Most food-borne diseases are caused by pathogenic bacteria, with *Staphylococcus aureus* as a leading cause of emesis and gastroenteritis. *S. aureus* usually contaminates food during preparation, processing, storage, or even packaging. The food easy to be contaminated includes most ready-to-eat products such as milk and cheeses, different types of meats, and various delicatessen items. Furthermore, *S. aureus* can cause food-borne poisoning via the host absorption of staphylococcal enterotoxins preformed in the food matrices even when staphylococcal cells have been killed. Thus control of *S. aureus* is needed for food products. One important control strategy that is suitable for food safety is the use of bacteriophages.

Bacteriophages (phages) are viruses that only infect and lyse bacterial cells. Their antibacterial activity was recognized initially by Hankin in 1896, Gameleya in 1898 and Twort in 1915, but in the past decade, worldwide interest and research on the exploitation of phages as antibacterial agents has resurfaced, with many pathogenic bacteria being targeted. One of the most relevant advances in the use of phages as biocontrol agents occurred in 2006 when the Food and Drug Administration approved the use of a six-phages cocktail designated LMP-102™ in ready-to-eat meat and poultry products, as well as in food-processing plants, to control *Listeria monocytogenes* contamination. Meanwhile, another phage product, Listex™ P100 was also approved for use in food products against *L. monocytogenes*. The application of other phages as antibacterial agents against experimental *Escherichia coli*, *Salmonella*, and *Campylobacter* contamination in foods has also
been researched\(^4\)-\(^6\). Therefore, using phages to reduce undesired bacteria has potential in food and food production-related environments.

In recent years, several bacteriophages against \(S.\ aureus\) have been isolated and their potential as biocontrol agents has been researched. Most of these phages are being considered as a way of treating \(S.\ aureus\) infection-related disease, such as phages \(\Phi M R 11, \Phi M R 25, CS1\) and \(DW 2\). Fewer researches were focused on the potential of anti-\(Staphylococcus\) bacteriophages as biocontrol agents in dairy products. A mixture of two phages, \(\Phi H 5\) and \(\Phi A 72\), inhibited \(S.\ aureus\) in Ultra-High-Temperature (UHT) and pasteurized whole-fat milk but was less active in semi-skimmed raw milk and whole, raw milk\(^10\). However, there are few reports focused on the biocontrol potential of \(S.\ aureus\) phages in other food products.

Herein, we discuss the isolation and characterization of a new \(S.\ aureus\) bacteriophage, JS01. The phage’s ability to lyse \(S.\ aureus\) ATCC 25923 was assessed. Phage application was performed to test the effectiveness of JS01 to inhibit \(S.\ aureus\) growth in three kinds of food samples. Our results support the potential use of this phage as a bio-preservation agent in foods.

**MATERIALS AND METHODS**

**Bacterial strains and growth media**

*Staphylococcus aureus* strains (ATCC 25923 and ATCC 6538) were used as hosts for the bacteriophage isolation and propagation. *S. aureus* was cultured in tryptic soy broth (TSB) at 37°C. The solid medium (TSA) contained 10 g L\(^{-1}\) agar.

**Bacteriophage enrichment, isolation and propagation**

Samples of effluent from two meat-processing plants and three milk factories were obtained and centrifuged at 1000 rpm for 5 min. The supernatant was filter-sterilised (0.22 µm-pore size) and subjected to a plaque assay using strains ATCC 25923 and ATCC 6538 as the host. The clear plaques were selected and isolated until a plaque with an identical morphotype was obtained. The final plaque, JS01, was propagated and stored at –72°C in SM buffer (50 mmol L\(^{-1}\) Tris-HCl, 2.0 g L\(^{-1}\) MgSO\(_4\), 7H\(_2\)O, 100 mg L\(^{-1}\) gelatin, 100 mmol L\(^{-1}\) NaCl, pH 7.5) containing 50% (v/v) glycerol. The bacteriophage was titrated using the agar overlay method as described\(^11\).

**Purification of phage particles**

Phage JS01 was purified by glycerol gradient ultracentrifugation following polyethylene glycol 6000-precipitation of phage lysates as described\(^7\). The purified phage suspension was divided into aliquots and stored at 4°C until used.

**Electron microscopy of phage JS01**

Purified phage was applied to a Formvar membrane and negatively stained with 10 g L\(^{-1}\) tungsten acetate. Electron micrograph was taken with a transmission electron microscope (Hitachi H-7000, Hitachi, Japan) at 80 kV.

**Phage DNA extraction, restriction and partially sequencing**

The phage JS01 DNA was extracted from a glycerol-purified phage stock solution as described\(^2\). The purified JS01 DNA was digested with EcoRI or HindIII and electrophoresed on a 1% horizontal agarose gel. The 4.1 kb fragment obtained from EcoRI digestion was inserted into the EcoRI restriction site of YEplac195 vector and sequenced by the Sangon Company (Shanghai, China) using universal pUC19 primers. The Sequence was analyzed using the NCBI BLAST.

**Biological characterization of phage JS01**

To examine the adsorption rate of phage, \(S.\ aureus\) cells grown to mid-log phase (4×10\(^9\) CFU mL\(^{-1}\)) were infected with phage JS01 at a multiplicity of infection (MOI) of 0.1 and incubated at 37°C. The titre of unabsorbed free phage virions was measured at 2, 5, 10, and 20 min. To determine the one-step growth of phage, \(S.\ aureus\) cells growing in TSB broth were mixed with phage JS01 at an MOI of 0.1 and allowed to absorb at room temperature for 5 min. Subsequently, phage-cell mixture was washed with SM buffer to remove unbound phages, then resuspended in fresh TSB medium and incubated at 37°C. The titre of the newly produced phages was measured every 30 min. The temperature stability of phage JS01 was determined by incubating phage suspensions at 30, 50, 70, and 90°C. The phage samples were removed at 0.5, 1, 2, and 4 h for phage titration. The pH stability of phage JS01 was determined over a pH range of 3.0–10.0. The phage was stored in 0.2 mol L\(^{-1}\) sodium phosphate-citrate buffer (pH 3.0–7.5), 0.05 mol L\(^{-1}\) Tris-HCl buffer (pH 7.5–9.0), or 0.05 mol L\(^{-1}\) glycine-NaOH buffer (pH 9.0–10.0) for
were isolated and propagated on ATCC 25923 and designated JS01. JS01 formed clear 3.2±0.3 mm-diameter plaques in a lawn of ATCC 25923 and also formed 3.0±0.2 mm-diameter plaques in a lawn of ATCC 6538. Pure phage stocks were prepared from glycerol gradient ultracentrifugation to achieve titres in excess of $10^{11}$ PFU mL$^{-1}$. Electron microscopy of purified phage JS01 demonstrates that it possesses an obvious tail and hence belongs to the order Caudovirales. The noncontractile tail of JS01 is 115±5 nm-long, while the isometric head is 65±5 nm-diameter (Fig. 1A).

JS01 DNA was digested with EcoRI and HindIII (data not shown), demonstrating the genome size of approximately 43 kb. The 4.1 kb fragment from EcoRI digestion was cloned into YEplac195 vector and sequenced. BLAST of this sequence indicates that it is partially similar with the sequences of four kinds of Staphylococcus phages of Siphoviridae family with different arrangement (Fig. 1B). It shows the highest identity (73%) to phage tp310-3 and several other Staphylococcus phages, with the five identical regions of A1–A5 sequentially arranged. The regions B1, B2, and B3 are identical to the corresponding regions of phage ΦMR11 and several other Staphylococcus phages. The regions C1 and C2 show the high identity to the corresponding sequence of phage 77 [13]. The region D shows identity (100%) to only one phage,
phage 37\textsuperscript{14}. Therefore, JS01 is a novel Staphylococcus phage and can be classified as belonging to the Siphoviridae family. JS01 was deposited into the China Center for Type Culture Collection (CCTCC) with accession number CCTCC M2011409.

**Biological study of phage JS01**

Biological study clarified other features of JS01. The optimal MOI of JS01 while infecting S. aureus ATCC 25923 (10\textsuperscript{8} CFU) was 0.1. With such an MOI, 8×10\textsuperscript{11} PFU JS01 was produced. During adsorption of JS01 to ATCC 25923, approximately 75% of phage was adsorbed to the host cells within the first 2 min, rising slowly to 95% at 20 min postinfection (Fig. 2A). The one-step growth curve of JS01 in ATCC 25923 indicated that the latent period and the beginning of the first burst was approximately 30 min. The average burst size was approximately 86 PFU per cell (Fig. 2B).

![Fig. 2.](image)

The stability of phage JS01 was tested at different temperatures. Incubation at 30°C and 50°C for 4 h produced similar phage titres confirming that phage JS01 is stable below 50°C. Incubation at 70°C significantly decreased the phage titre. However, more than 10\textsuperscript{4} PFU mL\textsuperscript{-1} phages remained active even after a 2-h incubation, indicating that phage JS01 will not be fully inactivated by current pasteurization processes. Only a 2-h incubation at 90°C resulted in the detection of no phage (Fig. 2C).

![Fig. 2.](image)

The stability of phage JS01 at different pH was then tested. JS01 keeping at pH 7.0–7.5 exhibited the highest phage titres (4.0×10\textsuperscript{10} PFU mL\textsuperscript{-1}). At pH 6.0 and 8.0, the phage titres decreased to about 10\textsuperscript{9} PFU mL\textsuperscript{-1}. While at pH lower than 6 and higher than 8, the phage titres decreased sharply. However, at pH lower than 5.0 and higher than 9.0, no phage particles could be survived (Fig. 2D), showing that phage JS01 was sensitive to acid and alkaline conditions.

**Bacterial challenge test**

The ability of phage JS01 to lyse S. aureus was assessed. JS01 was added into an early exponential culture of ATCC 25923 (OD\textsubscript{600}=0.25) at an MOI of 0.1 and incubated at 37°C. 1 h after
Phage applied, the bacterial number was reduced 10^5-fold (5.0 log units) compared with the no-phage control (Fig. 3A). More than 1 h after the addition of phage, the bacterial titres began to increase. At 4 h after phage challenge, the bacterial number increased to 4.4×10^5 CFU mL^{-1}, 3.9 log units lower than the no-phage control. When JS01 was applied into a diluted suspension of ATCC 25923 (4.8×10^3 CFU mL^{-1}) at an MOI of 10^5, the bacterial number decreased to zero at 2 h after phage treated, kept at zero until 6-h post-treatment, but increased to 1.4×10^4 CFU mL^{-1} at 8 h after treated, which was still 5.8 log units lower than the no-phage control (Fig. 3B).

Application of phage JS01 to control **S. aureus** in the food system

The effect of phage JS01 on the growth of **S. aureus** in three different foods was investigated. UHT whole-fat milk was experimentally contaminated with 10^4–10^6 CFU mL^{-1} of **S. aureus** ATCC 25923 and protected simultaneously with 10^5–10^8 PFU mL^{-1} of phage JS01 before incubation at 4°C or 22°C. No phage was added to the controls. **S. aureus** counts (4.2×10^4–5.6×10^6 CFU mL^{-1}) in the non-protected control increased by 0.2–1.2 log units after 6 h of incubation at 22°C and 0.5–0.6 log units after 3 d of incubation at 4°C (Fig. 4A-4D). While in the JS01-treated samples, all four phage concentrations evaluated were able to significantly inhibit the growth of **S. aureus**, in a concentration-dependent manner with the higher phage concentration producing higher inhibition efficacy, whatever at 4°C or 22°C. Application of 10^8 or 10^7 PFU mL^{-1} of phage JS01 was sufficient to completely eliminate viable **S. aureus** over time (Fig. 4A-4D), while 10^6 and 10^5 PFU mL^{-1} resulted in a 3.6–4.0 and 1.7–2.4 log units reduction of viable CFU counts after 6 h of incubation at 22°C (Fig. 4A, 4C), and 3.8–4.6 and 2.0–3.2 log units reduction after 3 d of incubation at 4°C (Fig. 4B, 4D), respectively. Even when the UHT whole-fat milk was contaminated with a much higher dose of **S. aureus** (10^8 CFU mL^{-1}), phage JS01 could reduce bacterial counts by 6.9, 3.4, and 1.8 log units at phage dose of 10^8, 10^7, and 10^6 PFU mL^{-1}, respectively, after 3 d of incubation at 4°C. The storage temperature applicable to UHT whole-fat milk, 4°C and 22°C, had little effect on the efficacy of phage.

Then, the anti-staphylococcal ability of phage JS01 in hot dogs was examined. Hot dogs were experimentally contaminated with **S. aureus** ATCC 25923, then treated with phage JS01 for 1 h and kept at 22°C for several days. **S. aureus** counts (1.5×10^5 CFU g^{-1}) in the non-protected control increased by 1.2 log units after 3 d of incubation (Fig. 5A). While applied with phage JS01, the viable **S. aureus** on the surface of hot dogs was completely eliminated during the first two days postapplication. However, after 3 d, the viable counts increased a little (16 CFU g^{-1}), but were still 4.0 log units lower than the non-protect control. Thus treatment of the contaminated hot dogs with JS01 significantly reduced the number of viable **S. aureus** cells on their surface.

Finally, the effect of phage treatment on the growth of **S. aureus** in yogurt was examined. Yogurt was experimentally contaminated with 10^4
**Fig. 4.** Effect of phage JS01 on growth inhibition of *S. aureus* ATCC 25923 in UHT whole-fat milk. Initially, bacterial cells (A, B: 10⁴ CFU mL⁻¹; C, D: 10⁶ CFU mL⁻¹) were added into each sample with 10⁵ PFU mL⁻¹ (circles), 10⁶ PFU mL⁻¹ (diamonds), 10⁷ PFU mL⁻¹ (triangles), or 10⁸ PFU mL⁻¹ (open squares) of phage JS01 or without phage JS01 (squares), and incubated at 22°C for up to 6 h (A, C) or at 4°C for up to 3 d (B, D). At each time point indicated, bacterial counts were monitored in triplicate.

**Fig. 5.** Effect of phage JS01 on the growth of *S. aureus* ATCC 25923 in hot dogs (A) and yogurt (B). At each time point indicated, bacterial counts were monitored in triplicate. Controls without phage JS01 (squares); with phage JS01 (diamonds).

or 10⁴ CFU mL⁻¹ of *S. aureus* ATCC 25923 and treated simultaneously with 10⁸ PFU mL⁻¹ of phage JS01 before incubation at 4°C. During the 7-d incubation, bacterial counts did not change obviously in both the phage treated and no-phage control tests, which was 1.0×10⁸~1.8×10⁷ CFU/ml when contaminated with 10⁸ CFU mL⁻¹ and 6.3×10³~1.0×10⁴ CFU mL⁻¹ when contaminated with 10⁸ CFU mL⁻¹ of *S. aureus* (Fig. 5B), indicating that phage JS01 produced no observable anti-bacterial effect in yogurt.

**DISCUSSION**

Bacteriophages have significant potential as antibacterial agents. Meanwhile, they are non-toxic to humans and are naturally present components in foods. Therefore, they should be
considered as safe for intentional application in foods and may be applied as bio-preservatives to preventing pathogen contamination in the food system\textsuperscript{19}. Phages may also be helpful in decontaminating food production-related environments. Our study isolated a novel Siphoviridae phage, JS01 from effluent of a meat-processing plant. Although the biological properties of JS01 were similar to that of other reported staphylococcal phages\textsuperscript{7,9}, it exhibited good stability at higher temperatures. Therefore, it is expected that phage JS01 might survive during the heating process, and thus, the phage would still be able to inhibit \textit{S. aureus} growth in food products even post pasteurization.

Phage JS01 was capable of reducing \textit{S. aureus} viable cell counts by 5.0 log units after 1 h application to the early exponential culture of ATCC 25923 and by 5.2–8.9 log units after 2–6 h application to the diluted culture of ATCC 25923. When applied to the commercial UHT whole-fat milk and hot dogs, phage JS01 was very effective in suppressing or preventing the growth of \textit{S. aureus} ATCC 25923. Thus JS01 can be used as a candidate bio-preservative for the control of \textit{S. aureus} contamination in these foods. Unfortunately, the phage could not inhibit the bacteria growth in \textit{S. aureus}-contaminated yogurt, likely due to the low pH (3.5–4.0) and high viscosity of yogurt, since no phage particles of JS01 could be survival at pH lower than 5.0. However, as most of foods easily to be contaminated by \textit{S. aureus} are nearly neutral, the contaminated \textit{S. aureus} might be effectively alleviated by the use of phage JS01.

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