Food Science of Animal Products

Research Article

Isolation and identification, biological characterization of *Staphylococcus aureus* phage and its application in milk

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Abstract: *Staphylococcus aureus* is one of the important pathogens that cause food contamination worldwide, and poses a great danger to people. The common treatment is antibiotic therapy; however, the misuse of antibiotics has led to the continuous emergence of drug-resistant strains. Therefore, new antibacterial methods need to be explored. In this study, a lytic *S. aureus* phage (named SP-CmSa-11) was isolated from dairy farms which belongs to the Myoviridae phage family. The optimal multiple of infection of SP-CmSa-11 is 0.1, and the host range included 7 strains of mastitis cow-derived *S. aureus*, 12 strains of animal-origin methicillin-resistant *S. aureus*, 1 strain of *S. xylosus*, 1 strain of *S. epidermidis* and 1 strain of *Enterococcus faecalis*. SP-CmSa-11's burst period was 40 min, and the burst size was about 130 PFU/cell. SP-CmSa-11 was inactivated after 10 min in a water bath at 70 °C, and the pH tolerance of SP-CmSa-11 ranges from 4 to 10 in 2 h. It's insensitive to chloroform and ultraviolet radiation. The total genome length of SP-CmSa-11 is 45 816 bp. The G + C content is 27.23%, and has 59 putative open reading frames. SP-CmSa-11 showed good antibacterial effects *in vitro* and milk. These results suggest that SP-CmSa-11 may be a promising alternative therapy for food contamination caused by *S. aureus*.

Keywords: *Staphylococcus aureus*; phage; biological properties; applications

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1 Introduction

Staphylococcus aureus is a gram-positive, foodborne pathogen that widely exists in the external environment, such as air, water and soil. Especially in the farms, *S. aureus* has been recognized as a major cause of bovine mastitis, causing 40% of mastitis cases in many countries around the world[1]. It's often transmitted through raw milk and dairy products, which caused great losses to the dairy industry and endangering human health. In Japan, a single outbreak led to more than 14 000 cases and one death in the summer of 2 000 due to the consumption of pasteurized products containing small amounts of staphylococcal enterotoxins (SEs)[2]. Of particular importance is the potential transmission of *S. aureus* between livestock and humans, as well as host-switching events leading to the emergence of new pathogenic or resistant clones[3]. It can causes human skin and soft tissue infections, as well as a variety of disease symptoms such as endocarditis, sepsis, toxic shock, and food poisoning[4–5]. Antibiotics are currently the most common treatment for *S. aureus* infections. However, with the long-term massive and unregulated use of antibiotics, the problem of bacterial drug resistance has become increasingly serious, in particular, the emergence of methicillin-resistant *S. aureus* (MRSA) strains and vancomycin-resistant *S. aureus* strains[6–7]. In previous studies, *S. aureus* isolated from dairy farms showed high resistance to oxacillin, penicillin, and vancomycin[8–9]. Currently, numerous reports on the effective sterilization technology have been widely used to prevent outbreaks associated with *S. aureus*, however, these traditional antimicrobial technology would has a large initial investment, potential damage to processing equipment, and a deleterious impact on organoleptic qualities (and possibly the nutritional value) of foods[10]. With the disadvantages of traditional antimicrobial agents, novel strategies are under development to fight multidrug-resistant strains. Among them, bacteriophages have been successfully exploited to treat some infections of *S. aureus*[11].

Phages are a class of viruses that infect bacteria in order to complete their own life cycle, which are an ideal alternative to antibiotic therapy with high specificity and ease of application[12]. Lytic phages infect their target bacterial cells through specific interaction with receptors followed by entry, hijack, and inhibition of host bacterium metabolism using it for its own progeny production leading to final cell lysis[13]. Lytic phages are preferred candidates for killing bacteria and they have indeed achieved great success in fighting against multidrug-resistant bacteria, with no negative impact on the normal flora and organismal cells in the animal[14]. Studies shown that the human immune system has well tolerance to phages, meaning that the host's immune level is not the main reason affecting the effectiveness of treatment[15]. Phages have been successfully applied to a variety of food matrices and have shown good results. There are also more and more phage products approved in food[16], which proves that phages have good application potential as a new strategy for food sterilization. Also, there have been studies on the application of lytic phages to inhibit *S. aureus* in milk and cheese, and *S. aureus* has been effectively inhibited under the action of phages[17–18]. However, the number of

lytic *S. aureus* phages is insufficient. According to the Pathosystems Resource Integration Center (PATRIC) Virginia Bioinformatics Institute database, only about 390 strains of *S. aureus* phages have been isolated and sequenced[19]. More phages that can be applied to food are urgently being discovered.

Therefore, the purpose of this study was to isolate and purify a phage that can lyse *S. aureus*, to characterize its features and to attempte to reduce *S. aureus* contamination in pasteurized milk. Its biological properties and lysis effects were evaluated to explore the potential of phage in food.

2 Materials and methods

2.1 Bacterial strains

A total of 44 strains were used in this study, including 16 strains of *S. aureus*, 17 strains of coagulase-negative *Staphylococcus*, 1 strain of *Enterococcus faecalis*, 1 strain of *E. hirae*, 1 strain of *Escherichia coli* which were isolated from dairy cow mastitis samples in a dairy farm of Sichuan (China). And 8 strains of animal-origin MRSA were preserved in the laboratory. These strains were stored at –80 °C.

2.2 Phage isolation and purification

Phage was isolated from the sewage samples of one dairy farm of Sichuan (China). Briefly, *S. aureus* CmSa-11 was used as the indicator strain for phage isolation. A 100 mL of the sewage sample was obtained, and the sample was centrifuged at 10 000 × *g* for 30 min at 4 °C (Eppendorf centrifuge 5810/5810R, Eppendorf, Germany). The supernatant was passed through a 0.22 μm filter (Millipore, Billerica, MA, USA). To confirm the presence of phage, the supernatant was serially diluted 10-fold, and a dotting assay was performed on LB agar (Hopebio, Qingdao, China) containing *S. aureus*. After drying for 15 min, the agar plate was incubated at 37 °C overnight, for observing the formed plaque. If a zone of lysis was observed, a lysate of a single phage was added to a 2 h culture of *S. aureus*, the mixture was incubated in a double-layer plate to obtain new phage plaques. The above steps were repeated 3–5 times to purify the phage.

2.3 Transmission electron microscopy (TEM) observation

The phage was amplified to a high titer (10^9 PFU/mL) and was sent to the Lilai Medical Experimental Center in Chengdu Hi-Tech District (Chengdu, China) for TEM observation. The phage suspension was placed on the surface of a copper mesh, and negative staining was performed using 2% phosphotungstic acid. The morphological characteristics of the isolated phage was analyzed using TEM (JEM-1400FLASH, JEOL, Tokyo, Japan) at an operating voltage of 80 kV. The phage was identified and classified according to the guidelines of the International Committee on Taxonomy of Viruses.

2.4 Host range analysis

The host range of the isolated phage was determined based on previous literature reports[20]. Briefly, 100 μL bacteria cultured to the logarithmic growth phase were coated on Mueller-Hinton Agar (MHA; Hopebio, Qingdao, China) plates. Then, 10 μL of phage suspension drops were placed on the bacteria plates, which were incubated at 37 °C for overnight. Lysis was observed and recorded.

2.5 Determination of optimal multiplicity of infection (MOI) of phage

The MOI of phage was determined following the method of Li et al.[21]. Briefly, 500 μL high-titer phage suspension (10^8 PFU/mL) was mixed with equal volume of *S. aureus* CmSa-11. Then the ratio of the concentration between phage and host bacteria was modulated to 0.001, 0.01, 0.1, 1, 10, 100, three replicates were performed for each MOI. Then the titer of phage was determined using methods mentioned above. The multiplicity of infection with the highest titer was the MOI of phage.

2.6 One-step growth curve determination

A one-step growth curve of isolated phage was determined according to the method of Liao et al.[22]. In brief, 0.2 mL of fresh overnight culture in LB was sub-cultured in 19.8 mL of sterile LB and incubated for 2 h at 37 °C to reach the log phase of bacterial growth. Then the bacterial culture was centrifuged at 3 000 × *g* for 10 min and the supernatant was pour off. The precipitated bacteria was resuspended with 20 mL LB broth, 1 mL of bacterial culture was mixed with 1 mL of phage suspension to obtain an ratio of the optimal MOI (0.1), and the mixture was cultured at 37 °C for 10 min, allowing phage adsorption onto the bacterial membranes. The phage-bacterial mixture was centrifuged at 3 000 × *g* for 10 min at 4 °C to discard the supernatant. After washing, the bacterial pellet was resuspended with 20 mL of fresh LB. The sample was then incubated at 37 °C for 180 min. And 100 μL of samples were taken out every 10 min up to 180 min and the titer of the samples at each time point was immediately measured by using the double-layer plaque assay. All tests were repeated three times. The burst size is calculated according to the following formula:

$$\text{Burst size} = \frac{N_1}{N_2}$$

Where N_1 was the phage titer at the end of the lysis (PFU/mL); N_2 was the amount of the host strain at the beginning of the infection (CFU/mL).

2.7 Phage stability assays

Temperature and pH stability of phage were evaluated using the methods of Li et al.[20]. To determine the phage survival at different temperatures (40, 50, 60, 70 °C) for 60 min, and then the phage titer was determined at 10-min intervals. The titer of phage incubated 2 h at different pH (2–12) was then determined. All tests were repeated three times.

Ultraviolet (UV) radiation stability of phage were determined following the method of Huang et al.[23], 4 mL of phage sample with a titer of 10^8 PFU/mL was spread on a sterilized petri dish (d = 90 mm) and placed on a bechtop (SW-CJ-2FD, Airtech, Suzhou Antai Airtech Co., Ltd.) at a vertical distance of 35 cm under UV light (400 mW/m^2). The samples were taken for titer determination every 10 min.

Chloroform stability of phage were determined following the method of Xiang et al.[24]. Briefly, pure phage cultures (10^8 PFU/mL) were mixed with final chloroform concentrations of 0%, 5%, 25%, 50%, and 75%, and incubated at 4 °C for 24 h. Then, the solution was centrifuged at 8 000 × *g* for 10 min. The supernatant was collected, and the pellet was discarded. After serial dilution, 100 μL of the diluted phage solution were mixed with 100 μL of the host bacterial cell and uniformly plated on the double-layer agar to measure phage titers. The experiment was performed in triplicate.

2.8 Genomic DNA isolation and sequencing

Phage DNA was extracted according to the method of Huang et al.[23] using the Norgen Biotek phage DNA isolation kit (Norgen Biotek, Canada). The DNA sample was sent to Beijing Tsingke Biotech Co., Ltd. for whole-genome sequencing analysis based on the Illumina HiSeq sequencing platform. The short reads were used for *de novo* genome assembly using SPAdes 3.13.0, and different *k*-mer lengths were selected for assembly using MEGAHIT (HKU-BGI, China) at the same time. Protein-coding genes were predicted by using the National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/) database. The extracted sequences were annotated with Prokka (Torsten Seemann, Australia). A circle map of the phage genome was generated with CG View. Phylogenetic relationships were based on the sequences of terminase large subunit comparisons with other phages in the NCBI and phylogenetic tree was drawn in MEGA 11 (Mega Limited, New Zealand).

2.9 *In vitro* antimicrobial activity test

S. aureus CmSa-11 was used to evaluate the antimicrobial activity of phage using the method of Liao et al.[22]. In brief, the host bacterial *S. aureus* CmSa-11 was cultured to the logarithmic growth phase and 1 mL of bacterial culture was inoculated into 48 mL of LB broth. At the same time, 1 mL phage was also added according to the ratio of the optimal MOI. The treatment groups were incubated at room temperature for 30 min and were cultured at 37 °C. For the control, saline magnesium (SM) buffer (NaCl 100 mmol/L, $MgSO_4{\cdot}7H_2O$ 8 mmol/L, Tris-HCl 50 mmol/L, pH 7.5) with the same volume as the phage used in the treatment, was also added to the bacterial solution. The control and treatment groups were both incubated at 37 °C, and the bacterial biomass was evaluated by measuring the optical density of 600 nm ($OD_{600\ nm}$) at 10 min intervals for 280 min. Simultaneously, the samples were continued to be cultured for 72 h to observe the continuous bactericidal efficacy of the phage.

2.10 Inhibitory effect of phage on *S. aureus* in milk

Artificial contamination of the milk matrix was simulated to observe whether the phage had inhibitory effects on *S. aureus* in milk based on the method of Tan et al.[25]. The 50 μL of host bacteria solution (1×10^8 CFU/mL) and the 50 μL of phage solution (1×10^7 PFU/mL) were mixed into 49.9 mL milk, the host bacteria suspension and SM buffer were mixed as a positive control. Phage-treated group and control group were incubated at room temperature for 30 min. After 30 min, the mixtures were cultured at 37 °C. Finally, aliquots of test samples and controls were collected for bacterial enumeration at 0, 4, 8, 12, 16, 20, 24, 28, 32, and 36 h of incubation. Each group was assessed in triplicate.

2.11 Statistical analysis

All statistical analysis were analyzed using Origin 2022 (OriginLab, USA). The significance of experimental data was assessed with *t*-test using SPSS 19.0 software (IBM, USA). The error bars represent the standard deviation (SD) of the mean.

3 Results and discussion

3.1 Isolation of a lytic phage against *S. aureus*

S. aureus CmSa-11 strain was used as the host, the phage, namely SP-CmSa-11, could produce small, round, and clear plaques, whose diameters ranged approximately from 0.5 to 1 mm, after overnight incubation at 37 °C on the top layer of a double-layered LB plate, as shown in Figure 1. Phage SP-CmSa-11 had an initial potency of 1.57×10^5 PFU/mL measured by double-layer plate method.

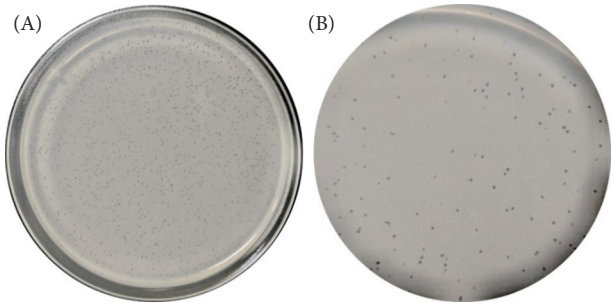


Figure 1 The formed clear plaques of phage SP-CmSa-11 on a double-plates. (A) Plaques before purification; (B) plaques after purification.

3.2 Morphology analysis of phage SP-CmSa-11

TEM was used to visualize the morphology of phage SP-CmSa-11 and for classification (Figure 2). It was found to be tadpole-shaped, with an icosahedral head of about 120 nm in length and 90 nm in width; with a retractable tail of about 140 nm in length; the head connected to the tail by a tail complex. The tail terminated by a baseplate and tail fibers with six short spines and six long whips. Based on the morphology and structural features of the phage virions, it is typical for members of Myoviridae phage family, according to the standard of the International Committee on Taxonomy of Viruses (ICTV).

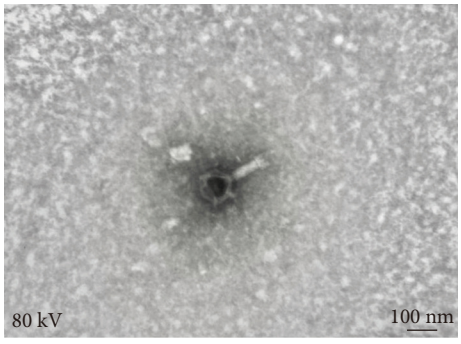


Figure 2 Morphology of phage SP-CmSa-11 by TEM (120 000 ×).

3.3 Phage host range

To determine the host range of phage SP-CmSa-11, 44 strains were tested (Table 1). Among them, phage SP-CmSa-11 could specifically infect 22 strains, including 7 strains of *S. aureus*, 12 strains of animal-origin MRSA, 1 strain of *S. xylosus*, 1 strain of *S. epidermidis*, and 1 strain of *E. faecalis*, which indicated that phage SP-CmSa-11 had a broad host range with high lysis ability.

Table 1 Host range of phage SP-CmSa-11.

Bacteria	Strain No.	Source	Spot test
S. aureus	CmSa-1	Mastitis milk	–
S. aureus	CmSa-2	Mastitis milk	+
S. aureus	CmSa-3	Mastitis milk	+
S. aureus	CmSa-5	Mastitis milk	–
S. aureus	CmSa-11	Mastitis milk	+

Table 1 (*Continued*)

Bacteria	Strain No.	Source	Spot test
S. aureus	CmSa-12	Mastitis milk	+
S. aureus	CmSa-13	Mastitis milk	+
S. aureus	CmSa-14	Mastitis milk	+
S. aureus	CmSa-15	Milking machine	+
S. aureus	CmSa-16	Milking machine	–
MRSA	CmSa-MRSA-4	Mastitis milk	+
MRSA	CmSa-MRSA-6	Mastitis milk	–
MRSA	CmSa-MRSA-7	Mastitis milk	+
MRSA	CmSa-MRSA-8	Mastitis milk	+
MRSA	CmSa-MRSA-9	Mastitis milk	+
MRSA	CmSa-MRSA-10	Mastitis milk	+
MRSA	CA-MRSA-1	Fresh pork	+
MRSA	CA-MRSA-2	Fresh pork	+
MRSA	CA-MRSA-3	Yak farm	+
MRSA	CA-MRSA-4	Yak farm	+
MRSA	CA-MRSA-5	Yak farm	–
MRSA	CA-MRSA-6	Yak slaughterhouse	+
MRSA	CA-MRSA-7	Yak slaughterhouse	+
MRSA	CA-MRSA-8	Yak slaughterhouse	+
S. sciuri	CmSsc-6	Farm ground	–
S. chromogenes	CmSc-3	Mastitis milk	–
S. arlettae	CmSar-9	Normal cow nipples swab	–
S. xylosus	CmSx-20	Mastitis milk	+
S. haemolyticus	CmSh-31	Mastitis milk	–
S. agnetis	CmSag-38	Normal milk sample	–
S. saprophyticus	CmSsa-44	Sick cow nipples swab	–
S. gallinarum	CmSg-55	Farm ground	–
S. simulans	CmSsi-100	Mastitis milk	–
S. cohnii	CmSco-102	Mastitis milk	–
S. warneri	CmSw-152	Mastitis milk	–
S. pasteuri	CmSp-153	Cow fur	–
S. epidermidis	CmSep-167	Mastitis milk	+
S. edaphicus	CmSed-252	Mastitis milk	–
S. hyicus	CmShy-424	Mastitis milk	–
S. rostri	CmSr-427	Cow fur	–
S. hominis	CmSho-606	Mastitis milk	–
E. faecalis	989	Normal milk sample	+
E. coli	736	Normal cow nipples swab	–
E. hirae	773	Farm ground	–

Note: + Represented clear lysis zone; – represented no lysis zone.

3.4 MOI

The MOI results for SP-CmSa-11 was shown in Figure 3. When using bacteria to amplify phage SP-CmSa-11, the production of phages is inversely proportional to the MOI during phage infection. As the MOI decreased from 100 to 0.1, the production of phages gradually increased. When the MOI is 0.1, the yield of phages reached the highest, up to 9.48 (lg (PFU/mL)), indicating that the MOI for phage SP-CmSa-11 was 0.1.

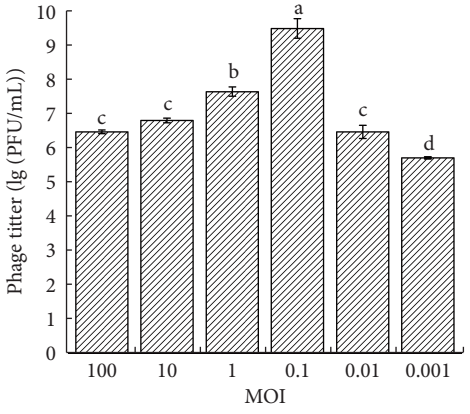


Figure 3 Optimal MOI determination for phage SP-CmSa-11. Different lowercase letters indicate significant differences ($P < 0.05$).

3.5 One-step growth curve

According to the one-step growth curve of phage SP-CmSa-11 (Figure 4), it was estimated from the growth curve that the latent period of phage SP-CmSa-11 is about 60 min. The titers of phage SP-CmSa-11 increased very quickly from the 60 min after initial phage infection, and reached the peak at the 160 min post phage infection, with the highest phage potency of 9.88 (lg (PFU/mL)). The burst size of phage SP-CmSa-11 is approximately 130 PFU/cells in *S. aureus* CmSa-11.

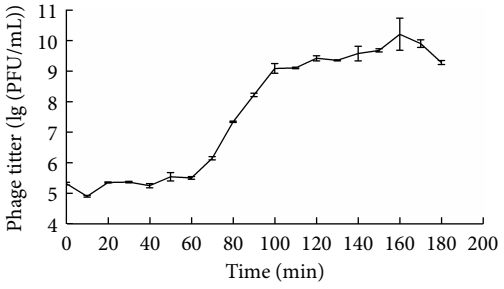
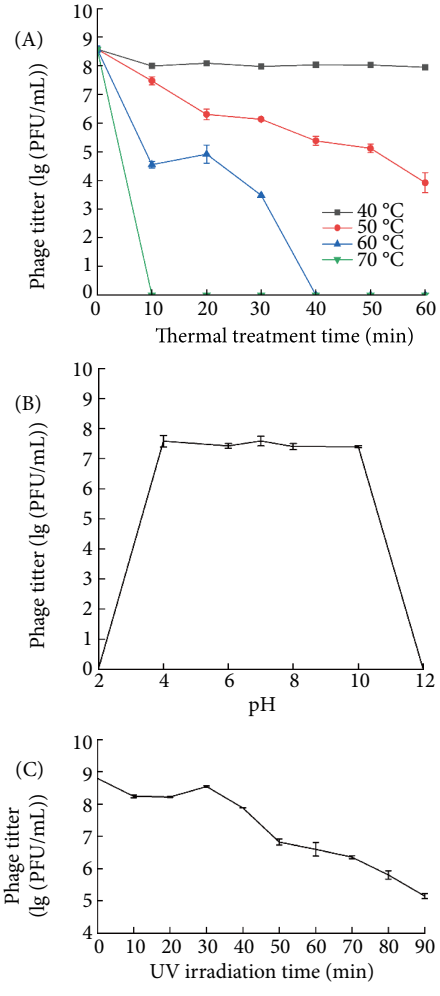


Figure 4 One-step growth curve of SP-CmSa-11.

3.6 Phage stability

The exploitation of the phage as an antibacterial agent requires a wide range of environmental adaptations. SP-CmSa-11 maintained stable activity at 40 °C (Figure 5A). However, the thermal stability of SP-CmSa-11 was gradually reduced at 50 °C for 1 h. An order of magnitude decrease occurred after 50 °C, especially at 60 °C, and it was inactivated after 10 min of incubation at 70 °C. SP-CmSa-11 maintained stable activity from pH 4 to 10, and lost their activity at pH 2 and 12. (Figure 5B). The results of UV tolerance experiments demonstrated that the phage titer decreased slowly after exposure to UV irradiation (Figure 5C). After 90 min of UV irradiation, the titer of SP-CmSa-11 decreased from 8.24 to 5.80 (lg (PFU/mL)). Similarly, SP-CmSa-11 exhibited good stability to chloroform (Figure 5D). As the chloroform concentration increased from 0% to 75%, the phage titer decreased about 1.5 (lg (PFU/mL)).



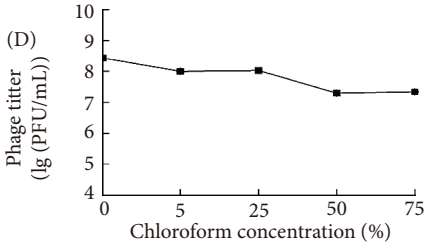


Figure 5 Biological characteristics of SP-CmSa-11 on (A) thermal stability, (B) pH, (C) UV irradiation, and (D) chloroform tolerance. Values are the mean of three determinations.

3.7 Genome sequencing and analysis of phage

The genomic characteristics of SP-CmSa-11 were elucidated using whole genome sequencing and a comparative genomic circle map of SP-CmSa-11 was shown in Figure 6. The gene structure of SP-CmSa-11 is a double-stranded DNA with a sequenced full length of 45 816 bp, with the longest contiguous sequence of 27 267 bp and a G + C content of 27.23%. The whole genome of SP-CmSa-11 encodes a total of 59 open reading frames (ORFs). Among 59 predicted ORFs, 41 encoded hypothetical proteins with unknown functions. Function proteins (18 ORFs) include endolysin, putative holin, putative tail fiber protein, putative peptidoglycan hydrolase, tail protein, minor structural protein, putative tape measure protein, major tail protein, putative head-tail joining, putative DNA packaging protein, major head protein, putative scaffold protein, head protein, putative portal protein, putative terminase large subunit, putative terminase small subunit, putative helicase, and putative ssDNA binding protein. No virulence genes, resistance genes, and integrase genes were found. Based on genome comparison, the whole-genome alignment shows that SP-CmSa-11 is most similar to *Staphylococcus* phage vB_SauS-philPLA88, which was similarly isolated from a milk source[26]. There is some similarity but not complete overlap between the whole genomes of SP-CmSa-11 and *Staphylococcus*

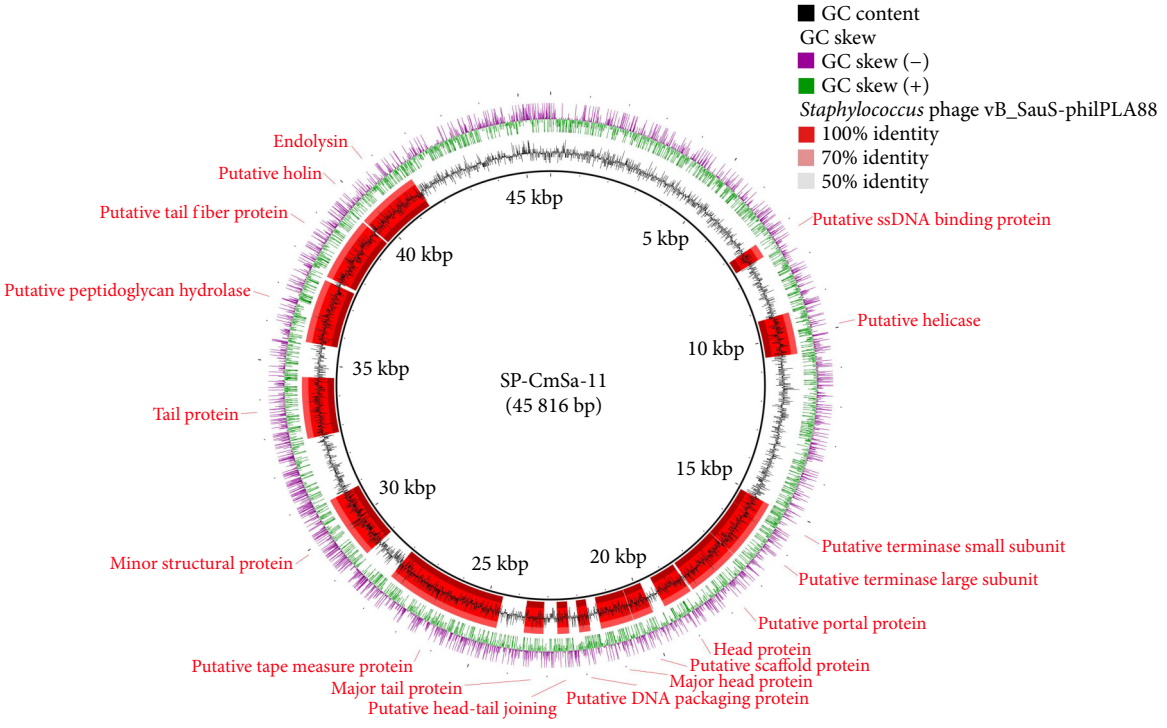


Figure 6 Functional annotation circle based on whole-genome analyses of *S. aureus* phages. The whole-genome circle diagram is annotated with gene annotation results in distinct colors, corresponding to functions in the legend.

phage vB_SauS-philPLA88, suggesting that SP-CmSa-11 is likely to be a newly discovered lytic *S. aureus* phage.

To further analyze the evolutionary origin of SP-CmSa-11, the sequences of terminase large subunit was analyzed using MEGA 11 software to construct a phylogenetic tree (Figure 7). According to the results, SP-CmSa-11 was also most closely related to *Staphylococcus* phage vB_SauS-philPLA88 (EU 861004.1).

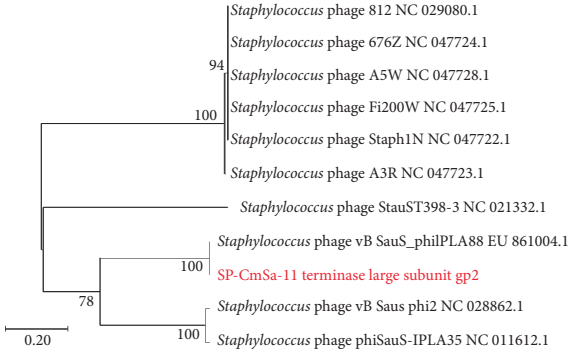


Figure 7 Phylogenetic tree constructed based on the sequences of the terminase large subunit of SP-CmSa-11. The genomic sequences of these phages were compared based on the ClustalW comparison algorithm, and a phylogenetic tree were analyzed using MEGA 11 software.

3.8 Phage bactericidal efficacy *in vitro*

The antimicrobial activity of SP-CmSa-11 against its host bacteria was evaluated in the LB broth (Figure 8A). The results showed that the bacterial levels were significantly reduced after 240 min of the phage treatment compared to the control group. After 72 h cultured, the broth in the phage-treated group was always clear, while the control group became very cloudy (Figure 8B). The good bactericidal activity revealed that SP-CmSa-11 is a strong candidate with potential for biological control of *S. aureus*.

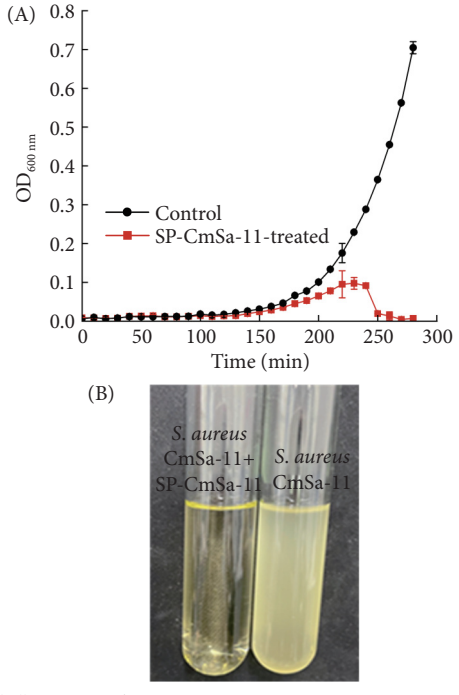


Figure 8 Challenge assay of SP-CmSa-11 against *S. aureus* CmSa-11 in (A) 280 min and (B) 72 h. Bacterial culture was infected with the phage at a MOI of 0.1 and incubated at 37 °C for 280 min. Phage was treated 30 min after microbial inoculation and optical density was measured at 600 nm. All experiments were performed in triplicate.

3.9 Phage bactericidal efficacy in milk

The efficacy of SP-CmSa-11 in reducing viable counts of *S. aureus* CmSa-11 in milk was shown in Figure 9. In the control group, viable counts of *S. aureus* CmSa-11 increased gradually and reached 11.53 (lg (CFU/mL)) after 8 h of incubation. Conversely, the application of SP-CmSa-11 obviously inhibited the host bacterial counts in the phage-treated group of incubation in milk at 37 °C for 24 h. Though the inhibitory effect of the SP-CmSa-11 was gradually weakened after 24 h, the viable counts was still less than the control group.

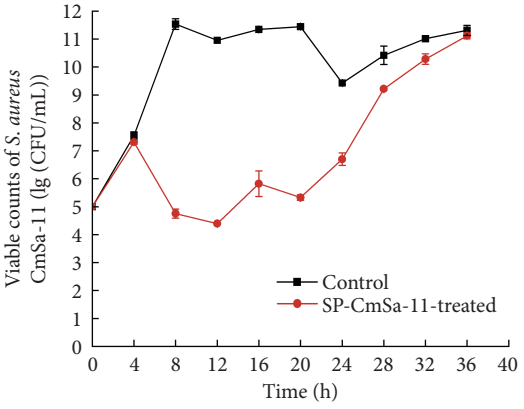


Figure 9 Antibacterial effect of SP-CmSa-11 in milk. The bacterial inhibition ability of SP-CmSa-11 was assessed against *S. aureus* in milk with the phage at a MOI of 0.1.

4 Discussion

Antibiotic resistance is considered one of the most serious threats to global public health security[27]. In this context, the biological control of foodborne pathogens based on phages has attracted more and more attention because of its safety and efficiency. In this study, SP-CmSa-11 was isolated and purified from the dairy farm, which can lyse *S. aureus*. As is known to all, mastitis caused by *S. aureus* and poor hygienicprocessing conditions are the most important sources of dairy product contamination. Growth of enterotoxigenic *S. aureus* in both raw milk and dairy products poses a potential health hazard to consumers[26]. Therefore, new biocontrol strategies to prevent growth of *S. aureus*, suitable to be applied in the food industry, are being explored. SP-CmSa-11 isolated in this study has stable properties and good bactericidal efficacy to *S. aureus*, which provides evidence for the subsequent application in food sterilization.

Notably, for treatment purposes, only virulent phages are utilized, as their replication invariably results in the lysis of bacterial cells. Virulent phages are distinct from temperate phages, as they are unable to integrate into the genome of the infected bacterium, which means they do not contribute to the horizontal transfer of genes[28]. According to the nomenclature of the ICTV, all currently known *S. aureus* phages belong to the order Caudovirales and are classified into 11 genera. Among these, four genera are classified under the Herelleviridae family (formerly a part of Myoviridae). Myoviridae phages generally have strong lytic[28], which has a an icosahedral capsid (diameter about 90 nm) and a long contractile tail (170–240 nm) ending in a basal plate[29]. The isolated SP-CmSa-11 in the present study matched the above morphological features.

In terms of growth factors, SP-CmSa-11 has a latent period of 60 min, which can infect 22 tested strains, indicating the host range of SP-CmSa-11 is rather broad. This relatively wider host range

suggests a potential high application of SP-CmSa-11 in different environments. Determining the growth curve of phages is crucial for understanding their biological characteristics and has practical implications for their applications. The short lysis period and large burst size allow phages to be used more efficiently. It has been reported that the burst size of *S. aureus* phage vB_SauS_JS02 was 52 PFU/cell[30], while the *S. aureus* phages SAJK-IND and MSP could only lysed (44 ± 3) and (25 ± 5) PFU/cell[31]. Compared with the reported phages, SP-CmSa-11 has a larger burst size of 130 PFU/cell, suggested that SP-CmSa-11 had robust replicative abilities and an extended duration of activity, which is one of the advantages of being used as a biocontrol agent because a high level of phage progenies will be produced per infection cycle for the subsequent infection[22].

The possible adverse environment factors of phage application as a biological agent for foodborne pathogens control are mainly temperature, pH, UV light, and organic solvent. As reported in previous literature, the first requirement for the successful application of phages for biocontrol of bacteria is phage stability[32]. High stability of phages means more application scenarios[33]. In this study, SP-CmSa-11 can maintain infectivity in a wide range of pH values (4.0–10.0), which had good acid tolerance compared to the *S. aureus* phage SauPS-28 (pH 6.0–9.0)[34]. The UV stability of SP-CmSa-11 was also very good, which means the phage is suitable for environmental application or storage. Similarly, SP-CmSa-11 exhibited relatively good stability to chloroform. SP-CmSa-11 was inactivated in 70 °C for 10 min or at 60 °C for 40 min. and this temperature is very suitable for pasteurization of milk. Therefore, its characteristic of temperatures no more than 60 °C, wide pH range stability, UV and organic solvent stability, and fast lysis capacity make it suitable for dairy farm environments and raw milk.

Phage can efficiently eliminate a certain type of bacteria in the environment without affecting other microorganisms. However, several environmental safety risks should be considered in phage application, especially the transfer or drift of antibiotic resistance genes, virulence genes, and other genetic factors[23]. The genomic analysis results showed that there was no tRNA, antibiotic resistance, virulence, or integrase genes contained in the SP-CmSa-11 genome, indicating that SP-CmSa-11 was safe and reliable in practical applications.

The risk of biological contamination of cow's milk derives mainly from cattle milking due to the exposure of udders to the environment, equipment, storage, dirty pipes, and others[35]. According to previous research studies, *S. aureus* has been detected in milk, and other food sources[36–37], posing a threat to human health. Due to its high antibiotic resistance, there is a need to reconsider the utilization of safe and environmentally friendly phage preparation for the prevention and control of *S. aureus*. In the present study, we investigated the potential application of SP-CmSa-11 in inhibiting the growth of *S. aureus* CmSa-11 in LB medium. SP-CmSa-11 showed good bactericidal ability *in vitro*, and was able to completely lyse the host bacteria at 280 min. And it can maintain bactericidal activity for at least 72 h. The results demonstrated that SP-CmSa-11 could effectively suppress the proliferation of *S. aureus* CmSa-11, indicating its strong infection potential. Furthermore, in milk, SP-CmSa-11 exhibited certain efficacy in inhibiting *S. aureus* CmSa-11 for 20 h at 37 °C. After 24 h, *S. aureus* in milk showed an increasing trend. The bactericidal effectiveness of SP-CmSa-11 decreased in milk than LB medium, which may be attributed to the organic substances such as proteins, fat content, inhibitory substances, and the conditions of food matrix and viscosity[38]. The basic mechanisms involved in the phage infection process in complex systems such as milk and dairy products still need to be clarified in the future.

5 Conclusion

In summary, we obtained a novel lytic phage, SP-CmSa-11, with lytic activity against *S. aureus*. Based on characterization experiments and genomic analysis, SP-CmSa-11 has a short latent period and good stability. It does not contain antibiotic resistance genes or virulence factors. SP-CmSa-11 could effectively reduce the abundance of *S. aureus* in LB medium and milk, indicating its potential to control *S. aureus* in contaminated milk and other foods. This study provided an important potential biocontrol agent for food industry.

Author contributions

Jinli Wang: data curation, visualization, investigation, writing-original draft preparation, writing-reviewing, and editing; **Yun Qu:** validation, investigation, writing-reviewing, and editing; **Hongmei Yin:** validation, writing-reviewing, and editing; **Anjian Liang:** methodology and investigation; **Yu Fu:** review, editing, and validation; **Chenglin Zhu:** conceptualization, methodology, writing-review, and editing; **Junni Tang:** conceptualization, writing-reviewing, editing, supervision, and funding acquisition.

Conflict of interest

The authors declare no conflicts of interest.

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