Phage JS02, A Putative Temperate Phage, A Novel Biofilm-Degrading Agent for *Staphylococcus Aureus*

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Abstract

Background: *Staphylococcus aureus* is a biofilm-producing organism that is frequently isolated from various environments worldwide. Because of the natural resistance of *S. aureus* biofilm to antibiotics, bacteriophages are considered as a promising alternative for its removal.

Results: The bacteriophage vB_SauS_JS02 was isolated from livestock wastewater and showed activity against multidrug-resistant (MDR) *S. aureus*. The phage vB_SauS_JS02 was morphologically classified as *Siphoviridae*; it had a broad host range (45 out of 81 strains, 55.6%) and high burst size (52 plaque-forming unit (PFU)/infected cell) and could survive in a pH range of 4 to 11 and a temperature range of 40 °C to 50 °C. Bioinformatics analysis showed that the phage genome contained a long double-stranded linear DNA genome of 46,435 base pairs with a G□C content of 33.1% and had 66 putative open reading frames (ORFs). The predicted protein products of the ORFs were clustered functionally into five groups as follows: replication/regulation, DNA packaging, structure/morphogenesis, lysis, and lysogeny. The phage vB_SauS_JS02 was a temperate phage with a higher inhibiting and degrading activity against planktonic cells (~86% reduction) and *S. aureus* biofilm (~68% reduction in biofilm formation). Moreover, the removal activity of the phage vB_SauS_JS02 against both planktonic cells and *S. aureus* biofilms was even better than that of the antibiotic (ceftazidime).

Conclusion: In summary, the present study introduced the phage vB_SauS_JS02 as a potential biocontrol agent against biofilm-producing *S. aureus*.

Background

Multidrug-resistant (MDR) *Staphylococcus aureus* is a clinical pathogen with worldwide occurrence that causes a wide range of health problems such as foodborne diseases, wound injuries, and urinary tract infections [1]. Methicillin-resistant *S. aureus* is frequently detected in contaminated dairy farms [2, 3]. Therefore, it is important to control the occurrence of *S. aureus* in foods in order to ensure food safety. Because *S. aureus* has a high ability to form biofilms on a wide range of surfaces, alternative and safe approaches are required for its biocontrol in foods.

Biofilms are aggregated structured communities of bacteria enclosed in a matrix (often referred to as extracellular polymeric substances), which is composed of proteins, DNA, and polysaccharides. During growth in biofilms, bacteria may evade host defenses and become tolerant to antimicrobial agents that can inhibit free-floating, single-cell (planktonic) bacteria, thereby making biofilms particularly difficult to eradicate [4].

Bacteriophages (phages) are viruses that infect only bacteria, and they replicate and propagate through host specificity and growth inhibition activity. Because antibiotics have been found to be ineffective against biofilms, there is a growing interest in the use of phages as a strategy for prevention and elimination of biofilm formation. On the basis of recent findings, bacteriophages are considered to play a crucial role in the prevention and elimination of biofilm-related infections [5].

In general, the life cycle of a phage enters either of the following two stages after infection of the host strain: a lytic cycle or a lysogenic cycle [6]. Although lytic phage seems to be an ideal candidate for controlling MDR *S. aureus* infections, more than 200 genome sequences of *Staphylococcal* bacteriophages are available in public genome databases, and most of these phages belong to the *Siphoviridae* family of temperate, tailed bacterial viruses [7]. Therefore, genetic engineering of the lysogen decision gene cluster in *S. aureus*-infecting *Siphoviridae* temperate phages to obtain virulent phages is a useful strategy for further applications to control *S. aureus*. In a previous study, a temperate phage in *Siphoviridae* family was transformed into a virulent one by random gene mutation using sodium pyrophosphate, and this virulent mutant phage showed rapid and long-lasting host cell growth inhibition activity; this suggested that this phage may have potential to serve as a novel biocontrol agent against *S. aureus* [1].

In the present study, a novel *Siphoviridae* phage, vB_SauS_JS02, was isolated and characterized to control MDR *S. aureus*. vB_SauS_JS02 is a temperate phage but has strong host inhibition activity. Moreover, the phage vB_SauS_JS02 showed a higher removal activity against planktonic cells of *S. aureus* than the antibiotic ceftazidime. The genome of this phage was sequenced and analyzed to determine the genes coding for PVL and integrase, which makes it unsuitable to be used as a bio-control agent. We therefore recommend genome engineering as an additional step to select candidate phages for potential application in phage therapy.

Results

Electron Microscopy Analysis of Bacteriophage

The isolated phage was found to form clear plaques on the host strain lawn culture. Transmission electron microscopy revealed a long hexagon head (80 ± 2 nm, n = 10) that was connected directly to a long noncontractile tail (300 ± 8 nm, n = 10) (**Figure 1A**). The morphology analysis clearly indicated that the phage belongs to the order *Caudovirales*, family *Siphoviridae*. On the basis of the classification and nomenclature rules of viruses and according to the morphological evidence, the phage was named as vB_SauS_JS02.

One-step Growth Curve Analysis and Thermal and pH Stability

The one-step growth curve of the phage vB_SauS_JS02 showed a latent period of 20 min and a burst size of 52 PFU/infected cell when infecting *S. aureus* SF16 (**Figure 1B**). The infectivity of phage was relatively stable at pH 6–9 and declined dramatically at lower or higher pH (**Figure 1C**). In addition, the infectivity of the phage vB_SauS_JS02 remained intact when heated to 40 °C or 50 °C and decreased rapidly above 60 °C (**Figure 1D**).

Phage Host Range and EOP Analysis

The hot spot assay revealed that most of the *S. aureus* strains (45 of 81 strains, 55.6%) were lysed by vB_SauS_JS02 (**Table 1**). The susceptible strains from the spot test were subjected to EOP analysis. A wide range of EOP (0.05 ± 0.02–0.94 ± 0.03) was obtained in the spot test (**Table 1**). Moreover, high (EOP ≥

0.5) and medium (0.1 ≤ EOP < 0.5) production levels were observed in approximately 16% and 36% of the tested strains, respectively (**Table 1**).

Genomic Sequencing and Comparative Genomic Analysis

The digestion pattern of the phage genome showed that the genome was digested by *Hind*Ⅲ, *Eco*RI, and *Eco*RV (**Figure S1**). The phage vB_SauS_JS02 contained a long double-stranded linear DNA genome of 46,435 base pairs with a G□C content of 33.1% (**Figure 2**). Bioinformatics analysis revealed that the phage vB_SauS_JS02 genome contained 66 putative ORFs (**Figure 2**). These 66 ORFs were similar to those of the genus deposited in the GenBank database with annotated functions. However, most of the annotated genes (39 of 66 ORFs) were hypothetical proteins (**Table S1**). The sequences were assigned to five functional clusters as follows: replication/regulation, DNA packaging, structure/morphogenesis, lysis, and lysogeny (**Figure 2**).

Bioinformatics analysis showed that the phage vB_SauS_JS02 genome was quite similar to that of the other members of *Triavirus* genus (**Table 2**). The ANIm value of the phage genome indicated that vB_SauS_JS02 shared 76.9–95.7% nucleotide sequence similarity to ΦSa2aw_st8, ΦSa2wa_st93, ΦSa2wa_st93mssa, Φ7401PVL, 47, 3A, 42e, ΦSauS-IPLA35, Φ12, and ΦSLT (**Figure 3**). Furthermore, the relatedness of the phage vB_SauS_JS02 with the *S. aureus* phages (phage ΦSa2aw_st8, ΦSa2wa_st93, ΦSa2wa_st93mssa, Φ7401PVL, 47, 3A, 42e, ΦSauS-IPLA35, Φ12, and ΦSLT) based on nucleotide and amino acid sequences was confirmed by EasyFig software and Gepard (**Figure S2**). The genome sections with similarities housed several gene groups, *e.g.*, major capsid, tail protein, and integrase (**Figure 4**). On the basis of the major capsid and the large subunit terminase protein sequences of the phage vB_SauS_JS02, it was confirmed that this phage is most related to phage ΦSa2aw_st8 (**Figure 5A and B**). Thus, vB_SauS_JS02 was classified as a member of the family *Siphoviridae*, genus *Triavirus*.

Antibacterial Activity of the Phage vB_SauS_JS02 Against Planktonic Cells

To study the effect of phage exposure on biofilm formation, we first followed bacterial growth in the planktonic phase in the presence of increasing MOIs (0.1, 1, 10, and 100) of the phage vB_SauS_JS02 and ceftazidime (16 µg/mL, sub-MIC) within 7 h. At all the MOIs tested, bacterial growth in the planktonic phase was equal to that of the nontreated control until 4 h post-treatment (**Figure 6A**). Growth of the bacteria infected with vB_SauS_JS02 at all the MOIs showed a significant reduction ($p < 0.001$) compared to that of the bacterial control from 5 to 7 h post-infection (**Figure 6A**). Additionally, the growth of the bacterial samples infected with vB_SauS_JS02 at all the MOIs was significantly reduced compared to that after ceftazidime treatment (sub-MIC, 16 µg/mL) at 7 h time point ($p < 0.05$, **Figure 6A**). At all MOIs (0.1, 1, 10, and 100), the phage vB_SauS_JS02 caused an ~86% reduction of *S. aureus* population at 7 h when compared with the control (without phage) in which 60% reduction was observed. The results of the bacterial reduction test indicated that the growth of bacterial cells was inhibited in the presence of vB_SauS_JS02 at different MOIs in the early phase culture of *S. aureus*.

Biofilm formation in the presence of increasing MOIs (0.1 to 100) of the phage vB_SauS_JS02 and ceftazidime (16 µg/mL, sub-MIC) at 24 h time point was also monitored by the standard CV assay. Similar results were obtained in the groups infected with different MOIs (**Figure 6B)**. Pretreatment with phage (MOI: 0.1) significantly inhibited biofilm formation ($p < 0.001$, **Figure 6B**). At MOIs of 0.1, 1, 10, and 100, the phage JS02 removed 62%, 70%, 69%, and 69% of the biofilm's biomass after 24 h, respectively. Ceftazidime at the concentration of 16 µg/mL reduced the biofilm's biomass by 43% after 24 h. The SEM technique was used to confirm the results obtained by the CV assay (**Figure 6C-E**). The results of SEM confirmed the abovementioned observations. Biofilm formation was suppressed in both the phage pretreated (MOI: 0.1) and the antibiotic (ceftazidime, 16 µg/mL, sub-MIC) pretreated plates (**Figure 6D, E**). Moreover, the effect of the phage treatment was better than that achieved after ceftazidime treatment (**Figure 6B, D, and E**). Thus, the bacteriophage vB_SauS_JS02 exhibited a good preventive effect on biofilm formation.

Discussion

In the present study, we report a *S. aureus* temperate phage isolated from sewage effluent that showed an efficient activity to control planktonic cell growth to prevent biofilm formation. We first discuss the experimental results that led to the selection of the phage vB_SauS_JS02, followed by genome findings, which demonstrated that it is a temperate phage, thus, making it unsuitable for phage therapy.

The primary experimental evidences such as clear plaque formation, broad host range, well-stability in different pHs and temperatures encouraged us to consider JS02 as a advantageous candidate to control *S. aureus* infection. Moreover, the results of the assay of JS02 activity against planktonic cells showed a significant reduction in absorbance measured at OD_{570} as compared to that noted for the ceftazidime (16 µg/mL, sub-MIC) treatment group. In addition, pretreatment with the phage JS02 remarkably inhibited biofilm formation of the SF16 strain. The *S. aureus* SF16 strain exhibited a biofilm-forming capacity and was identified as a high-risk pathogen based on its resistance to various antibiotics such as tetracycline, ciprofloxacin, penicillin, ampicillin, clindamycin, and azithromycin. Phage-bacteria interaction is highly dependent on both the particular bacterial strain and the phage. For instance, similar to planktonic cells, the response of the biofilm to the phage was strain-dependent; the temperate phage ΦPan70 produced a reduction of 99.9% to ~ 99.999% depending on the strain [8]. Although the mechanism of phage-biofilm interaction is still unclear, it is believed that phage-derived enzymes degrade the major exopolysaccharide protective layer of the biofilm matrix, and consequently, phages can reach and kill bacterial cells in the biofilm [9]. Furthermore, phages can diffuse through the pores and channels in the biofilm to reach different layers of the biofilm [10].

Most *S. aureus* isolates carry multiple bacteriophages in their genome (prophage), and based on our knowledge, all the known *Staphylococcal* temperate phages belong to the family *Siphoviridae* [11]. On the basis of the morphological characteristics, the results indicated that the phage vB_SauS_JS02 belongs to the *Siphoviridae* family, which belonged to serogroup A with a tail length of more than 200 nm and a head with a long hexagon head [12]. According to the one-step growth curve analysis, the latent period for the phage JS02 was approximately 20 min, and the burst size was approximately 52 PFU/infected cell, which were similar to those observed for the drug-resistant *S. aureus* phage S1 that belonged to serogroup A [12]. Bioinformatics analysis of the phage genome showed that this phage could be categorized as an unclassified species within *Triavirus* genus (Table 2). The JS02 genome contained 46,435 bp with a G□C content of 33.1% (Fig. 2). Genome analysis of the phage revealed 66 predicted ORFs; however, most of the annotated genes (39/66 ORFs) were

hypothetical proteins (**Table S1**). The module for lysis included amidase and holin. ORF63 and ORF64 encoded proteins that were nearly identical to amidase (N-acetylmuramidase), and ORF62 encoded a protein identical to holin. In the lysogeny modules, the predicted proteins of ORF02 and ORF03 were identified as integrase and showed highest similarity to that of *S. aureus* ΦSa2aw_st8 [13]. Finally, the predicted proteins of ORF65 and ORF66 showed similarity to *S. aureus* Φ7401PVL, with 100% identity [14]. In some cases, it is found as a part of the Panton-Valentine leucocidin (PVL) group of genes, which encode members of the leucocidin group of bacterial toxins that attack leukocytes by creating pores in cell membrane. PVL was found to be encoded in diverse prophages of *S. aureus*, and it appears to be a virulence factor associated with several human diseases[13].

It is widely known that virulent phages are suitable to be developed as biocontrol agents. Although the JS02 phage showed very efficient *S. aureus* growth inhibition activity, the existence of lysogeny associated genes makes it a little riskful to introduce as a suitable biocontrol agent for some reasons. For instance, the insertion of phage genome into host cell chromosome makes these cells potentially resistant to the closed phages. In addition, some undesirable genes such as genes encoding toxins and virulence factors are transferred by lysogenic phages.

For vB_SauS_JS02, the following aspects indicated its high performance to control *S. aureus* infection: absolutely clear plaque formation, high titer progeny, broad host range, and adequate inhibitory effect on planktonic cells and biofilms of *S. aureus*. A previous study reported that mutations induced in the lysogeny module by using sodium pyrophosphate led to the loss of lysogenic capability, which enabled to select lytic variants [15]. Another study showed that the temperate phage SA13 when transformed into a virulent phage by gene mutations acted as an antimicrobial agent against *S. aureus* [1].

The present study demonstrates the vital role of phage genome sequencing of the candidates agents to be used as alternative therapy. While vB_SauS_JS02, showed almost all lytic phage characteristics and seemed to be an ideal candidate for controlling *S. aureus* infection, was harbored lysogenic-mediated genes and could be introduces as a temperate phage based on genome analysis. To our knowledge temperate phages might be avoided as antibacterial agents because of their numerous demerit. However, mutant temperate phages could be incapable of both entering the lysogeny cycle and effectively killing host cells that have been lysogenized by related temperate phages [16]. Isolation or engineering of mutants is an additional step to the development of a therapeutic phage that would be necessary if obligatory lytic phages are selected.

Conclusions

In summary, this study presents the phage vB_SauS_JS02 as a bacteriophage that can effectively control *S. aureus* infection through inhibition of planktonic cell growth and biofilm formation. Bioinformatics analysis showed that this phage could be categorized as an unclassified species in the genus *Triavirus*, family *Siphoviridae*. Although temperate phages are not recommended for phage therapy, selecting gene mutations or engineering of genes coding for PVL and integrase as an additional step could enable to develop temperate phages for phage therapy.

Methods

Host Strains

From 2010 to 2019, 81 *S. aureus* strains were recovered from several sewage samples, Jiangsu province, China. The isolates were kept in Luria-Bertani (LB) broth supplemented with 30% glycerol at -80 ºC. In addition to the wild isolates above, four reference strains, namely *S. aureus* CMCC 26001, CMCC 26003, ATCC 29213, and ATCC 25923 were also used as host in some steps. All bacteria were routinely cultured in tryptic soy broth (TSB, Qingdao Hope Bio-Technology Co., Ltd, Qingdao, China) or tryptic soy agar (TSA).

Bacteriophages Isolation

S. aureus SF16 (nonlysogenic strain) was used as the primary host to isolate bacteriophage in the present study. Phage isolation was done based on the conventional protocol as described previously with slight modification [17]. Shortly, the exponential phase culture of the *S. aureus* strain was inoculated with sewage effluent collected from a local livestock industry in Jiangsu province, China Before incubation at 37 ºC for 24 h. Then, the mixture was centrifuged for 20 min at 10,000 × *g* and filtered through a 0.22-µm pore size membrane filter. The presence of specific phages in sample was determined by the spotting assay on lawn culture of the host bacteria [18]. Pure phage was obtained by the three repetitions of the single-plaque isolation method as described elsewhere [19]. At the end, to obtain the high-purified phage particle, the filtered phage solution was subjected to ultracentrifugation through CsCl gradient [17]. The purified phage solution were stored at 4 ºC till further experiments.

Transmission Electron Microscopy

The purified phage lysate was transferred onto a carbon-coated copper grid (Ted Pella Inc., USA) and then, negatively stained using 2% phosphotungstic acid (PTA) as described previously [20]. The phage particle was observed using a Zeiss transmission electron microscope (TEM) EM902 (Zeiss, Oberkochen, Germany) at an accelerating voltage of 100 kV..

One-step Growth Curve Analysis

To determine the latent period and phage burst size, one-step growth curve analysis was carried out as described elsewhere with some modifications [21]. Briefly, the early-exponential growth phase of host cells (*S. aureus* SF16) were harvested by centrifugation (8.000 ×g, 10 min) and resuspended in fresh TSB. The phage was added at a multiplicity of infection (MOI) of 1 and incubated at 37 ºC for 10 min. Then, the mixture was centrifuged at 6,000 × *g* for 10 min, and the pellet was suspended in TSB, followed by incubation at 37 ºC with constant shaking of 100 rpm. Samples were taken at 10-min intervals (up to 90 min) and phage titers were immediately determined by the double-layer agar plate method (DLA). This experiment was done triplicate.

Thermal and pH Stability

For thermo-stability, the phage was incubated at 40 ºC, 50 ºC, 60 ºC, 70 ºC, and 80 ºC, and sample were collected after 20, 40, and 60 min for phage titration using the DLA method. For pH stability, the phage were added to tubes containing SM buffer of different pH values (2–12) and then incubated for 1 h at 37 ºC before phage titration was performed by the DLA method [17].

Host Range Analysis

Host range of the phage was assessed against reference strains and wild isolates using the spotting assay [22]. Briefly, 10 µl of phage suspension (10^7 PFU/ml) was spotted individually onto the surface of bacterial lawn culture plates and incubated at 37 ºC. The plates were checked for clear plaque formation after 18–24 h. On note, the appearance of clear plaque was considered as positive result.

Efficiency of Plating (EOP)

The efficiency of plating (EOP) was defined as the ratio of phage titer on the target host to phage titer on the reference host bacteria (*S. aureus* SF16) and also was used to determine the effectiveness of phage to cause productive infection in a different *S. aureus* isolates. EOPs were only calculated for the *Staphylococcus* isolates that showed a clear zone in the spoting test. The experiment was performed triplicate and EOP of each phage/bacterial strain combination was classified as high (EOP ≥ 0.5), medium (0.1 ≤ EOP □ 0.5), low (0.001 □ EOP □ 0.1), or no (EOP ≤ 0.001) production level according to the mean ± SD score of EOP [23].

Bacteriophage DNA extraction

Bacteriophage genome was extracted based on Chang et al. method. [21]. The phage DNA was digested using restriction enzyme *Hind*□, *Eco*R, and *Eco*RV (TaKaRa, Tokyo, Japan) according to the manufacturer's instructions. After digestion, the fragments were visualized using electrophoresis in 0.8% agarose containing ethidium bromide (0.5 µg/ml).

Genome Sequencing and Genomic Analysis

The preparation of gene library and whole-genome sequencing were performed by Benagen Biotech Co., Ltd. (Wuhan, China) using the Genome Sequencer Illumina NovaSeq System. The open reading frames (ORFs) were predicted using Glimmer 3.02 [24], and nucleotide and protein sequences were scanned for homologs with the alignment search tools (BLASTP, BLASTX, and BLASTN search) available at the NCBI database (http://blast.stva.ncbi.nlm.nih.gov/Blast.cgi). Protein motif search was conducted using HMMER (https://www.ebi.ac.uk/Tools/hmmer/search/phmmer), and annotations were made by referring to published genomes of other phages with at least 98% similarity. The genome was screened for tRNA-encoding genes by using tRNAscan-SE [25]. The Average Nucleotide Identity distance matrix (ANIm) value was calculated for classifying and identifying the phage genome [26]. EasyFig (V 3.2) was used to compare the whole genome with related phage genomes showing the highest similarity at nucleotide and amino acid levels [27, 28]. Phylogenetic trees were constructed using MEGA 7.0 to investigate the evolutionary relationship of the phage. The complete genome sequence of the *S. aureus* phage was deposited in GenBank under accession number LC541428. Pangenome analysis of the isolated phage was performed by CLC genomics workbench 12 (QIAGEN, Aarhus, Denmark) and compared with all phages belong to *Triavirus* and unclassified *Triavirus* genus using the neighbor joining construction method with Kimura 80 as nucleotide distance measure. Bootstrap analysis was performed using 1000 replicates. The complete coverage and identity similarity of the isolated bacteriophage against all phages in *Triavirus* and unclassified *Triavirus* genus was constructed using the default parameter of the database [29]. **Susceptibility of Planktonic Cells**

The *S. aureus* solution (OD_{600} = 0.1) was seeded onto a 96-well sterile polystyrene microtiter plate. To determine the effect of the phage and the antibiotic (ceftazidime) on planktonic culture simultaneously, the bacterial culture was mixed with the phage at different ratios (MOIs of 0.1, 1, 10, and 100) and with ceftazidime at the sub-minimum inhibitory concentration (MIC) of 16 µg/ml. Following incubation, the absorbance of the different samples was measured at OD_{570} at appropriate time points (1–7 h). Wells containing bacteria without phage and antibiotic were used as controls. This experiment was repeated three times [30].

Prevention of Biofilm Formation

To establish the potential of the isolated phage to prevent biofilm formation, *S. aureus* cells were treated with different concentration of the phage to obtain MOIs of 0.1, 1, 10, and 100 with or without antibiotic (16 µg/ml, sub-MIC value) in a 96-well sterile polystyrene microtiter plate. After incubation at 37 ºC for 24 h, the content of the wells was removed, and the wells were gently washed twice with 0.9% NaCl, dried in the inverted position before stained with 1% crystal violet for 10 min. The plates were washed again using distilled water and 200 µl of 0.9% NaCl solution was added to each well. The optimal absorbance at 570 nm was measured in an ELISA plate reader. This experiment repeated three times and the percentage of biofilm removal was calculated in compare to the well contained only bacteria (control) [32].

Examination of Biofilm by Scanning Electron Microscopy

S. aureus biofilms treated with the isolated phage and the antibiotic were also studied by scanning electron microscopy (SEM). For SEM, coverslips were fixed in 2.5% glutaraldehyde solution for 4 h and dehydrated using a serial concentration of ethanol as follows 10 min in 50%, 10 min in 70%, 15 min each in 80%, 15 min in 90%, and 20 min in absolute ethanol. The samples were dried before examined using a scanning electron microscope (ZEISS EVO-LS10, Germany) operating at 5 kV.

Statistical Analysis

The obtain data were analyzed by one-way or two-way analysis of variance (ANOVA) using GraphPad PRISM software (version 5.02). A *p*-value of <0.05 was considered to indicate a statistically significant difference.

Abbreviations

MDR: Multidrug-resistant; ATCC: American Type Culture Collection; CMCC: National Center for Medical Culture Collections; CFU: Colony forming units; MIC: Minimum inhibitory concentration; EOP: Efficiency of plating; MOI: Multiplicity of infection; PFU: Plaque forming units.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article [and its supplementary information files].

Competing interests

There are no conflicts of interest to report.

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Authors' Contributions

LZ, KS, and RW planned the experiments; KS and ASD contributed to genome analysis; LZ, HD, HW, LS, HT, and RCW performed the experiments; LZ wrote the manuscript; KS and RW edited the manuscript for intellectual content. All authors read and approved the final manuscript.

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Tables

Table 1
Lytic activity of the phage vB_SauS_JS02 against *Staphylococcus* strains

Bacterial Strain	Infectivity (spot test)	Efficacy of plating (EOP) (mean ± SD)
ATCC25923	+	0.34 ± 0.04
CMCC26001	+	0.42 ± 0.02
CMCC26003	+	0.44 ± 0.07
ATCC29213	+	0.24 ± 0.03
A14	+	0.07 ± 0.03
A15	+	0.08 ± 0.02
A16	+	0.35 ± 0.05
J7	+	0.43 ± 0.04
J28	+	0.48 ± 0.06
M9	+	0.05 ± 0.02
MP00036	-	0
MP00037	-	0
MP00038	-	0
MP00049	-	0
MP00050	-	0
MP00051	-	0
MP00065	-	0
MP00077	-	0
MP00079	-	0
MP00086	-	0
MP00088	-	0
MP00090	-	0
MP00094	-	0
MP00098	-	0
MP00107	-	0
S7	+	0.12 ± 0.02
SD5	+	0.42 ± 0.04
SD6	+	0.47 ± 0.04
SF15	+	0.77 ± 0.05
SF16	+	1.00
SF22	+	0.33 ± 0.06
SF29	+	0.21 ± 0.03
SF30	+	0.16 ± 0.05
SF34	+	0.11 ± 0.03
SF36	+	0.74 ± 0.04
SF37	+	0.39 ± 0.05
SF38	+	0.75 ± 0.06
SF39	+	0.28 ± 0.03
SF46	+	0.73 ± 0.04

, clear plaque; -, no plaque

Bacterial Strain	Infectivity (spot test)	Efficacy of plating (EOP) (mean ± SD)
SF49	+	0.42 ± 0.06
SF50	+	0.28 ± 0.06
SF54	+	0.64 ± 0.04
SF55	+	0.47 ± 0.03
SF56	+	0.42 ± 0.02
SF60	+	0.44 ± 0.07
SM9	+	0.65 ± 0.02
TQ1	+	0.94 ± 0.03
X5-1	+	0.38 ± 0.06
XB1	-	0
XG17	+	0.11 ± 0.05
XG19	+	0.18 ± 0.04
XG25	+	0.33 ± 0.03
XG26	+	0.13 ± 0.02
YN1	+	0.74 ± 0.04
YN2	+	0.31 ± 0.02
YN3	+	0.27 ± 0.04
YN4	+	0.29 ± 0.03
YN5	+	0.63 ± 0.02
YZ12	+	0.57 ± 0.04
YZ15	-	0
YZ16	+	0.63 ± 0.02
YZ17	-	0
YZ19	+	0.67 ± 0.06
YZ20	-	0
YZ27	-	0
YZ29	-	0
YZ30	-	0
YZ34	-	0
YZ35	-	0
YZ37	-	0
YZ38	-	0
YZ41	-	0
YZ42	-	0
YZ43	-	0
YZ48	-	0
YZ49	-	0
YZ50	-	0
YZ52	-	0
YZ53	-	0
YZ54	-	0
□, clear plaque; -, no plaque		

Bacterial Strain	Infectivity (spot test)	Efficacy of plating (EOP) (mean ± SD)
YZ56	-	0
, clear plaque; -, no plaque		

Table 2
Comparison of the basic characteristics of the phage vB_SauS_JS02 and other similar phages

Phage name	vB_SauS_JS02	ΦSa2wa_st8	ΦSa2wa_st93	ΦSa2wa_st93mssa	Φ7401PVL	47	3A	42e	ΦSauS-IPLA35
Genome length (bp)	46435	45914	45913	45913	47252	44777	43095	45861	45344
GC content (%)	33.2	33.1	33.1	33.1	33	33.5	33.5	33.7	33.2
ICTV	No	No	No	No	No	Yes	Yes	Yes	Yes
Quary coverage (%)	-	88	88	88	98	80	82	80	80
Identity (%)	-	98.6	98.6	98.6	99.0	99.3	97.6	99.2	97.7
Isolation country	China	Australia	Australia	Australia	Japan	Canada	USA	Canada	Spain
Accession number	LC541428	MK940809	MG029517	MG029516	AP012341	AY954957	NC_007053	AY954957	EU861005

Figures

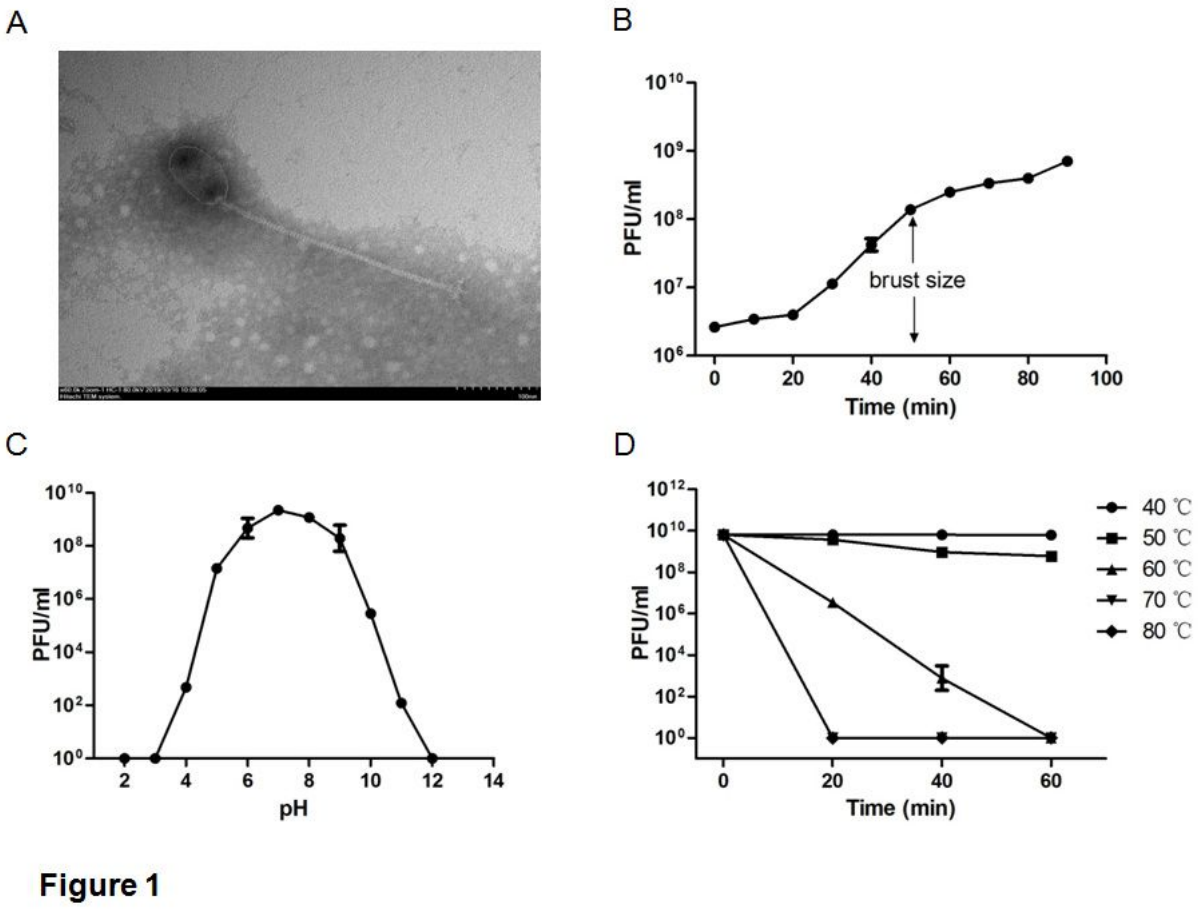

Figure 1

Figure 1

Electron micrograph (A), one-step growth curve (B), pH stability (C), and thermostability (D) of the phage vB_SauS_JS02.

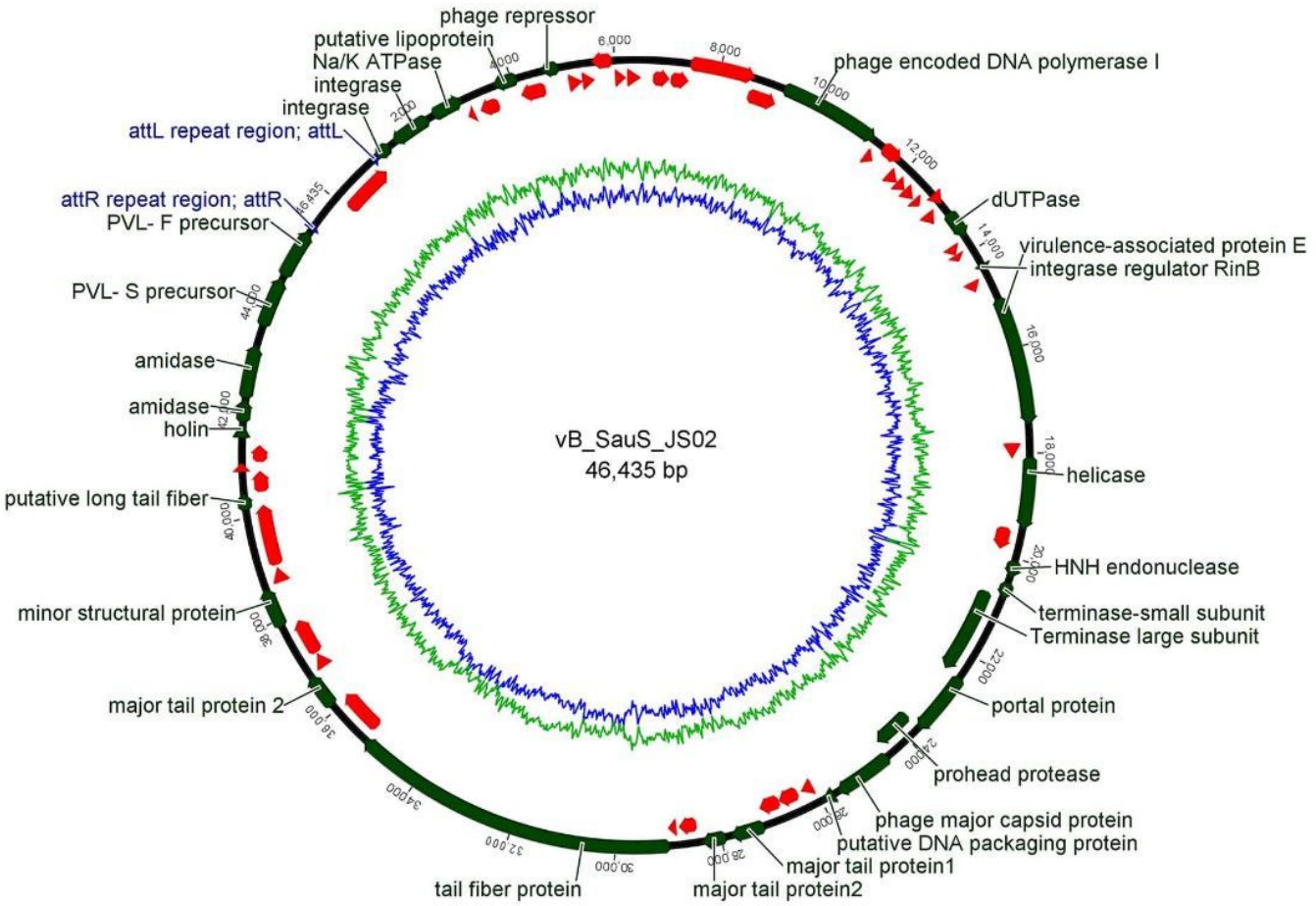

Figure 2

Figure 2

Schematic representation of the dsDNA genome of the temperate phage vB_SauS_JS02. The 66 putative ORFs are represented by arrows, with colors indicating their predicted functions if known. The proposed modules are based on the predicted functions.

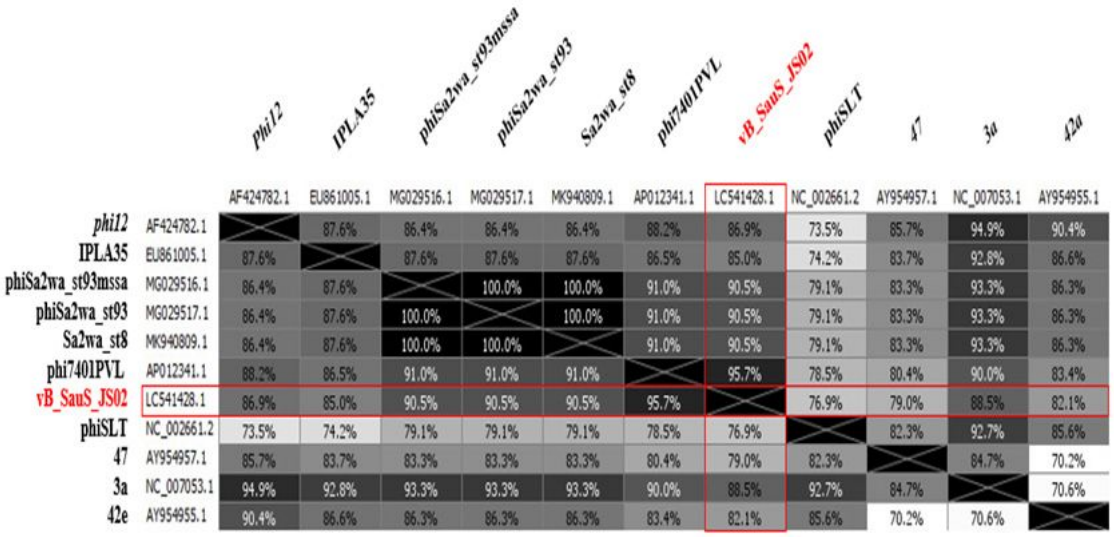

Figure 3

Figure 3

Average nucleotide identity (ANI) distance matrix of vB_SauS_JS02 and the other phages of the genus Triavirus.

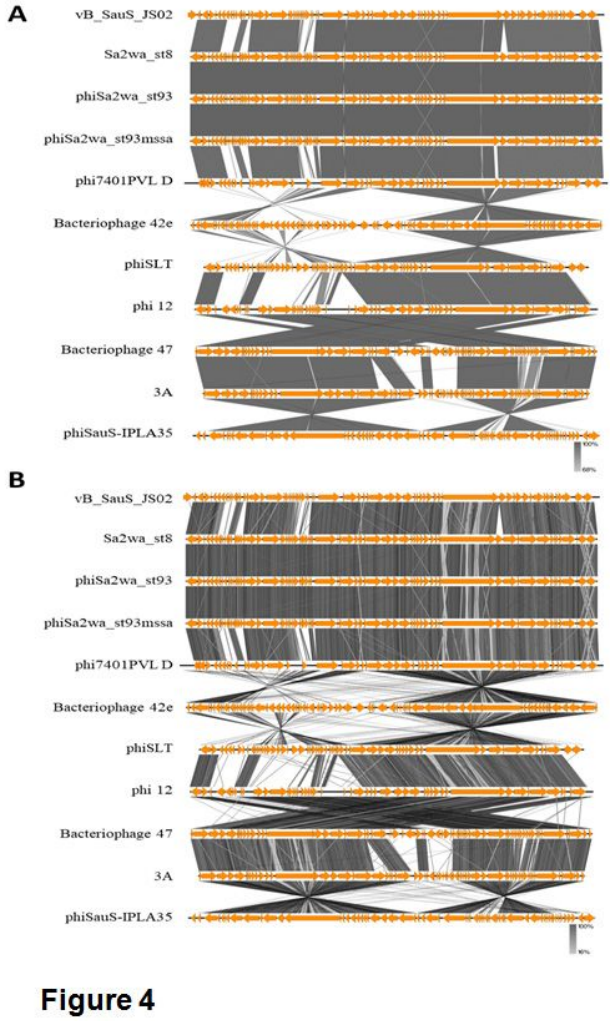

Figure 4

Figure 4

Comparison of the genome of the phage vB_SauS_JS02 with those of the closest related Staphylococcus aureus phages (phage Sa2aw_st8, ΦSa2wa_st93, ΦSa2wa_st93mssa, Φ7401PVL, 47, 3a, 42e, IPLA35, Φ12, and ΦSLT) at the nucleotide (A) and amino acid levels (B).

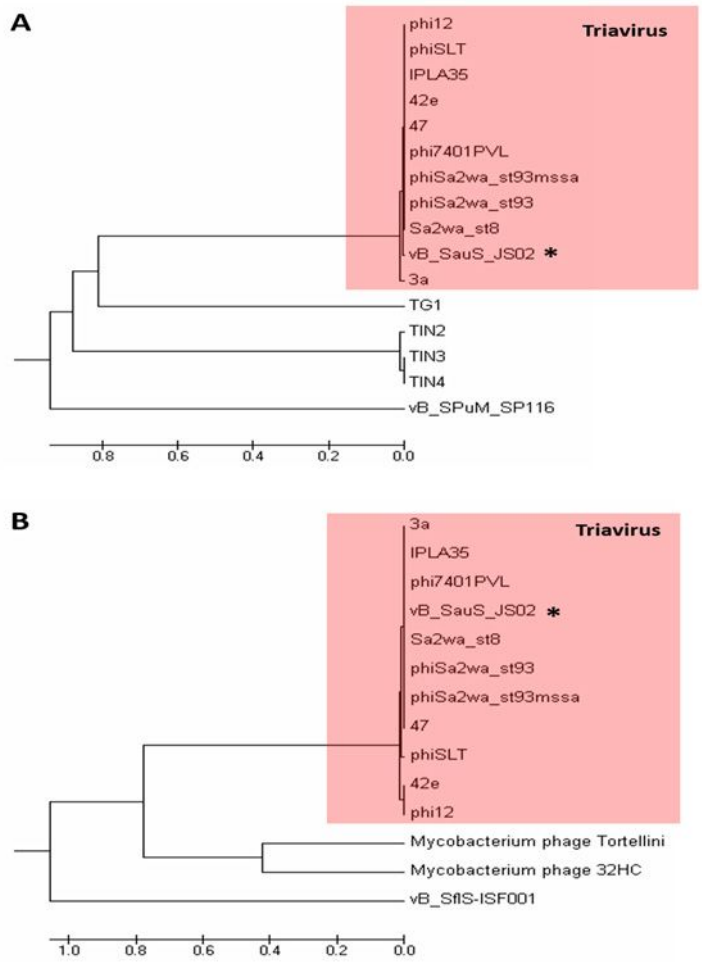

Figure 5

Figure 5

Phylogenic relationship of the phage vB_SauS_JS02 was investigated using the UPGMA method with 2000 bootstrap replications based on amino acid sequences of major capsid (A) and terminase (large subunit) proteins. The sequences of these proteins in phages vB_SPuM_SP116 [33] and vB_SflS-ISF001 [34] were used as outgroups.

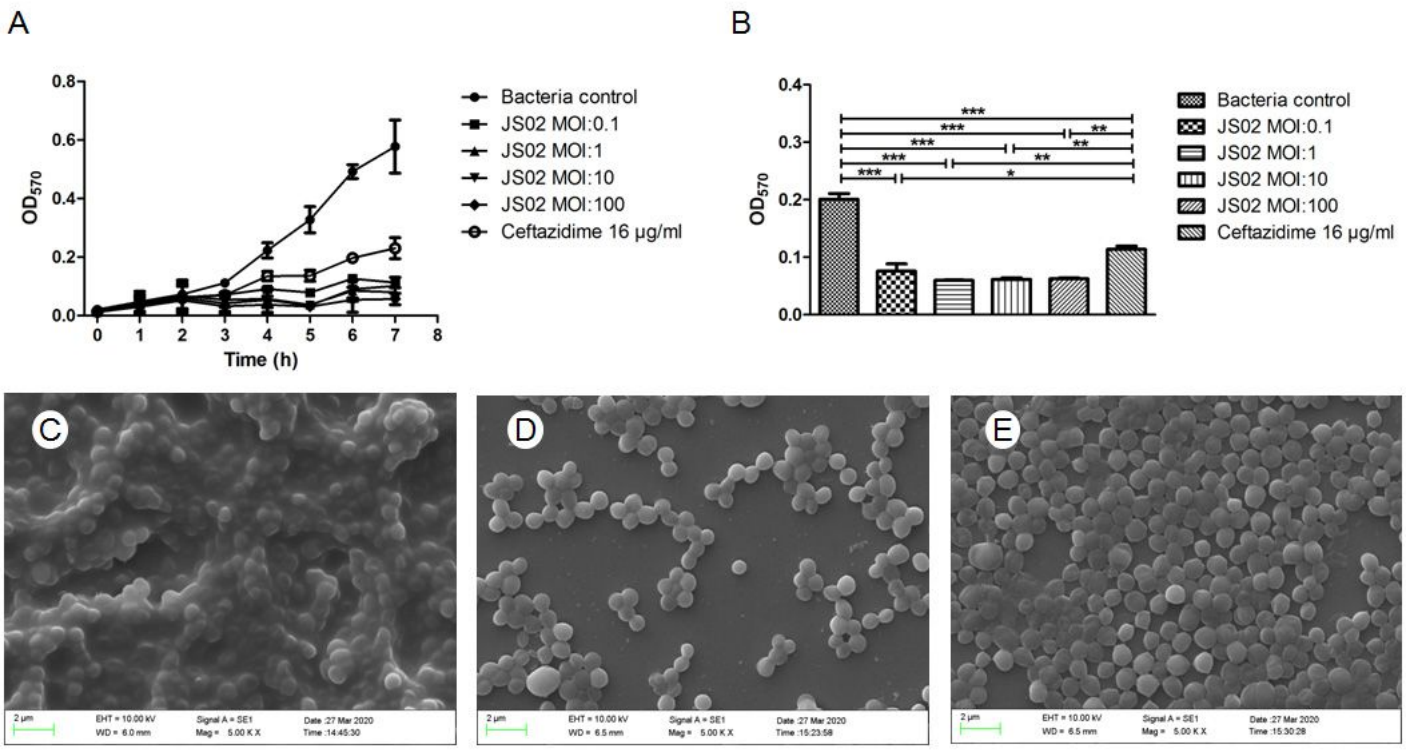

Figure 6

Figure 6

Effects of treatment with the phage and the antibiotic (ceftazidime) on the absorbance of planktonic cells of Staphylococcus aureus in terms of OD (A). Inhibition of biofilm formation by pretreatment of planktonic cells with the phage vB_SauS_JS02 and ceftazidime (16 µg/mL, sub-MIC) at 24 h time point (B). Absorbance was measured at OD570 after CV staining. Scanning electron micrographs: (C) Micrograph of an untreated surface after biofilm formation by Staphylococcus aureus for 24 h. The plate's surface was pretreated with the phage vB_SauS_JS02 (MOI = 0.1) (D) and with ceftazidime (16 µg/mL, sub-MIC) (E) for inhibiting biofilm formation after 24 h. All values are expressed as mean of three determinations ± SD. MOI, multiplicity of infection.

Supplementary Files

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