Isolation and characterization of a broad-spectrum phage SapYZU11 and its potential application for biological control of *Staphylococcus aureus*

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Received: 16 November 2022; Accepted: 15 January 2023; Published: 1 April 2023

RESEARCH ARTICLE

Abstract

Staphylococcus aureus, a prominent pathogen, is frequently encountered in clinical and food-processing settings. Given its ability to develop antimicrobial resistance, effective control strategies are required to ensure microbial safety. In this study, four lytic *S. aureus* phages (SapYZU10, SapYZU11, SapYZU12, and SapYZU13) were isolated from sewage samples in Yangzhou, China. Their biological characteristics and bactericidal effect against *S. aureus* isolates *in vitro* and in milk and fresh pork were evaluated. Their activities remained relatively stable under stressful conditions (-80–70°C, pH 3.0–12.0). Notably, SapYZU11 (100%, 53/53) effectively lysed all 53 *S. aureus* strains, followed by SapYZU12 (90.57%, 48/53), SapYZU13 (79.25%, 42/53), and SapYZU10 (71.70%, 38/53). Among the phages with short latent periods (10–20 min), SapYZU11 had a larger burst size (152.00 plaque forming units [PFU]/mL) and no genes related to antibiotic resistance and virulence. Furthermore, SapYZU11 effectively eradicated *S. aureus* and its cocktail (YZUsa1, YZUsa4, YZUsa12, YZUsa14, and methicillin-resistance *S. aureus* (MRSA) JCSC 4744) in Luria–Bertani broth and both food items. Particularly in milk, SapYZU11 with a multiplicity of infection (MOI) of 100 inhibited MRSA JCSC 4744 strain and *S. aureus* cocktail with maximum reduction levels of 5.03 log (Lg) colony-forming unit (CFU)/mL and 2.80 Lg CFU/mL, respectively. Conversely, contaminated pork treated with three MOIs of SapYZU11 at 25°C and 4°C resulted in reductions of 0.29–1.29 Lg CFU/mL and 0.11–0.32 Lg CFU/mL, respectively. Therefore, SapYZU11 proved as a promising biocontrol agent against *S. aureus* in different food production settings.

Keywords: bacteriophage; biocontrol; food security; milk; *Staphylococcus aureus*

Introduction

In this fast-paced era, animal-derived food products are widely popular among consumers (Mahros *et al.*, 2021). However, foods such as milk and meat, which are rich in proteins and nutrients, promote stubborn bacterial growth, potentially contaminating food products (Iyer *et al.*, 2021). Such frequent and uncontrolled bacterial contamination causes a significant economic burden and poses a threat to public health (Zhou *et al.*, 2018b). *Staphylococcus aureus* is a Gram-positive pathogen that causes a variety of diseases, including skin infections, respiratory infections, and food poisoning in animals and humans (Su *et al.*, 2020). Moreover, because of antibiotic overuse, *S. aureus* has gradually evolved as a pathogen, such as methicillin-resistance *S. aureus* (MRSA), with the ability to cause life-threatening infections spreading rapidly worldwide (Samir *et al.*, 2022). A recent conducted survey of food products, covering most of the provincial capitals of China, revealed that high prevalence of *S. aureus* was found in meat products as well as pasteurized milk (Zhang *et al.*, 2022a). Furthermore, *S. aureus*

ISSN 1757-837X online, DOI 10.15586/qas.v15i2.1238

accounts for approximately 11.26% of outbreaks caused by important foodborne pathogens in China (Wu *et al.*, 2018). Considering that the use of antibiotics and chemical agents affects the flavor of food or even has adverse effects (Nikolic *et al.*, 2020; Ravindran and Jaiswal, 2019) and that the development of new antibiotics is difficult, it is necessary to find alternative options to control *S. aureus* in food applications.

Bacteriophages (phages), which are ubiquitous viruses, can be isolated from river water, soil, farms, and domestic sewage and can effectively lyse bacterial cells (Bhetwal *et al.*, 2017). In recent years, phages have gradually come to be known as natural and effective bactericidal substitutes because of the low efficiency of potent antibiotics in treating bacterial diseases, and hence play an important role in the prevention and control of foodborne pathogens (Pang *et al.*, 2019; Santos and Azeredo, 2019). Some advantages of phages include target specificity protecting the microbiota of the host, capacity to multiply at the site of infection, and low production costs (Monteiro *et al.*, 2019). The safety and efficacy characteristics of phages were proven in several human and animal models with bacterial infections (Miedzybrodzki *et al.*, 2012; Takemura-Uchiyama *et al.*, 2014). Nonetheless, the isolation and screening of phages having a broad-spectrum effect and high lysis ability remains one of the primary paths to control *S. aureus* in food products.

In this study, highly lytic *S. aureus* phages were isolated to analyze their biological characteristics. To confirm the potential of phages against *S. aureus*, their bactericidal effects were evaluated *in vitro* and food matrices (milk and fresh pork).

Materials and Methods

Strains and culture conditions

This study used 62 bacterial strains of different genera (Table 1). A total of 47 *S. aureus* strains (YZUsa 1–47) were isolated from 267 nonduplicate samples collected from three swine farms, two slaughter houses, and four markets (Yangzhou, China) from October 2019 to May 2020. The 267 samples included swine stool swabs (n = 30), swine nose swabs (n = 30), dust swabs (n = 30), swine carcass swabs (n = 40), and pork swabs (n = 137). All swab samples were collected using sterile dry swabs (Amies Agar Transport Swabs, Copan, Italy) as described previously and transported to laboratory within 2 h (Zhou *et al.*, 2018a). All samples were screened for *S. aureus* isolates as described by Li *et al.* (2015) and confirmed by polymerase chain reaction (PCR) sequencing of products targeting the Staphylococcus-specific 16S ribosomal RNA (rRNA). Following other strains were used in this study: seven standard isolates, namely, *S. aureus* American Type Culture Collection (ATCC) 25923 and ATCC 29213, *Staphylococcus epidermidis* ATCC 12228, *Escherichia coli* China Center of Industrial Culture Collection (CICC) 10664, *Salmonella* E*nteritidis* CICC 21513, *Listeria monocytogenes* ATCC 1911, and *Vibrio mimicus* CICC 21613; four clinical MRSA isolates, namely, N315, MRSA85/2082, JCSC 4744, and WZ153; and three Staphylococcal strains, namely, *Staphylococcus caprae* SD2 and *S. epidermidis* YIC and SD1. All strains were obtained from the Key Laboratory of Prevention and Control of Biological Hazard Factors, Yangzhou University (Jiangsu, China).

Isolation and propagation of phages

S. aureus phages were isolated from sewage samples collected between December 2019 and May 2020 from Yangzhou, China, using the previously described method (Zhou *et al.*, 2021). In brief, 50 mL of the collected sewage was separated at 8,000 × *g* for 10 min, and the supernatant liquid was collected through 0.45- and 0.22-μm membranes (Sangon Biotech Co. Ltd., Shanghai, China). A 3-mL phage filtrate and a 100-μL host bacteria with stable growth were suspended in a double Luria–Bertani (LB) broth (2 ×LB; Hope Bio-Technology Co. Ltd., Qingdao, China), and incubated at 120 revolutions per minute (rpm) and 37°C for 24 h. The presence of phage was confirmed using the spot assay, and the high-titer phage suspensions of at least three enrichment processes were purified. Phages were diluted at different concentrations using saline magnesium (SM) dilution buffer. The host bacteria were mixed with phages and added to 5 mL of soft-top agar (LB containing 0.4% [w/v] agar), followed by pouring of the mixture into solid nutritional agar plates (LB containing 1.5% [w/v] agar) for overnight incubation. Large and round transparent patches were mixed in the buffer and a double layer was created; a pure phage solution was obtained after several cycles. The phage titers were determined in plaque forming units (PFU/mL) as described by Zhou *et al.* (2021).

Transmission electron microscopy analysis of phages

Transmission electron microscopy (TEM; Tecnai 12; Tecnai, Eindhoven, Netherlands) was used to examine the morphology of phages (Zhou *et al.*, 2021). The phages were concentrated by isokinetic centrifugation using the cesium chloride density gradient method and incubated in a copper mesh. After 5 min, 2% phosphotungstic acid (Sigma-Aldrich Trading Co. Ltd., Shanghai, China) was added and allowed to dry naturally.

Table 1. Host range of SapYZU10, SapYZU11, SapYZU12, and SapYZU13.

No.	Strain	Species	Location	Source	Lysis performance			
					SapYZU10	SapYZU11	SapYZU12	SapYZU13
1.	YZUsa1	*S. aureus*	Swine farm	Stool	+++	+++	+++	+++
2.	YZUsa2	*S. aureus*	Swine farm	Stool	–	+++	+	–
3.	YZUsa3	*S. aureus*	Swine farm	Stool	+++	+++	+++	+++
4.	YZUsa4	*S. aureus*	Swine farm	Stool	+++	+++	+++	+++
5.	YZUsa5	*S. aureus*	Swine farm	Dust	++	+++	++	+
6.	YZUsa6	*S. aureus*	Swine farm	Nose	+++	+++	+++	+++
7.	YZUsa7	*S. aureus*	Swine farm	Nose	–	+	+++	+
8.	YZUsa8	*S. aureus*	Slaughter house	Nose	–	+++	–	–
9.	YZUsa9	*S. aureus*	Slaughter house	Nose	–	+	+	–
10.	YZUsa10	*S. aureus*	Slaughter house	Nose	–	+	+	–
11.	YZUsa11	*S. aureus*	Slaughter house	Nose	–	+++	++	–
12.	YZUsa12	*S. aureus*	Slaughter house	Nose	+++	+++	+++	+++
13.	YZUsa13	*S. aureus*	Slaughter house	Nose	–	+	++	+
14.	YZUsa14	*S. aureus*	Slaughter house	Nose	+++	+++	+++	+++
15.	YZUsa15	*S. aureus*	Slaughter house	Nose	–	+++	+	–
16.	YZUsa16	*S. aureus*	Slaughter house	Nose	+++	+++	+++	+++
17.	YZUsa17	*S. aureus*	Slaughter house	Nose	+++	+++	+++	+++
18.	YZUsa18	*S. aureus*	Slaughter house	Nose	+++	+++	+++	+++
19.	YZUsa19	*S. aureus*	Slaughter house	Nose	+++	+++	+++	+++
20.	YZUsa20	*S. aureus*	Slaughter house	Nose	+++	+++	+++	+++
21.	YZUsa21	*S. aureus*	Slaughter house	Nose	+++	+++	+++	+++
22.	YZUsa22	*S. aureus*	Slaughter house	Carcass	++	+++	++	+++
23.	YZUsa23	*S. aureus*	Slaughter house	Carcass	+++	+++	+++	+++
24.	YZUsa24	*S. aureus*	Market	Pork	+++	+++	+++	+++
25.	YZUsa25	*S. aureus*	Market	Pork	+	+++	+	+
26.	YZUsa26	*S. aureus*	Market	Pork	+	+++	+	+
27.	YZUsa27	*S. aureus*	Market	Pork	–	+	–	+
28.	YZUsa28	*S. aureus*	Market	Pork	–	++	+++	–
29.	YZUsa29	*S. aureus*	Market	Pork	+	+	++	+
30.	YZUsa30	*S. aureus*	Market	Pork	–	+	–	–
31.	YZUsa31	*S. aureus*	Market	Pork	–	+	++	+
32.	YZUsa32	*S. aureus*	Market	Pork	+++	+	+++	+
33.	YZUsa33	*S. aureus*	Market	Pork	+++	+	+++	+
34.	YZUsa34	*S. aureus*	Market	Pork	++	+	+++	+
35.	YZUsa35	*S. aureus*	Market	Pork	+++	+	++	+
36.	YZUsa36	*S. aureus*	Market	Pork	+++	+	++	+
37.	YZUsa37	*S. aureus*	Market	Pork	+++	+	+++	+
38.	YZUsa38	*S. aureus*	Market	Pork	+++	+++	+++	+++
39.	YZUsa39	*S. aureus*	Market	Pork	–	+	–	–
40.	YZUsa40	*S. aureus*	Market	Pork	+++	+++	+++	+++
41.	YZUsa41	*S. aureus*	Market	Pork	++	+++	+++	+++
42.	YZUsa42	*S. aureus*	Market	Pork	+++	+	+++	+
43.	YZUsa43	*S. aureus*	Market	Pork	+	+++	+++	+++
44.	YZUsa44	*S. aureus*	Market	Pork	+	+	+++	+
45.	YZUsa45	*S. aureus*	Market	Pork	+++	++	+++	++
46.	YZUsa46	*S. aureus*	Market	Pork	++	+++	+	+

(*Continues*)

Table 1. Continued.

No.	Strain	Species	Location	Source	Lysis performance			
					SapYZU10	SapYZU11	SapYZU12	SapYZU13
47.	YZUsa47	*S. aureus*	Market	Pork	+	++	+++	+++
48.	MRSA N315	*S. aureus*	Hospital	Human	+++	++	++	++
49.	MRSA 85/2082	*S. aureus*	Hospital	Human	–	++	++	–
50.	MRSA JCSC 4744	*S. aureus*	Hospital	Human	+	+++	++	++
51.	MRSA WZ153	*S. aureus*	Hospital	Human	+	++	++	+
52.	ATCC 25923	*S. aureus*	-	-	–	+	–	–
53.	ATCC 29213	*S. aureus*	-	-	+	+++	+	+
54.	ATCC 12228	*S. epidermidis*	-	-	–	+	–	–
55.	YIC	*S. epidermidis*	-	Pickle	–	–	–	–
56.	SD1	*S. epidermidis*	-	Pickle	–	–	–	–
57.	SD2	*Staphylococcus caprae*	-	Pickle	–	–	–	–
58.	CICC 10664	*Escherichia coli*	-	CICC	–	–	–	–
59.	Eh-YZU05	*Enterobacter hormaechei*	-	Pork	–	–	–	–
60.	CICC 21513	*Salmonella*	-	CICC	–	–	–	–
61.	ATCC 1911	*Listeriamonocytogenes*	-	ATCC	–	–	–	–
62.	CICC 21613	*Vibrio mimicus*	-	CICC	–	–	–	–

"+++" indicates clear and translucent plaque.
"++" indicates a slightly turbid plaque.
"+" indicates plaque turbidity.
"–" indicates no plaque.
ATCC: American Type Culture Collection; CICC: China Center of Industrial Culture Collection.

Multiplicity of infection of phages

As described by Luo *et al.* (2021), the phage solution diluted into different multiples was dispersed in host bacterial suspension (multiplicity of infection [MOI] = 100, 1, 0.1, 0.01, and 0.001), and the phage dilution ratio with a relatively high titer was used as the best MOI for the phage.

One-step growth curve

The experimental method for phage growth in host bacteria is based on previous studies with some adjustments (Luo *et al.*, 2021). Phages stock dilutions (100 µL, 10^7 PFU/mL) were dispersed onto the pre-prepared *S. aureus* ATCC 29213 suspension (100 µL, 10^8 colony-forming unit [CFU]/mL) and were allowed to rest for 10 min. The adsorbent was centrifuged using the same procedure, and the precipitate was resuspended in 5-mL LB broth. The cultures were aspirated at 0 min, and then at 10, 20, 30, 40, 50, 60, 75, 90, 105, 120, 135, 150, 165, 180, 200, 220, and 240 min to determine their titers. The burst size was calculated by dividing the titer of the stably growing phage by the average of the original titer.

Thermal and pH stabilities

The phages were studied for their ability to survive at different temperatures and pH values. To measure thermal stability, 100 µL of phage suspension (10^9 PFU/mL) was transferred into 900-µL fresh LB broth, incubated at –80, –40, –20, 40, 50, 60, and 70°C and collected after 20 min, 40 min, and 1 h.

For estimating pH stability, the phage suspension was added to fresh LB broth with different pH values (pH was adjusted from 2.0 to 12.0 using HCl or NaOH) to a final concentration of 10^8 PFU/mL. Subsequently, the culture was incubated at 37°C for 1 h. After respective temperature and pH experiments, the phage titer was also determined using the double-layer agar method.

Lytic spectra of phages

Bacterial strains (n = 62) were dispersed in a double-layer dish, solidified to allow each phage solution (10^8 PFU/mL, 10 µL) to be dropped vertically onto the surface. Sodium chloride, Magnesium sulphate, and gelatine (SM) buffer was selected as a blank control and incubated at 37°C.

The resulting plaque brightness was divided into four different grades (Li *et al.*, 2021b) as shown in Table 1: clear and translucent (+++), slightly turbid (++), turbidity (+), and no plaque (–).

Bactericidal activity of phage against *S. aureus* in LB broth

Phage activity in the bacterial challenge test was determined by the 96-well plate culture method (Alves *et al.*, 2014). The concentration of each of the *S. aureus* strain suspension (ATCC 29213, YZUsa1, YZUsa4, YZUsa12, YZUsa14, and MRSA JCSC 4744) was adjusted to 10^6 CFU/mL, and five *S. aureus* strains (YZUsa1, YZUsa4, YZUsa12, YZUsa14, and MRSA JCSC 4744) were selected to be mixed in equal volumes to prepare *S. aureus* cocktail. Thereafter, *S. aureus* or *S. aureus* cocktail was thoroughly mixed with phage (MOI = 0.01, 1, and 100) in equal proportions to make phages and bacterial mixtures. A 96-well plate containing 198-μL LB broth was supplemented with 2 μL of phages and bacterial mixtures, and statically cultured at 37°C for 24 h; the absorbance was measured at 600 nm every 3 h.

Genomic analysis of phage SapYZU11

The extracted phage genomic DNA was sequenced on an Illumina sequencer (Illumina, San Diego, CA, USA), and the sequencing results were processed according to the method described by (Zhou *et al.* (2021). Open reading frames (ORFs) were predicted using software packages (FGENESB, Glimmer, and GeneMarkS) and annotated based on the "NCBI Prokaryotic Genome Automatic Annotation Pipeline" and "eggNOG" functions. Transfer RNA (tRNA)-encoding genes were screened using tRNA Scan-SE. The completed phage genome sequence of SapYZU 11 was deposited in GenBank (accession number MW864250).

Bactericidal activity of phage against S. aureus in skimmed milk

The effect of purified phage on the growth of *S. aureus* in skimmed milk was measured according to previously described methods with certain modifications (Li *et al.*, 2021a). Ultra-high temperature (UHT) skimmed milk was used in the current study to evaluate the effect of phage on *S. aureus*. Skimmed milk, 10 mL, was inoculated with 100-μL MRSA JCSC 4744 or *S. aureus* cocktail (10^6 CFU/mL), followed by treatment with 100 μL of phage solution (MOI = 0.01, 1, and 100) or SM buffer. This was then incubated with a static culture at 25°C or 4°C. Cultures (1 mL) were removed for bacterial counting at 0, 3, 6, 9, 12, 24, and 48 h (25°C) or on daily basis (7 days, 4°C).

Bactericidal activity of phage against *S. aureus* in fresh pork

The method as described by Li *et al.* (2021b) with minor modifications was used to measure the effect of phages on the growth of *S. aureus* in fresh pork. In order to prevent the interference of microbiota, fresh pork samples of 1 × 1 × 1 cm size were washed with 70% ethanol and treated with ultraviolet radiation (UV) for 1 h. The *S. aureus* cocktail (10^6 CFU/mL, 100 μL) was evenly distributed on the surface of each meat piece, followed by a stable attachment and addition of the same amount of phage solution (MOI = 0.01, 1 and, 100) in the same position. Finally, the samples were cultured in constant temperature incubators at 25°C and 4°C. A standard plate count was performed at 0, 3, 6, 9, 12, 24, and 48 h (25°C) or on daily basis (7 days, 4°C) to quantify the surviving cells.

Statistical analysis

All tests in this study were performed in triplicate. Results are presented as mean values, and variances were determined by standard deviation from mean values (Zhou *et al.*, 2021). The SPSS software with two-way analysis of variance (IBM-Armonk, New York, USA) was used to calculate the significance of data, and performed to determine differences between control and treatment groups (thermal and pH stability, inhibition assays *in vitro*, and food matrix). $P < 0.05$ was considered statistically significant.

Results

Isolation and morphological analysis of phages

Four phages (SapYZU10, SapYZU11, SapYZU12, and SapYZU13) infecting the host *S. aureus* ATCC 29213 were obtained from the sewage samples of Yangzhou, China. TEM results (Figures 1A–1D) indicated that the head diameters of SapYZU10 and SapYZU11 phages measured 41.61 ± 3.39 nm and 26.75 ± 2.69 nm, respectively. SapYZU10 and SapYZU11 phages had similar short tails (13.56 ± 1.96 nm and 28.55 ± 2.03 nm, respectively), and belonged to the *Podoviridae* family. Phages SapYZU12 and SapYZU13 had an icosahedral head (75.47 ± 2.36 nm and 74.77 ± 5.32 nm, respectively) attached to a contracted tail (182.33 ± 11.26 nm and 104.05 ± 9.23 nm, respectively). These morphological characteristics indicated that phages SapYZU12 and SapYZU13 belonged

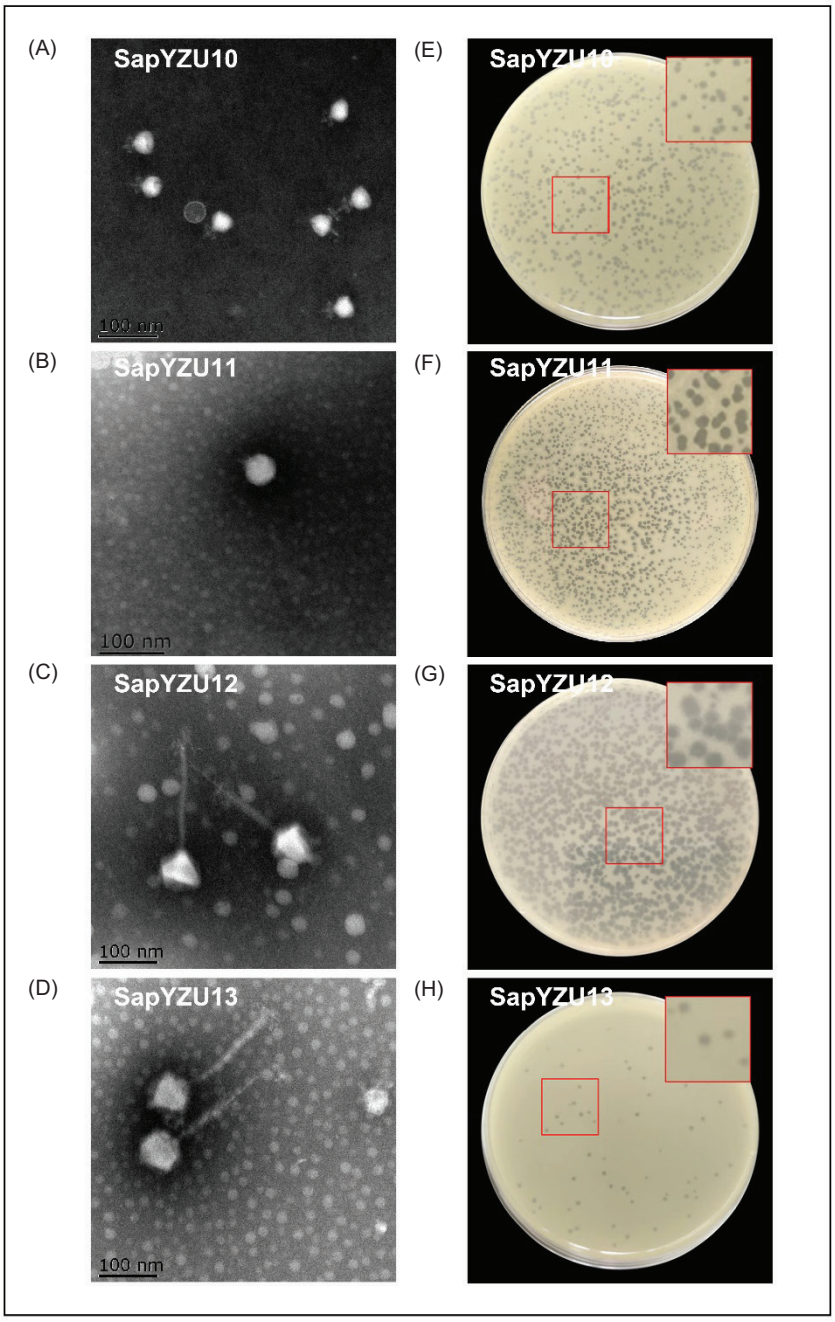

Figure 1. (A) Electron microscopy and (B) plaque morphology of *S. aureus* phages (SapYZU10, SapYZU11, SapYZU12, and SapYZU13). Images of phage plaques are magnified three times and shown with red squares.

to the *Myoviridae* family. Additionally, phage patches formed by the four mentioned phages were different, their diameters were more than 1 mm, and their sizes were uniform (Figures 1E–1H).

Optimal MOI and one-step growth of phages

When the four phages were in an optimal MOI, the titers produced were above 10^8 PFU/mL, especially for SapYZU11, in which titer was as high as 1.6×10^9 PFU/mL (Figures 2A–2D). As shown in Figures 2E–2H, the incubation periods of phages SapYZU10, SapYZU11, and SapYZU12 were 20 min, but their burst sizes were different. Compared to SapYZU10 (63.01 PFU/infected cell) and SapYZU12 (61.23 PFU/infected cell), SapYZU11 had the highest burst size of 152.00 PFU/infected cell. Although SapYZU13 had a short incubation period (10 min), its burst size (59.42 PFU/cell) was lowest of the four phages.

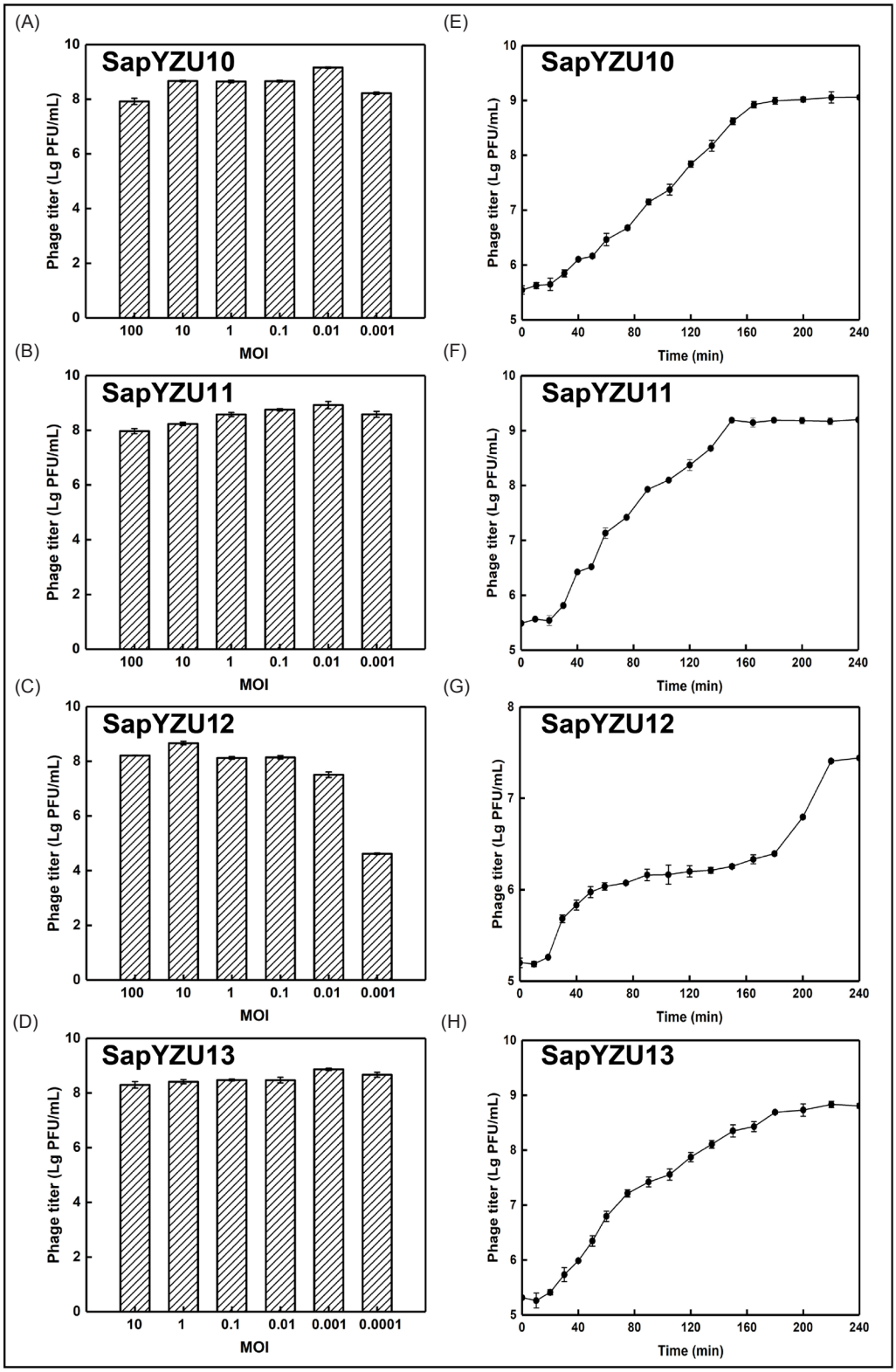

Figure 2. (A) Titer change of S. aureus phage under six multiplicity of infection (MOIs). (B) One-step growth curve of phages with an MOI of 0.1 with *S. aureus* ATCC 29213 as host bacterium.

Thermal and pH stability of phages

The tolerance to distinct temperatures and pH conditions of phages are shown in Figure 3. Results showed no reduction in titers when the four phages were incubated between −80°C and −20°C, remaining at 8.85, 8.92, 8.64, and 7.79 log (Lg) PFU/mL. The titers of phages SapYZU10, SapYZU11, and SapYZU12 decreased by 0.74, 0.62, and 0.67 Lg PFU/mL, respectively, when incubated between 40°C and 50°C (P< 0.05) whereas the titers of SapYZU13 stabilized at 7.7 Lg PFU/mL. With the temperature rising to 60°C, activity of the four phages continually reduced by 1.26, 1.99, 1.86, and 2.40 Lg PFU/mL, respectively (P < 0.05). The thermal inactivation kinetics of SapYZU11 and SapYZU13 were similar when incubated at 70°C for 20 min and their titers decreased to 4.90 Lg PFU/mL and 2.95 Lg PFU/mL, respectively, whereas phages SapYZU12 and SapYZU13 were inactivated at 70°C. However, SapYZU11 was inactivated after incubation at 70°C for 40 min and longer, and only SapYZU10 retained a fraction of its activity after 1-h incubation, with a titer of 3.89 Lg PFU/mL (Figures 3A–3D). In addition, the results of acid–base solution on the growth of four phages showed that they retained a stable titer (8.13, 8.20, 7.91, and 7.52 Lg PFU/mL) from pH 3.0 to pH 12.0 for 1 h (Figures 3E–3H).

Lytic spectrum of phages

Upon testing the phages against 62 common bacterial strains, including 53 *S. aureus* (Table 1), the results showed that SapYZU11 had the widest lytic spectrum, lysing 100% of the tested *S. aureus* strains and one *S. epidermidis* strain. Notably, 29 *S. aureus* (+++) strains were sensitive to SapYZU11, six *S. aureus* strains (++) showed slightly turbid plaques, and the remaining phage plaques were very cloudy (+). Compared to phage SapYZU12 with a lysis rate of 90.57% (48/53), SapYZU13 and SapYZU10 lysed only 79.25% (42/53) and 71.70% (38/53) of *S. aureus* strains, respectively. However, all four phages were unable to lyse bacteria other than *S. aureus* and *S. epidermidis*.

Phage SapYZU11 treatment in LB broth

The effects of four phages on *S. aureus* ATCC 29213 in LB broth are shown in Figures 4A, 4B, and Supplementary Figures S1 and S2. The optical density (OD_{600nm}) value of *S. aureus* ATCC 29213 without phage treatment was stable at 0.10 in the first 6 h, increased sharply to 0.91 at 6–15 h, and finally rose slowly at 15–24 h before stabilizing at 1.04. When the MOIs were 1 and 0.01, SapYZU11 reduced the OD_{600nm} value of *S. aureus* to 0.45 (at 15 h, P < 0.05), which was significantly lower than that of the control group (0.98). However, SapYZU11 with an MOI of 100 showed better inhibitory effects, and the maximum reduction was 0.65 (at 15 h, P < 0.001). Compared to SapYZU11, phages SapYZU10, SapYZU12, and SapYZU13 showed maximum reduction in OD_{600nm} values of *S. aureus* when the MOI was 100, which were 0.17 (at 24 h), 0.17 (at 24 h), and 0.06 (at 12 h), respectively (P < 0.05).

In addition, consistent with the expected results, following treatment with phage SapYZU11 at different MOI values (1 and 100) within 24 h, the $OD_{600\ nm}$ values of five *S. aureus* strains, i.e., YZUsa1, YZUsa4, YZUsa12, YZUsa14, and MRSA JCSC 4744, were stabilized at 0.14, 0.12, 0.14, 0.15, and 0.16, respectively. By contrast, when MOI was 0.01, SapYZU11 only decreased the growth inhibition of *S. aureus* YZUsa12, and the OD_{600nm} value was 0.36 at 24 h (P < 0.05; Figures 4C and S3–S6). Furthermore, when the MOI values were 1 and 100, SapYZU11 inhibited the growth of *S. aureus* cocktail for 24 h, and reduced their OD_{600nm} values at 24 h by 0.41 and 0.43, respectively. However, the control effect of SapYZU11 with an MOI of 0.01 on *S. aureus* cocktail decreased slightly, and the best effect was achieved at 18 h, with the OD_{600nm} value decreased by 0.33 (P < 0.05).

Phage SapYZU11 treatment in milk

The effect of SapYZU11 on the viability of *S. aureus* in milk was examined at 25°C and 4°C (Figures 5A and 5B). At 25°C, SapYZU11 with an MOI of 100 effectively inhibited the growth of MRSA JCSC 4744 and *S. aureus* cocktail for up to 24 h, thus reducing their counts by 5.03 Lg CFU/mL and 2.80 Lg CFU/mL (compared to the untreated milk sample), respectively (P < 0.001). Moreover, when the MOI values were 1 and 0.01, the growth of MRSA JCSC 4744 was significantly inhibited, and its count decreased by 3.97 Lg CFU/mL and 2.03 Lg CFU/mL, respectively (P < 0.001). At the same MOI values, the *S. aureus* cocktail counts were 7.50 Lg CFU/mL and 8.41 Lg CFU/mL at 48 h, respectively, whereas the count was 8.90 Lg CFU/mL for the untreated milk sample.

The results of SapYZU11 on *S. aureus* in milk at 4°C are shown in Figures 5C and 5D. SapYZU11 at an MOI of 100 resulted in maximum reductions of MRSA JCSC 4744 and *S. aureus* cocktail at 4 days to 0.33 Lg CFU/mL and 0.29 Lg CFU/mL, respectively (P < 0.05). After phage treatment at MOIs of 0.01 and 1, the counts of MRSA JCSC 4744 decreased by 0.25 Lg CFU/mL (at 6 days) and 0.27 Lg CFU/mL (at 3 days), respectively, whereas the *S. aureus* cocktail counts at MOIs of 0.01 and 1 decreased by 0.11 Lg CFU/mL (at 2 days) and 0.16 Lg CFU/mL (at 3 d), respectively.

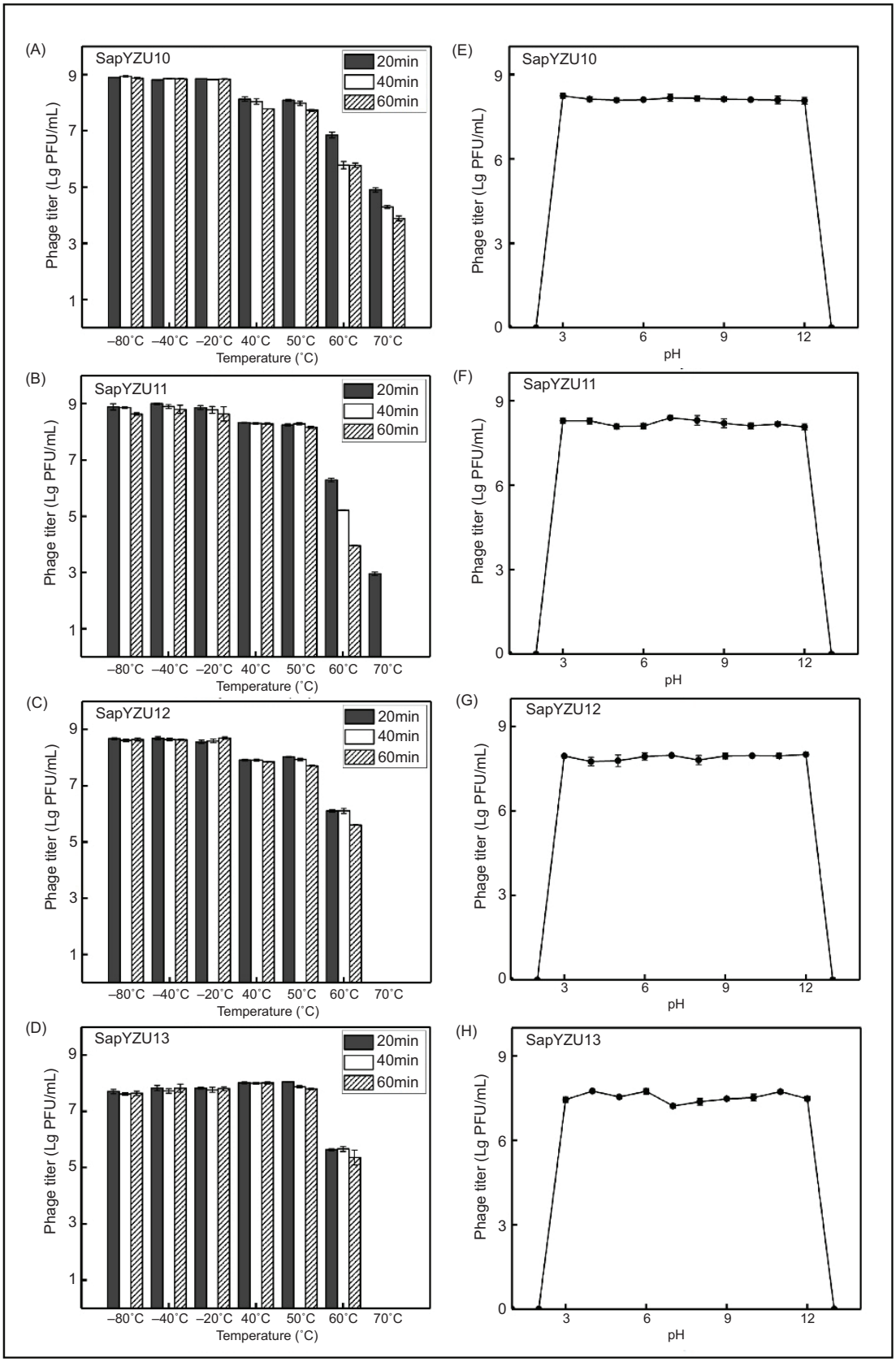

Figure 3. (A) Stability of *S. aureus* phages were maintained under different temperatures (–80°C, –40°C, –20°C, 40°C, 50°C, 60°C, and 70°C) for 2 h. (B) Stability of *S. aureus* phages was maintained under acid–base solution conditions (2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 11.0, and 12.0) for 2 h.

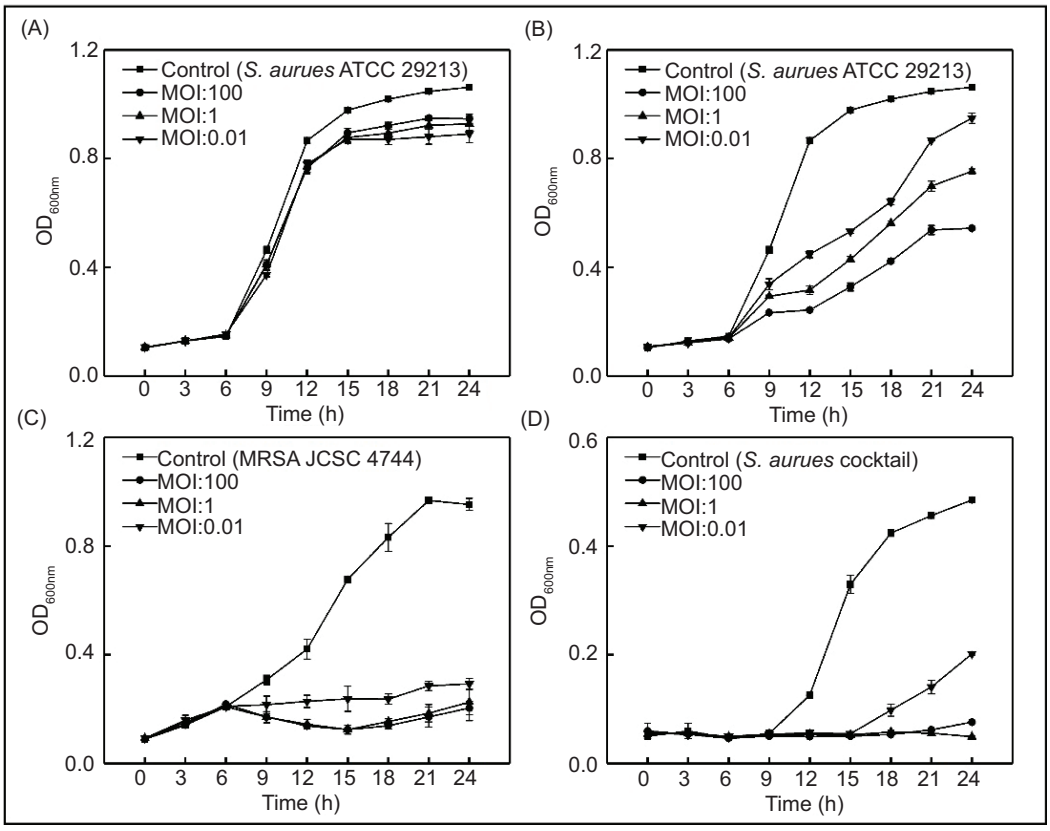

Figure 4. ***In vitro*** **lytic activity of (A) phage SapYZU10, and (B) SapYZU11 against** ***S. aureus*** **ATCC 29213 in LB broth.** ***In vitro*** **lytic activity of phage SapYZU11 against (C) MRSA JCSC 4744 and (D)** ***S. aureus*** **cocktail (YZUsa1, YZUsa4, YZUsa12, YZUsa14, and MRSA JCSC 4744) in LB broth.**

Phage SapYZU11 treatment in fresh pork

In order to test the bactericidal potential of SapYZU11 in meat products, fresh pork was artificially inoculated with *S. aureus* cocktail. When administered at MOIs of 1 and 100, SapYZU11 reduced the counts of viable *S. aureus* at 25°C by 0.98 Lg CFU/mL (at 48 h) and 1.29 Lg CFU/mL (at 48 h), respectively ($P < 0.05$; Figure 6A). On the other hand, at MOIs of 1 and 100, *S. aureus* decreased by 0.16 Lg CFU/mL (at 3 days) and 0.32 Lg CFU/mL (at 4 days) at 4°C, respectively ($P < 0.05$; Figure 6B). However, when using SapYZU11 at an MOI of 0.01 at 25°C, the maximum reduction of *S. aureus* was demonstrated to be 0.30 Lg CFU/mL at 48 h ($P < 0.05$). By contrast, the count of *S. aureus* was reduced by 0.11 Lg CFU/mL (at 2 days) following phage SapYZU11 treatment at 4°C.

Discussion

In the food industry, phages with specific characteristics are considered natural antimicrobials and potential tools against multi-drug-resistant bacteria (Lewis and Hill, 2020). In this study, four virulent *S. aureus* phages were isolated, and their inhibition against *S. aureus* in LB broth and food was analyzed. It is reported that latent period and burst size of phages are indicators of potential antibacterial capabilities (Yang *et al.*, 2020). A phage with a larger burst size can have a practical advantage in therapy, because the phage population increases therapy's initial dose by several hundred folds within a short time period (Amarillas *et al.*, 2017). Previously, studies reported that the latent period of phages pSal-SNUABM-04 (Kwon *et al.*, 2020) and SaGU1 (Shimamori *et al.*, 2021) infecting *S. aureus* was 60 min and 40 min, respectively. In comparison, our data from the one-step growth curve showed that SapYZU11 and three other phages had shorter latent periods. Despite exhibiting a shorter latent period, SapYZU13 was not considered the best candidate to be a biocontrol agent because of its lower burst size. Another study reported that phages ME18 and ME126, which infect *S. aureus*, displayed a burst size of 114.00 PFU/cell and 140.00 PFU/cell, respectively (Gharieb *et al.*, 2020). In contrast, SapYZU11 has a larger burst size, which is critical of efficacy as a biological antibacterial agent. These results indicated that SapYZU11 could be used as an effective biocontrol agent against *S. aureus* contamination in the food industry.

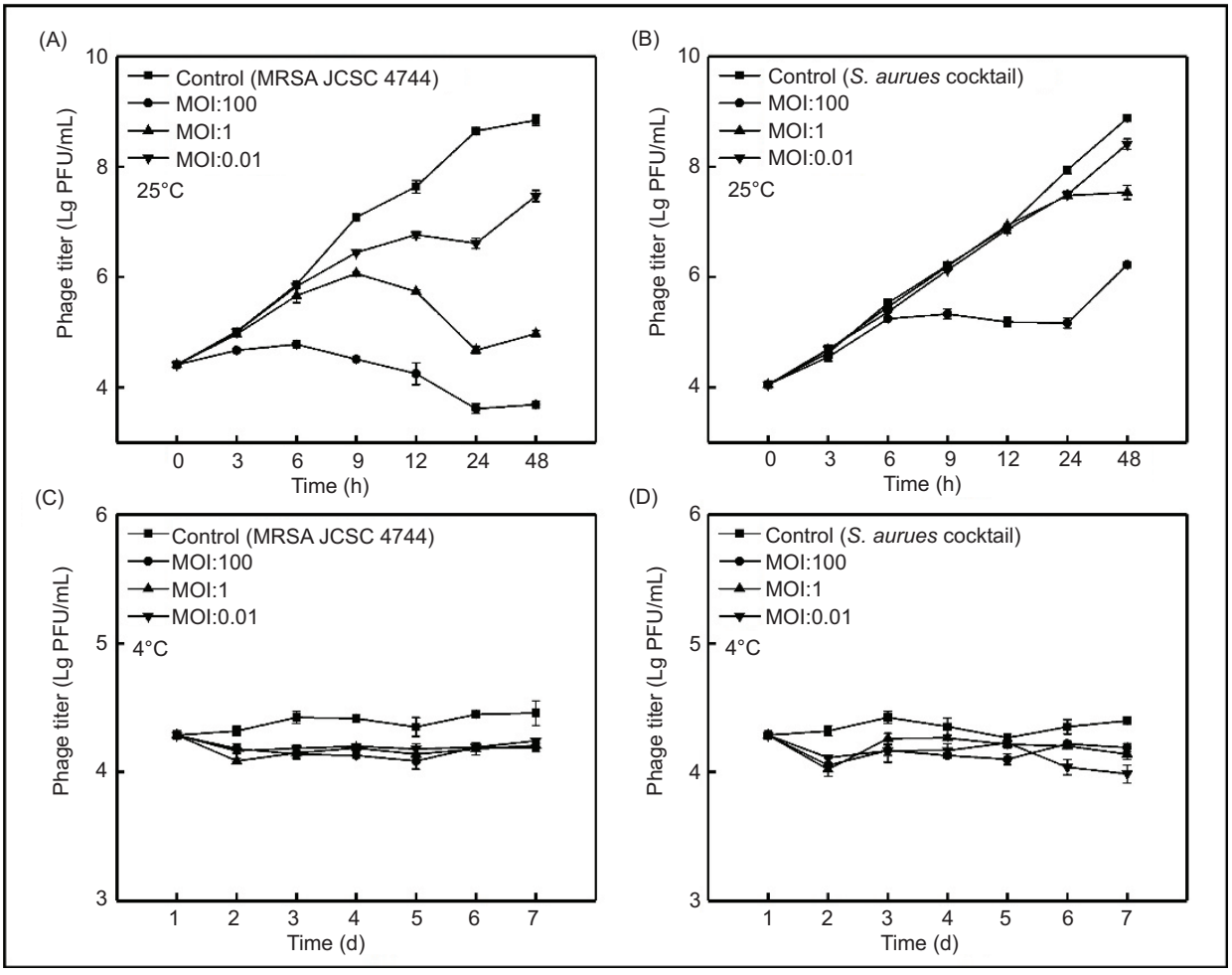

Figure 5. Effectiveness of phage SapYZU11 in reducing (A and C) MRSA JCSC 4744, and (B and D) *S. aureus* cocktail (10^6 CFU/mL) in milk at 4°C and 25°C. Phage SapYZU11 with three MOIs (0.01, 1, and 100) was mixed with *S. aureus* in milk, and the number of bacteria was determined at the indicated time points (4°C: 1, 2, 3, 4, 5, 6, and 7 day; 25°C: 0, 3, 6, 9, 12, 24, and 48 h).

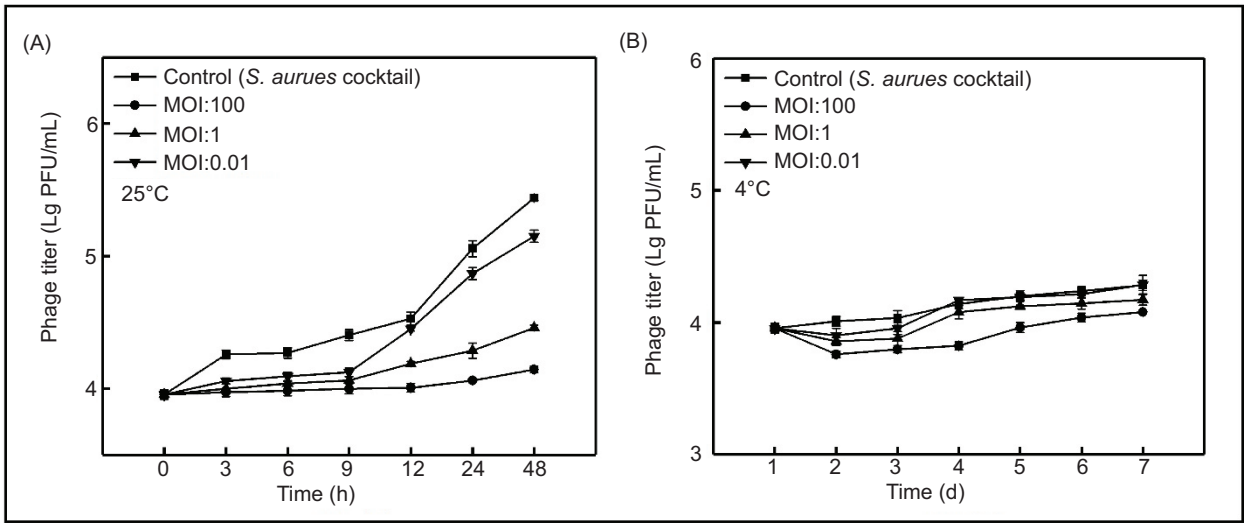

Figure 6. Effectiveness of phage SapYZU11 in reducing *S. aureus* cocktail in fresh pork at (A) 4°C and (B) 25°C. *S. aureus* (10^6 CFU/mL) was divided into several parts and added to fresh pork and phage SapYZU11 at three MOIs (0.01, 1, and 100) at the same position after drying. The number of bacteria was determined at the indicated time points (4°C: 1, 2, 3, 4, 5, 6, and 7 day; 25°C: 0, 3, 6, 9, 12, 24, and 48 h).

Temperature and pH values have significant effects on phage stability, and are considered important characteristics to be applied in the food industry (Cao *et al.*, 2021). Isolated phages sustained for 1 h at temperatures below 50°C exhibited a stable titer. However, at 60°C, the activity of phages decreased, and after incubation at 70°C for 20 min, they gradually became inactive, similar to the *S. aureus* phage vB_SauS_JS02 (Zhang *et al.*, 2022b). However, the temperature tolerance of SapYZU11 and other three phages was generally higher, compared to the four *Staphylococcus* phages Stab20–Stab23 isolated in Albania (Oduor *et al.*, 2020). Moreover, SapYZU11 exhibited higher pH tolerance and stability than some previously reported phages, such as phage JD419 (Feng *et al.*, 2021). Notably, SapYZU11 showed the highest activity at pH 12.0 for 2 h upon persistent incubation, allowing for its utilization in distinct food matrices and food production conditions with different pH values.

A phage with a wide range of hosts is a primary criterion of application in the food industry to ensure maximal bacterial infection, especially in fermented foods or farm animals (Meaden and Koskella, 2013). In a study involving 21 *S. aureus* strains as host spectra, phage vB_SauM_CP9 could lyse only 10 strains effectively (Kitamura *et al.*, 2020). In another study involving four phages in the host spectrum, phage Trsa205 infected 37.50% (21/56) of *S. aureus* strains and lysed 66.07% (37/56) when combined with Trsa207, Trsa220, and Trsa222 phages (Abdurahman *et al.*, 2021). In comparison, the isolated *S. aureus* phage SapYZU11 showed a broad range of hosts, with ability to infect a full range of tested *S. aureus* strains. Analysis of complete genome revealed that SapYZU11 harbored two genes encoding tail fiber protein (Figure S7 and Supplementary Table S1), which shared 83.60% and 97.60% amino acid sequence identity with phage CSA13 (Cha *et al.*, 2019). Phage CSA13 had a broad host spectrum and lytic activity against strains, including methicillin-susceptible *S. aureus* (MSSA), MRSA, local *S. aureus* isolates, and non-aureus Staphylococci. Therefore, a broad host spectrum of SapYZU11 could be due to the existence of unique tail fiber proteins. Its genome did not encode any gene related to tRNA, drug resistance, lysogenicity, and virulence. These results identified that SapYZU11 had a potential application value as a biocontrol agent in food. However, the future research must elucidate the efficiency of plating (EoP), resistance, and safety of phage SapYZU11 for a more accurate and effective phage therapy in food applications.

In control of *S. aureus* in LB broth by phages, the ability of SapYZU11 to significantly inhibit the growth of all six *S. aureus* strains (ATCC 29213, YZUsa1, YZUsa4, YZUsa12, YZUsa14, and MRSA JCSC 4744) was reflected. This lytic activity was similar to the one reported by Ma *et al.* (2021), where the phage cocktail (LSA2366 and LSA2308) was found to decrease the content of *S. aureus* BM001 in LB broth within 24 h. Notably, in LB broth with *S. aureus* cocktail, SapYZU11 with MOIs of 1 and 100 displayed a high level of infective potential similar to phage LPSTLL (Guo *et al.*, 2021). However, the optical density of *S. aureus* cocktail culture significantly decreased within 15 h of incubation with SapYZU11 at an MOI of 0.01, possibly because of the development of phage resistance or complex interactions between bacteria as reported by Zhou *et al.* (2021). Further research is required to understand the exact mechanism for developing bacterial resistance against phage SapYZU11.

Phage SapYZU11 had excellent characteristics and biological safety supporting its possible application in the food industry. In the present study, milk and fresh pork were used as representatives of food products frequently contaminated with *S. aureus* to evaluate the efficacy of SapYZU11 in controlling these pathogens in food matrices. The experimental results of artificial contamination of *S. aureus* in milk sample showed that when controlling the growth of MRSAJCSC 4744 at 25°C, the inhibitory rate of SapYZU11 at MOIs of 1 and 100 was more than 99.99%. This lytic activity was higher than that reported in a study conducted by Ma *et al.* (2021), showing an inhibitory rate of 99.97% against *S. aureus* in milk sample at 24 h following treatment with LSA2308 at an MOI of 10,000. Phage SapYZU11 exhibited greater efficiency than phage LSA2308, with the inhibition rate for *S. aureus* reaching 99.05% with SapYZU11 at an MOI of 0.01 in milk. In addition, SapYZU11 displayed a high level of infective potential against *S. aureus* cocktail at 25°C, and exhibited lytic activity in fresh pork samples. Their inhibitory effect was more apparent with growing numbers of MOIs at 25°C, as reported by Zhou *et al.* (2021). Similar results were also reported by Zhou *et al.* (2022), suggesting that addition of a high dosage of SapYZU11 could remove bacterial contamination from food products with greater efficiency.

At 4°C, concentration in *S. aureus* and *S. aureus* cocktail after 7-day SapYZU11 treatment was closely compared to the initial bacterial concentration (3.92–4.15 Lg CFU/mL) in milk and fresh pork samples whereas concentration in the control group was 4.38–4.51 Lg CFU/mL, which is consistent with the lytic activity of phages SPHG1 and SPHG3 (Esmael *et al.*, 2021). In fact, phages require a stable host to reproduce and propagate; therefore, in low-temperature conditions both bacterial growth and phage activity are slow, consequently diminishing the outcome of phage treatment (Abhisingha *et al.*, 2020). It was reported that some phages showed similar lytic activity at 4°C; however, the bactericidal mechanisms of phages at low temperature is still unclear (Witte *et al.*, 2022). Accordingly, the temperature must be considered prior to the treatment with phage. Additionally,

the data showed that inhibition of *S. aureus* by phage SapYZU11 in milk had a more significant effect than that in fresh pork, regardless of whether the phage was applied at 25°C or 4°C. These results revealed that aside from phage type and concentration, the food item could play a vital role in determining the efficacy of a bacteriophage intervention. This could have possibly resulted from the inability of phage particles to move and reach their host on the surface of a solid food or/and the emergence of phage resistance (Duc *et al.*, 2020). Greater inhibition of *S. aureus* in milk could be attributed to the mobility of liquid milk, as the free movement offered in a liquid system would aid phage–bacteria engagement, increasing interactions between them.

Although SapYZU11 was proved to be effective in removing pathogen contamination from milk and fresh pork, one limitation of the current study was that the viable concentration of host bacteria could only be reduced to the initial level at the most but not below the detection limit (<1 Lg CFU/mL). This limitation also suggests how it was not be possible for one phage to control all pathogenic *S. aureus*. Therefore, further research is required wherein multiple combinations of phages, other food-grade antimicrobials, or higher concentration of phages could be used to improve their antibacterial effectiveness. In addition, in this study, the lysing activity of SapYZU11 was evaluated only in milk and pork. The future studies must include *in vitro* analysis in more food matrices and could focus on the stability, effectiveness, and safety of phage SapYZU11.

Conclusions

In conclusion, the four phages isolated in this study demonstrated high activity at a wide range of temperatures and pH values, and were able to lyse more than 60% of *S. aureus*. Notably, SapYZU11 lysed 100% of the tested *S. aureus* and revealed greater burst size (152.00 PFU/cell), compared to those of the other three phages. Moreover, SapYZU11 was more effective against *S. aureus* in LB broth, and significantly decreased the content of *S. aureus* cocktail in milk and fresh pork at 25°C and 4°C. These results suggested that phage SapYZU11 could be used as a novel natural biocontrol agent against *S. aureus* in food products.

Funding

This research was supported by the National Natural Science Foundation of China (grant numbers: 32102100 and 32001661) and China Postdoctoral Science Foundation (grant number: 2022M712695).

Conflicts of interest

The authors had no competing interests to declare.

Author contributions

Study conception and design: Wen-yuan Zhou; Acquisition of data: Hua Wen, Wen-yuan Zhou, Lei Yuan, Xuan Li, Jun-hang Ye, and Ya-jie Li; Analysis and interpretation of data: Hua Wen, Wen-yuan Zhou; Drafting of manuscript: Hua Wen, Wen-yuan Zhou and Lei Yuan; Critical revision: Hua Wen, Zhen-quan Yang, and Wen-yuan Zhou.

References

Abdurahman, M.A., Tosun, I., Durukan, I., Khorshidtalab, M. and Kilic, A.O., 2021. Four temperate bacteriophages from methicillin-resistant *Staphylococcus aureus* show broad bactericidal and biofilm removal activities. Kafkas Universitesi Veteriner Fakultesi 27: 1. https://doi.org/10.9775/kvfd.2020.24680

Abhisingha, M., Dumnil, J. and Pitaksutheepong, C., 2020. Efficiency of phage cocktail to reduce *Salmonella Typhimurium* on chicken meat during low temperature storage. Food Science and Technology (LWT) 129: 109580. https://doi.org/10.1016/j.lwt.2020.109580

Alves, D.R., Gaudion, A., Bean, J.E., Perez, E.P., Arnot, T.C., Harper, D.R., et al. 2014. Combined use of bacteriophage K and a novel bacteriophage to reduce *Staphylococcus aureus* biofilm formation. Applied and Environmental Microbiology 80: 6694–6703. https://doi.org/10.1128/AEM.01789-14

Amarillas, L., Rubi-Rangel, L., Chaidez, C., Gonzalez-Robles, A., Lightbourn-Rojas, L. and Leon-Felix, J., 2017. Isolation and characterization of phiLLS, a novel phage with potential biocontrol agent against multidrug-resistant *Escherichia coli*. Frontiers in Microbiology 8: 1355. https://doi.org/10.3389/fmicb.2017.01355

Bhetwal, A., Maharjan, A., Shakya, S., Satyal, D., Ghimire, S., Khanal, P.R. et al. 2017. Isolation of potential phages against multidrug-resistant bacterial isolates: promising agents in the rivers of Kathmandu, Nepal. BioMed Research International 2017: 3723254. https://doi.org/10.1155/2017/3723254

Cao, Y., Zhang, Y., Lan, W. and Sun, X., 2021. Characterization of vB_VpaP_MGD2, a newly isolated bacteriophage with biocontrol potential against multidrug-resistant *Vibrio parahaemolyticus*. Archives of Virology 166: 413–426. https://doi.org/10.1007/s00705-020-04887-x

Cha, Y., Chun, J., Son, B. and Ryu, S., 2019. Characterization and genome analysis of *Staphylococcus aureus* Podovirus CSA13 and its anti-biofilm capacity. Viruses (Basel) 11(1): 54. https://doi.org/10.3390/v11010054

Duc, H.M., Son, H.M., Yi, H., Sato, J., Ngan, P.H., Masuda, Y., et al. 2020. Isolation, characterization and application of a polyvalent phage capable of controlling *Salmonella* and *Escherichia coli*

O157:H7 in different food matrices. *Food Research International* 131: 108977. https://doi.org/10.1016/j.foodres.2020.108977

Esmael, A., Azab, E., Gobouri, A.A., Nasr-Eldin, M.A., Moustafa, M., Mohamed, S.A., e al. 2021. Isolation and characterization of two lytic bacteriophages infecting a multi-drug resistant *Salmonella* Typhimurium and their efficacy to combat salmonellosis in ready-to-use foods. Microorganisms 9(2): 423. https://doi.org/10.3390/microorganisms9020423

Feng, T., Leptihn, S., Dong, K., Loh, B., Zhang, Y., Stefan, M.I., et al. 2021. JD419, a *Staphylococcus aureus* phage with a unique morphology and broad host range. Frontiers in Microbiology 12: 602902. https://doi.org/10.3389/fmicb.2021.602902

Gharieb, R.M.A., Saad, M.F., Mohamed, A.S. and Tartor, Y.H., 2020. Characterization of two novel lytic bacteriophages for reducing biofilms of zoonotic multidrug-resistant *Staphylococcus aureus* and controlling their growth in milk. Food Science and Technology (LWT) 124: 109145. https://doi.org/10.1016/j.lwt.2020.109145

Guo, Y., Li, J., Islam, M.S., Yan, T., Zhou, Y., Liang, L., et al. 2021. Application of a novel phage vB_SalS-LPSTLL for the biological control of *Salmonella* in foods. *Food Research International* 147: 110492. https://doi.org/10.1016/j.foodres.2021.110492

Iyer, K.R., Revie, N.M., Fu, C., Robbins, N. and Cowen, L.E., 2021. Treatment strategies for cryptococcal infection: challenges, advances and future outlook. Nature Reviews Microbiology 19: 454–466. https://doi.org/10.1038/s41579-021-00511-0

Kitamura, N., Sasabe, E., Matsuzaki, S., Daibata, M. and Yamamoto, T., 2020. Characterization of two newly isolated *Staphylococcus aureus* bacteriophages from Japan belonging to the genus Silviavirus. Archives of Virology 165: 2355–2359. https://doi.org/10.1007/s00705-020-04749-6

Kwon, J., Kim, S.G., Kim, H.J., Giri, S.S., Kim, S.W., Lee, S.B., et al. 2020. Isolation and characterization of *Salmonella* jumbo-phage pSal-SNUABM-04. Viruses (Basel) 13(1): 27. https://doi.org/10.3390/v13010027

Lewis, R. and Hill, C., 2020. Overcoming barriers to phage application in food and feed. Current Opinion in Biotechnology 61: 38–44. https://doi.org/10.1016/j.copbio.2019.09.018

Li, Y., Chen, H., Shu, M., Zhong, C., Bi, Y., Yang, H., et al. 2021b. Isolation, characterization and application of an alkaline resistant virulent bacteriophage JN01 against *Escherichia coli* O157:H7 in milk and beef. Food Science and Technology (LWT) 144: 111266. https://doi.org/10.1016/j.lwt.2021.111266

Li, G., Wu, C., Wang, X. and Meng, J., 2015. Prevalence and characterization of methicillin susceptible *Staphylococcus aureus* ST398 isolates from retail foods. International Journal of Food Microbiology 196: 94–97. https://doi.org/10.1016/j.ijfoodmicro.2014.12.002

Li, H., Yang, X., Zhu, X., Gao, L., Rao, S., Yuan, L., et al. 2021a. Isolation and characterization of broad host-range of bacteriophages infecting *Cronobacter sakazakii* and its biocontrol potential in dairy products. Quality Assurance and Safety of Crops & Foods 13: 21–44. https://doi.org/10.15586/QAS.V13I3.890

Luo, D., Li, C., Wu, Q., Ding, Y., Yang, M., Hu, Y., et al. 2021. Isolation and characterization of new phage vB_CtuP_A24 and application to control *Cronobacter* spp. in infant milk formula and lettuce. *Food Research International* 141: 110109. https://doi.org/10.1016/j.foodres.2021.110109

Ma, F., Ning, Y., Wan, Q., Zou, L., Liu, Y., Chen, S., et al. 2021. Bacteriophages LSA2308 and LSA2366 infecting drug-resistant *Staphylococcus aureus*: isolation, characterization and potential application for milk safety. Food Science and Technology (LWT) 152: 112298. https://doi.org/10.1016/j.lwt.2021.112298

Mahros, M.A., Abd-Elghany, S.M. and Sallam, K.I., 2021. Multi-drug-, methicillin-, and vancomycin-resistant *Staphylococcus aureus* isolated from ready-to-eat meat sandwiches: an ongoing food and public health concern. International Journal of Food Microbiology 346: 109165. https://doi.org/10.1016/j.ijfoodmicro.2021.109165

Meaden, S. and Koskella, B., 2013. Exploring the risks of phage application in the environment. Frontiers in Microbiology 4: 358. https://doi.org/10.3389/fmicb.2013.00358

Miedzybrodzki, R., Borysowski, J., Weber-Dabrowska, B., Fortuna, W., Letkiewicz, S., Szufnarowski, K., et al. 2012. Clinical aspects of phage therapy. Advances in *Virus Research* 83: 73–121. https://doi.org/10.1016/B978-0-12-394438-2.00003-7

Monteiro, R., Pires, D.P., Costa, A.R. and Azeredo, J., 2019. Phage therapy: going temperate? Trends in Microbiology 27: 368–378. https://doi.org/10.1016/j.tim.2018.10.008

Nikolic, P., Mudgil, P. and Whitehall, J., 2020. The *in vitro* antibacterial effect of permethrin and formaldehyde on *Staphylococcus aureus*. MicrobiologyOpen 9: e1054. https://doi.org/10.1002/mbo3.1054

Oduor, J., Kadija, E., Nyachieo, A., Mureithi, M.W. and Skurnik, M., 2020. Bioprospecting *Staphylococcus* phages with therapeutic and bio-control potential. Viruses (Basel) 12(2): 133. https://doi.org/10.3390/v12020133

Pang, Z., Raudonis, R., Glick, B.R., Lin, T.J. and Cheng, Z., 2019. Antibiotic resistance in *Pseudomonas aeruginosa*: mechanisms and alternative therapeutic strategies. Biotechnology Advances 37: 177–192. https://doi.org/10.1016/j.biotechadv.2018.11.013

Ravindran, R. and Jaiswal, A.K., 2019. Wholesomeness and safety aspects of irradiated foods. Food Chemistry 285: 363–368. https://doi.org/10.1016/j.foodchem.2019.02.002

Samir, S., El-Far, A., Okasha, H., Mahdy, R., Samir, F. and Nasr, S., 2022. Isolation and characterization of lytic bacteriophages from sewage at an egyptian tertiary care hospital against methicillin-resistant *Staphylococcus aureus* clinical isolates. Saudi Journal of Biological Science 29: 3097–3106. https://doi.org/10.1016/j.sjbs.2022.03.019

Santos, S.B. and Azeredo, J., 2019. Bacteriophage-based biotechnological applications. Viruses (Basel) 11(8): 737. https://doi.org/10.3390/v11080737

Shimamori, Y., Pramono, A.K., Kitao, T., Suzuki, T., Aizawa, S.I., Kubori, T., et al. 2021. Isolation and characterization of a novel phage SaGU1 that infects *Staphylococcus aureus* clinical isolates from patients with atopic dermatitis. Current Microbiology 78: 1267–1276. https://doi.org/10.1007/s00284-021-02395-y

Su, Y.C., Hung, W.W., Lin, J.M., Chang, C.C., Chen, Y.H., Lai, Y.L., et al. 2020. Tracking the evolution of the two successful CC59 methicillin-resistant *Staphylococcus aureus* clones in Taiwan: the divergence time of the two clades is estimated to be the

1980s. International Journal of Antimicrobial Agents 56: 106047. https://doi.org/10.1016/j.ijantimicag.2020.106047

Takemura-Uchiyama, I., Uchiyama, J., Osanai, M., Morimoto, N., Asagiri, T., Ujihara, T., et al. 2014. Experimental phage therapy against lethal lung-derived septicemia caused by *Staphylococcus aureus* in mice. *Microbes* & *Infections* 16: 512–517. https://doi.org/10.1016/j.micinf.2014.02.011

Witte, S., Huijboom, L., Klamert, S., van de Straat, L., Hagens, S., Fieseler, L., et al. 2022. Application of bacteriophages EP75 and EP335 efficiently reduces viable cell counts of *Escherichia coli* O157 on beef and vegetables. Food Microbiology 104: 103978. https://doi.org/10.1016/j.fm.2022.103978

Wu, Y.N., Liu, X.M., Chen, Q., Liu, H., Dai, Y., Zhou, Y.J., et al. 2018. Surveillance for foodborne disease outbreaks in China, 2003 to 2008. Food Control 84: 382–388. https://doi.org/10.1016/j.foodcont.2017.08.010

Yang, M., Liang, Y., Huang, S., Zhang, J., Wang, J., Chen, H., et al. 2020. Isolation and characterization of the novel phages vB_VpS_BA3 and vB_VpS_CA8 for lysing vibrio parahaemolyticus. Frontiers in Microbiology 11: 259. https://doi.org/10.3389/fmicb.2020.00259

Zhang, L., Shahin, K., Soleimani-Delfan, A., Ding, H., Wang, H., Sun, L., et al. 2022b. Phage JS02, a putative temperate phage, a novel biofilm-degrading agent for *Staphylococcus aureus*. Letters in Applied Microbiology 75: 643–654. https://doi.org/10.1111/lam.13663

Zhang, F., Wu, S., Lei, T., Wu, Q., Zhang, J., Huang, J., et al. 2022a. Presence and characterization of methicillin-resistant *Staphylococcus aureus* co-carrying the multidrug resistance genes *cfr* and *lsa* (E) in retail food in China. International Journal of Food Microbiology 363: 109512. https://doi.org/10.1016/j.ijfoodmicro.2021.109512

Zhou, Y., Li, L., Han, K., Wang, L., Cao, Y., Ma, D., et al. 2022. A polyvalent broad-spectrum *Escherichia* phage *Tequatro* virus EP01 capable of controlling *Salmonella* and *Escherichia coli* contamination in foods. Viruses (Basel) 14. https://doi.org/10.3390/v14020286

Zhou, W.Y., Li, X., Osmundson, T., Shi, L., Ren, J. and Yan, H., 2018a. WGS analysis of ST9-MRSA-XII isolates from live pigs in China provides insights into transmission among porcine, human and bovine hosts. Journal of Antimicrobial Chemotherapy 73: 2652–2661. https://doi.org/10.1093/jac/dky245

Zhou, W.Y., Li, X., Shi, L., Wang, H.H. and Yan, H., 2018b. Novel SCCmec type XII methicillin-resistant *Staphylococcus aureus* isolates identified from a swine production and processing chain. *Veterinary Microbiology* 225: 105–113. https://doi.org/10.1016/j.vetmic.2018.09.007

Zhou, W.Y., Sun, S.F., Zhang, Y.S., Hu, Q., Zheng, X.F., Yang, Z.Q., et al. 2021. Isolation and characterization of a virulent bacteriophage for controlling *Salmonella Enteritidis* growth in ready-to-eat nixed-ingredient salads. *Journal* of *Food Protection* 84: 1629–1639. https://doi.org/10.4315/JFP-20-460

Supplementary

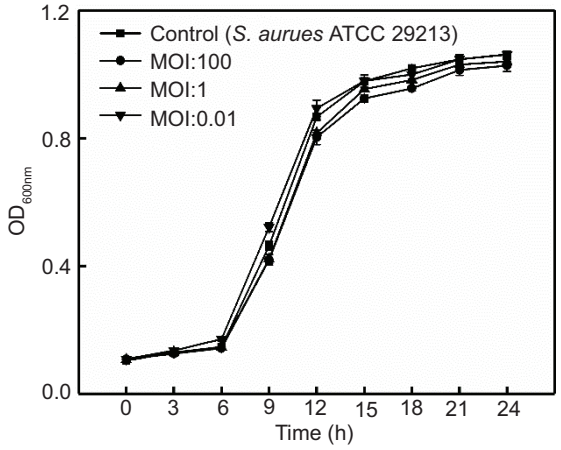

Figure S1. ***In vitro*** **lytic activity of phage SapYZU12 against** ***S. aureus*** **ATCC 29213 in LB broth.**

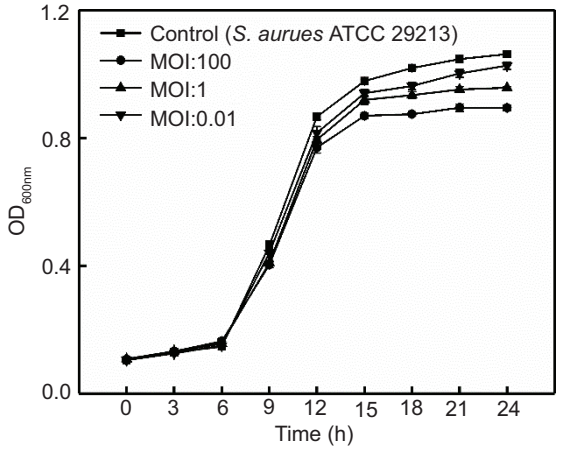

Figure S2. ***In vitro*** **lytic activity of phage SapYZU13 against** ***S. aureus*** **ATCC 29213 in LB broth.**

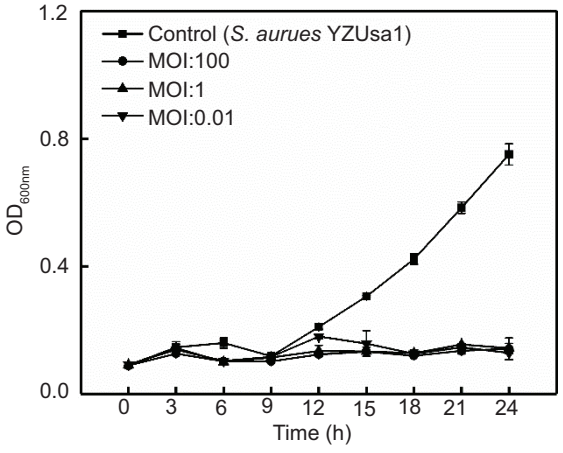

Figure S3. ***In vitro*** **lytic activity of phage SapYZU11 against** ***S. aureus*** **YZUsa1 in LB broth.**

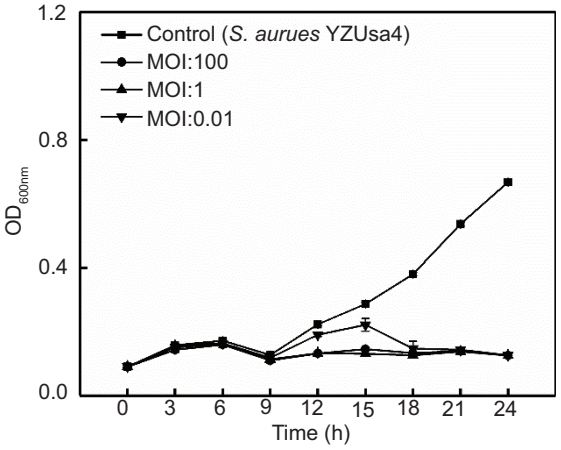

Figure S4. ***In vitro*** **lytic activity of phage SapYZU11 against** ***S. aureus*** **YZUsa4 in LB broth.**

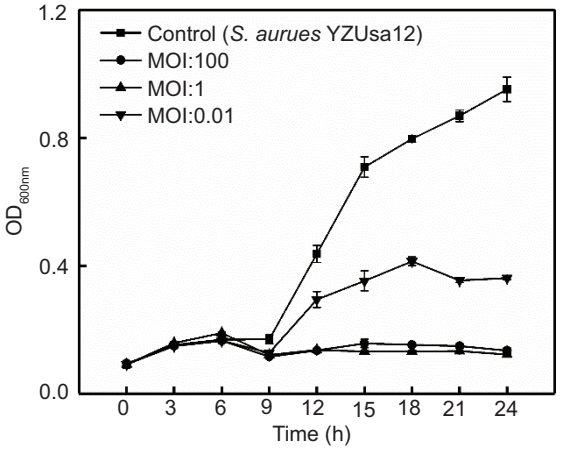

Figure S5. ***In vitro*** **lytic activity of phage SapYZU11 against** ***S. aureus*** **YZUsa12 in LB broth.**

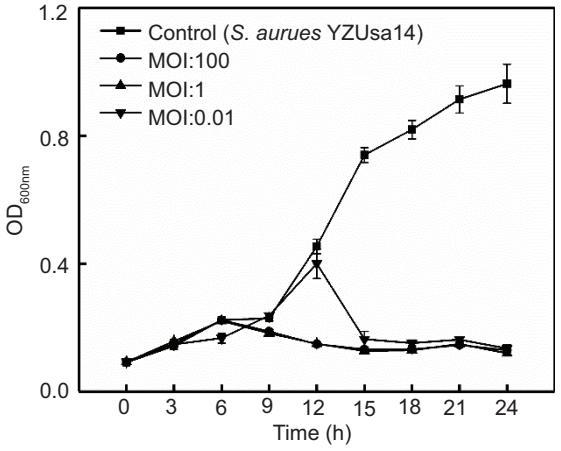

Figure S6. ***In vitro*** **lytic activity of phage SapYZU11 against** ***S. aureus*** **YZUsa14 in LB broth.**

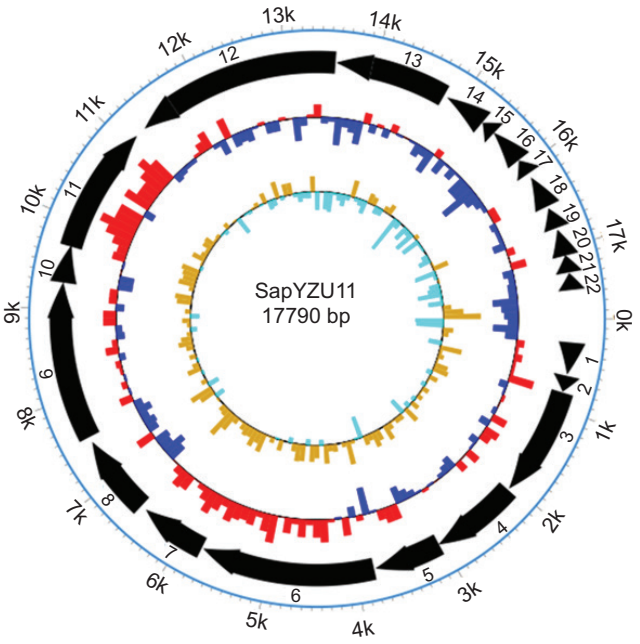

Figure S7. Whole genome map of phage SapYZU11.

Table S1. Genomic function annotation of SapYZU11.

ORFs	Start	End	Strand	Length (bp)	Protein (aa)	Putative function	Best phage homolog	Identity (%)	Accession No.
ORF1	266	625	+	360	120	Hypothetical protein	Staphylococcus phage SA03-CTH2	77.87	QDH84802.1
ORF2	638	820	+	183	61	Hypothetical protein	Staphylococcus phage SAP-2	100.00	YP_001491544.1
ORF3	827	2053	+	1,227	409	Major head protein	Staphylococcus phage SAP-2	98.53	YP_001491544.2
ORF4	2068	3051	+	984	328	Upper collar protein	Staphylococcus phage CSA13	98.17	YP_009819982.1
ORF5	3044	3799	+	756	252	Lower collar protein	Staphylococcus phage SLPW]	98.41	YP_009278569.1
ORF6	3812	5755	+	1,944	648	Minor structural protein	Staphylococcus phage SAP-2	97.99	YP_001491544.2
ORF7	5767	6519	+	753	251	Phage lysin	Staphylococcus phage GRCS	97.60	YP_009004305.1
ORF8	6582	7478	+	897	299	Tail fiber protein	Staphylococcus phage LSA2366	94.16	QQO38231.1
ORF9	7538	9301	+	1,764	588	Tail fiber protein	Staphylococcus phage LSA2366	99.66	QQO38232.1
ORF10	9303	9722	+	420	140	Holin	Staphylococcus phage LSA2366	100.00	QQO38233.1
ORF11	9700	11139	+	1,440	480	Phage lysin	Staphylococcus phage Pabna	99.37	YP_009816546.1
ORF12	11253	13538	–	2,286	762	DNA polymerase	Staphylococcus phage SCH1	97.11	YP_009787907.1
ORF13	13554	14801	–	1,248	416	DNA encapsidation protein	Staphylococcus phage Pabna	99.52	YP_009816544.1
ORF14	14849	15328	–	480	160	Hypothetical protein	Staphylococcus phage GRCS	96.05	YP_009004298.1
ORF15	15331	15501	–	171	57	Hypothetical protein	Staphylococcus phage S24-1	96.36	YP_004957422.1
ORF16	15494	15907	–	414	138	Hypothetical protein	Staphylococcus phage BP39	91.97	YP_009283981.1
ORF17	15910	16089	–	180	60	Hypothetical protein	Staphylococcus phage SLPW	100.00	YP_009278557.1
ORF18	16140	16508	–	369	123	Single-stranded DNA binding protein	Staphylococcus phage Pabna	98.10	NC_048107.1
ORF19	16532	16768	–	237	79	Hypothetical protein	Staphylococcus phage SLPW	97.44	YP_009278555.1
ORF20	16786	17088	–	303	101	Hypothetical protein	Staphylococcus phage SLPW	99.00	YP_009278554.1
ORF21	17081	17278	–	198	66	Hypothetical protein	Staphylococcus phage GRCS	95.38	YP_009004291.1
ORF22	17282	17473	–	192	64	Hypothetical protein	Staphylococcus phage BP39	87.76	YP_009283976.1