Huang J, Feng X, Sun C, Du C, Zuo J, Li Y, Du T, et al. LysGH15, a novel bacteriophage Lysin, protects a murine bacteremia model efficiently against lethal methicillin-resistant Staphylococcus aureus infection. J CLIN MICROBIOL. 2011;49(1):111–7.
27. Xu J, Chen M, He L, Zhang S, Ding T, Yao H, Lu C, Zhang W. Isolation and characterization of a T4-like phage with a relatively wide host range within Escherichia coli. J BASIC MICROB. 2016;56(4):405–21.
28. Wang H, Shen Y, LI P, Xiao Y, Li Y, Hu X, Wang Z, Cheng Z, Wang Z, Liu Q et al. Characterization and genomic analysis of a demerecviridae phage SP76 with lytic multiple-serotypes of Salmonella. ARCH MICROBIOL 2022, 204(3).
29. Kong X, Wang H, Guo G, Li P, Tong P, Liu M, Ma X, Dong C, Li Y, Zhang H, et al. Duck sewage source coliphage P762 can lyse STEC and APEC. Virus Genes. 2022;58(5):436–47.
30. Wick RR, Judd LM, Gorrie CL, Holt KE. Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. PLOS COMPUT BIOL. 2017;13(6):e1005595.
31. Seemann T. Prokka: rapid prokaryotic genome annotation. Bioinformatics. 2014;30(14):2068–9.
32. Sullivan MJ, Petty NK, Beatson SA. Easyfig: a genome comparison visualizer. Bioinformatics. 2011;27(7):1009–10.
33. Waterhouse A, Bertoni M, Bienert S, Studer G, Tauriello G, Gumienny R, Heer FT, de Beer TAP, Rempfer C, Bordoli L, et al. SWISS-MODEL: homology modelling of protein structures and complexes. NUCLEIC ACIDS RES. 2018;46(W1):W296–303.
34. Li Y, Shi X, Xu J, Huang X, Feng J, Huang Y, Liu K, Yu F. Proteomics-based analysis on the stress response mechanism of bidens Pilosa L. under cadmium exposure. J HAZARD MATER. 2024;462:132761.
35. Adams MC, Schiltz CJ, Sun J, Hosford CJ, Johnson VM, Pan H, Borbat PP, Freed JH, Thomason LC, Court C, et al. The crystal structure of bacteriophage lambda RexA provides novel insights into the DNA binding properties of Rex-like phage exclusion proteins. NUCLEIC ACIDS RES. 2024;52(8):4659–75.
36. Gao D, Ji H, Li X, Ke X, Li X, Chen P, Qian P. Host receptor identification of a polyvalent lytic phage GSP044, and preliminary assessment of its efficacy in the clearance of Salmonella. MICROBIOL RES. 2023;273:127412.
37. Tung C, Alvarado-Martínez Z, Tabashsum Z, Aditya A, Biswas D. A highly effective Bacteriophage-1252 to control multiple serovars of Salmonella enterica. FOODS. 2023;12(4):797.
38. Zhao J, Lin Y, Wang C, Zayda M, Maung AT, Mohammadi TN, Duc HM, Yu P, Ma M, Gong D, et al. Biocontrol of Salmonella typhimurium in milk, lettuce, Raw pork meat and ready-to-eat steamed-chicken breast by using a novel bacteriophage with broad host range. INT J FOOD MICROBIOL. 2023;402:110295.
39. Kropinski AM, Mazzocco A, Waddell TE, Lingohr E, Johnson RP. Enumeration of bacteriophages by double agar overlay plaque assay. Methods Mol Biol. 2009;501:69–76.
40. Azari R, Yousefi MH, Taghipour Z, Wagemans J, Lavigne R, Hosseinzadeh S, Mazloomi SM, Vallino M, Khalatbari-Limaki S, Berizi E. Application of the lytic bacteriophage Rostam to control Salmonella enteritidis in eggs. INT J FOOD MICROBIOL. 2023;389:110097.
41. SWANSTROM M, ADAMS MH. Agar layer method for production of high titer phage stocks. Proc Soc Exp Biol Med. 1951;78(2):372–5.
42. Park H, Kim J, Kim H, Cho E, Park H, Jeon B, Ryu S. Characterization of the lytic phage MSP1 for the Inhibition of multidrug-resistant Salmonella enterica serovars Thompson and its biofilm. INT J FOOD MICROBIOL. 2023;385:110010.
43. Kosznik-Kwaśnicka K, Grabowski A, Grabski M, Kaszubski M, Górniak M, Jurczak-Kurek A, Węgrzyn G, Węgrzyn A. Bacteriophages vB_Sen-TO17 and vB_Sen-E22, newly isolated viruses from chicken feces, specific for several Salmonella enterica strains. Int J Mol Sci. 2020;21(22):8821.
44. Łobocka M, Dąbrowska K, Górski A. Engineered bacteriophage therapeutics: rationale, challenges and future. BioDrugs: Clin Immunotherapeutics Biopharmaceuticals Gene Therapy. 2021;35(3):255–80.
45. Tétart F, Repoila F, Monod C, Krisch HM. Bacteriophage T4 host range is expanded by duplications of a small domain of the tail fiber adhesin. J MOL BIOL. 1996;258(5):726–31.
46. Tetart F, Desplats C, Krisch HM. Genome plasticity in the distal tail fiber locus of the T-even bacteriophage: recombination between conserved motifs swaps adhesin specificity. J MOL BIOL. 1998;282(3):543–56.
47. Trojet SN, Caumont-Sarcos A, Perrody E, Comeau AM, Krisch HM. The gp38 adhesins of the T4 superfamily: a complex modular determinant of the phage's host specificity. GENOME BIOL EVOL. 2011;3:674–86.
48. de Jonge PA, von Meijenfeldt F, Costa AR, Nobrega FL, Brouns S, Dutilh BE. Adsorption Sequencing as a Rapid Method to Link Environmental Bacteriophages to Hosts. ISCIENCE. 2020;23(9):101439.

49. Heller K, Braun V. Polymannose O-antigens of Escherichia coli, the binding sites for the reversible adsorption of bacteriophage T5 + via the L-shaped tail fibers. J VIROL. 1982;41(1):222–7.
50. Nobrega FL, Costa AR, Kluskens LD, Azeredo J. Revisiting phage therapy: new applications for old resources. TRENDS MICROBIOL. 2015;23(4):185–91.
51. Tao C, Yi Z, Zhang Y, Wang Y, Zhu H, Afayibo DJA, Li T, Tian M, Qi J, Ding C et al. Characterization of a Broad-Host-Range lytic phage SHWT1 against Multidrug-Resistant Salmonella and evaluation of its therapeutic efficacy in vitro and in vivo. FRONT VET SCI 2021, 8.
52. Nobrega FL, Costa AR, Santos JF, Siliakus MF, van Lent JW, Kengen SW, Azeredo J, Kluskens LD. Genetically manipulated phages with improved pH resistance for oral administration in veterinary medicine. SCI REP-UK. 2016;6:39235.
53. Mahichi F, Synnott AJ, Yamamichi K, Osada T, Tanji Y. Site-specific recombination of T2 phage using IP008 long tail fiber genes provides a targeted method for expanding host range while retaining lytic activity. FEMS MICROBIOL LETT. 2009;295(2):211–7.
54. Walker N, Li S, Strauss H, Pokharel S. Salmonella typhimurium DT 104 response to lytic bacteriophage and lactobionic acid on Raw chicken breast. FOOD MICROBIOL. 2021;100:103862.
55. de Klerk JN, Robinson PA. Drivers and hazards of consumption of unpasteurised bovine milk and milk products in high-income countries. PEERJ. 2022;10:e13426.
56. Ajose DJ, Abolarinwa TO, Oluwarinde BO, Montso PK, Fayemi OE, Aremu AO, Ateba CN. Whole genome sequence analysis of multi-drug resistant and biofilm-forming Staphylococcus haemolyticus isolated from bovine milk. BMC MICROBIOL. 2024;24(1):426.
57. Brewster JD, Paul M. Short communication: improved method for centrifugal recovery of bacteria from Raw milk applied to sensitive real-time quantitative PCR detection of Salmonella spp. J DAIRY SCI. 2016;99(5):3375–9.
58. Braz M, Pereira C, Freire C, Almeida A. Potential of bacteriophage phT4A as a biocontrol agent against Escherichia coli in food matrices. INT J FOOD MICROBIOL. 2024;424:110847.
59. Zhang Y, Zou G, Islam MS, Liu K, Xue S, Song Z, Ye Y, Zhou Y, Shi Y, Wei S, et al. Combine thermal processing with polyvalent phage LPEK22 to prevent the Escherichia coli and Salmonella enterica contamination in food. FOOD RES INT. 2023;165:112454.
60. Li J, Li Y, Ding Y, Huang C, Zhang Y, Wang J, Wang X. Characterization of a novel siphoviridae Salmonella bacteriophage T156 and its microencapsulation application in food matrix. FOOD RES INT. 2021;140:110004.

Publisher's note