

Isolation and Characterization of Staphylococcus Aureus Phage and Its Anti-Biofilm Activity Individually or Collaborative with Streptomycin

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Research

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Abstract

Background: *Staphylococcus aureus* was a widespread of Gram-positive pathogen bacteria which causes a wide range of symptoms. Bacteria biofilm was the multicellular community of microorganisms that attached to non-biological and biological surfaces.

Method: Here, we aimed to isolation and characterization of *S. aureus* phage and research its bactericidal activity that individually or collaborative with streptomycin.

Results: In this study, virulent phage WX was isolated from slaughter house in Jiangsu, China. It's belonged to the *Siphoviridae* family and optimal growth temperature was 37 °C, the pH of optimal preservation buffer was 6~7, optimal multiplicity of infection (MOI) was 0.01 and the genome size was 141,342 bp. Phage WX has can sterilize most clinical strains of *S. aureus* which was isolated from clinical patients in the first people's hospital of Yunnan province laboratory. Streptomycin has better anti-biofilm effect than phage WX in low concentration culture of bacteria, nonetheless, phage WX has better anti-biofilm effect than streptomycin in high concentration culture of bacteria. Collaboration of phage WX and streptomycin have better anti-biofilm effect than alone of WX or streptomycin in low concentration culture of bacteria and phage WX have better anti-biofilm effect than streptomycin in high concentration culture of bacteria.

Conclusion: The data of this study provided a strong evidence of application phage for reduce the growth of *S. aureus* biofilm, this study was important for clinic and replace antibiotics in some extent.

Introduction

Staphylococcus aureus was a widespread of Gram-positive pathogen bacteria that causes a wide range symptoms of gastroenteritis, subclinical and clinical mastitis, skin and soft tissue, osteoarticular, pleuropulmonary, bacteremia, mastitis, anterior nares, nosocomial infections, infective endocarditis and device related infections [1–6]. With the broader application and abusing of methicillin, vancomycin and trimethoprim-sulfamethoxazole that leading emergence of many resistant bacteria [7, 8]. *S. aureus* wound infection were strongly associated with the formation of biofilm communities [9]. Furthermore, human organs and medical devices were also the medium for biofilm. Fortunately, phages have a strong ability to clear biofilm [10, 11].

Bacterial biofilm was the multicellular community of microorganisms that embedded self-produced extracellular matrix that attached to highly hydrated extracellular matrix, biological surfaces and non-biological [12–14]. The extracellular polymeric substances matrix of biofilm acts as a barrier that reduces the penetration of antimicrobial agents into the interior of biofilm [15, 16]. Biofilm were highly resistant to desiccation, heat, antibiotics and acidic condition [17]. Bacteria in biofilm were approximately 10 to 1000 times less sensitive to antimicrobial agents than planktonic bacteria, on account of extracellular polymeric substances of the biofilm that prevent contact with antimicrobial agents [18, 19]. Which makes totally eliminate of biofilm among husbandry, food industry and clinic were scarcely possible [20].

With the aggravation abuse of antibiotic resulting the problem of multiple resistant bacteria (MRB). In 2003, there have 80,000 death annually which caused by abuse of antibiotics in China [21]. Phages and their derivatives were ideal candidates for replacing or compensating of antibiotic problems in the future [22]. Phage has the ability of sterilize bacteria [23,24]. Due to ability of kill bacteria, which appear to be a good alternative to antimicrobials and disinfectants. Above all, phage only infect bacteria and not be harmful to humans, making them safe for apply in clinic and food products [25]. Recent study found that phage has high efficiency in reducing and control of bacterial biofilms on various surfaces that formed by *Escherichia coli*, *Salmonella*, *Listeria monocytogenes* and *Pseudomonas aeruginosa* [26–30].

In this article, we have isolated and characterized a lytic *S. aureus* phage WX. Prevention and control of contamination that caused by *S. aureus* biofilm has great importance for economic point and medical. Afterwards, we have studied the basic features of *S. aureus* phage WX. Therefore, the purpose of this study was to reduce *S. aureus* biofilm formation or control mature biofilms through phage WX. Our aim was to determine whether phage could be used as an alternative therapeutic agent against multidrug-resistant bacterial strains, specifically for *S. aureus* strains.

Materials And Methods

Bacterial strains and growth conditions

S. aureus was isolated from a clinic patient in Yunnan first people's hospital, China, it was used as host bacteria for isolate phages. The host strain and phage host range determination strains were grown aerobically on BHI plates or in BHI broth (Difco, Detroit, MI, USA) and incubated in 37 °C. Soft top agar containing of 0.5% (w/w) agar in BHI broth for phage plaque confirmation and BHI agar plates containing of 1.8% (w/w) agar. All *S. aureus* strains were stored in – 80 °C in BHI broth (Difco, Detroit, MI, USA) containing of 20% (v/v) glycerol.

Phage isolation and purification

S. aureus targeting phages were isolated from pig slaughter house. The phages isolate method was modified as follows [31]. Briefly, 10 g of pig farm trash can was mixed with 20 mL of sterile normal saline (0.9% NaCl) buffered in 50 mL sterile centrifuge tube and then shocked incubator of 200 rpm for 2 h in room temperature. Then, samples were centrifuged at 5000 × g for 15 min and filtered with 0.22 µm filter membrane. 10 mL of each filtering medium was added to 30 mL of BHI broth that containing of 1% (v/v) of overnight culture of the host strain and then incubated for 48 h. After that, Cultures were centrifuged for 8 000 × g, 15 min and the supernatant was filtered with 0.22 µm filter membrane. The filtrate was diluted 10 times in series and mixing with 5 mL of molten BHI soft agar that containing of *S. aureus* (2×10^8 cfu/mL), and immediately added to BHI plate. Overnight culture and plaque formation was observed. Single phage plaque was selected for phage purification and repeated for three times.

The pH, thermotolerance, MOI, growth curve and TEM of isolated phage Optimum pH-MOI and thermotolerance

The phage WX stock was diluted to 1×10^8 pfu/mL with BHI broth. Take of 0.99 mL liquid buffer for pH of 3, 4, 5, 6, 7, 8, 9, 10 and 11 (50 mmol/L Citrate buffer, pH 3, pH 4, pH 5; 50 mmol/L phosphate buffer, pH 6, pH 7, pH 8; 50 mmol/L Tris-HCl buffer, pH 9; 50 mmol/L Sodium carbonate buffer, pH 10, pH 11) in 1.5 mL sterile centrifuge tube, added 0.01 mL of diluted phage WX for titer of 1×10^6 pfu/mL to each tube. Place at room temperature for 1 h then detection the titer of phage WX in different pH buffer. The experiments were repeated for three times. Thermotolerance detection were placed of 1 mL diluted phage WX in temperature controller of 4 °C, 25 °C, 37 °C, 42 °C, 50 °C, 60 °C and 90 °C for 1 h, respectively. Multiplicity of infection (MOI) was the ratio of phages to host bacteria of initial infection. According to the MOI of 0.0001, 0.001, 0.01, 0.1, 1, 10 and 100 added phage WX stocks and *S. aureus* culture, cultured in 37 °C for 8 h. The culture were centrifuged at $10\,000 \times g$ for 15 min in 4 °C, then supernatant was filtered with 0.22 µm filter and the titer of phage increment solution were determined through double plate method, the experiment was repeated for three times. For growth curve measure, 1×10^8 pfu/mL of phage WX were added to BHI culture containing of 1/250 *S. aureus* seed culture according the optimum MOI and shacked culture in 37 °C, intermittent sampling was used to determine the titer of phage.

Transmission electron microscopy

The morphology of the phage particles was observed by transmission electron microscopy (TEM). Briefly, each phage stock dilution (approximately 2×10^8 to 2×10^9 pfu/mL) was deposited on copper grids with carbon-coated Formvar films, stained with 2% uranyl-acetate (pH 4.0). Phage samples were imaged using a Philips EM 300 electron microscope, operated at 80 kV in Jiangnan university (Wuxi, China). Phage was classified and identified referring to the International Committee on Taxonomy of Viruses (Rodhain et al., 1995).

Phage genome DNA extraction, sequencing and bioinformatics analysis

Firstly, phage was purified from concentrated to a high titer stock with 10 kDa filter (about 10^9 to 10^{10}). Purified phage was treated with RNase and DNase in 37 °C for 1 h. Then, Takara minibest viral RNA/DNA Extraction kit (Cat#9766) was carried out to obtain purified phage genome DNA. Restriction endonuclease *EcoRI*, *Hind III*, *Not I* and *Xho I* were used for phage genome digestion, respectively. Extracted phage genomic DNA was sequenced using a Illumina Hiseq (Sangon Biotech, China). The original sequencing data were evaluated by FastQC and assembled with SPAdes assembler software. The NCBI Blast compare with multiple databases of CDD, KOG, COG, NR, NT, PFAM, Swissprot and TrEMBL were used for function annotation information of gene protein sequence.

Phage lytic spectrum and antimicrobial susceptibility of *S. aureus*

The host range of the phage WX was determined by the spot test method [32]. The reference strains (All strains were isolated from clinical patients) were tested for susceptibility of phage WX. Generally, each of 200 μL reference strains (10^9 cfu/mL) was added to 5 mL of liquefied BHI soft agar (BHI broth with 0.5% (w/w) agar), and poured over to BHI 1.8% (w/w) agar plate. Three minutes later, single drops of phage suspension were added and incubated in 37 °C for 24 h. Antibiotic sensitivity of *S. aureus* strains were tested against seventeen antibiotics by minimal inhibitory concentration (MIC) method. The antimicrobials tested were Penicillin, Streptomycin, Kanamycin sulfate, Gentamicin, Ciprofloxacin, Levofloxacin, Rifampicin, Vancomycin, Erythromycin, Teicoplanin and Tetracycline.

The different effects of phage and antibiotics on biofilms

Make first-phase preparations, A 48 - well cell slide was placed into a 24 - well plate. Seed solution was inoculated into 100 mL BHI culture solution at the rate of 4‰. Inoculate 1 mL of bacterial solution into 24-well plate. One group, added phage WX, streptomycin, and mixtures of streptomycin and phage WX, respectively, nothing added as control (The addition amount of phage was MOI = 1, The final concentration of streptomycin was 10 $\mu\text{g}/\text{ml}$), Incubation (37 °C, 24 h). The other group, firstly, host culture for 12 hours, after that, added phage, streptomycin, and mixtures of streptomycin and phage, respectively, nothing added as control (The addition amount of phage was MOI = 1, the final concentration of streptomycin was 10 $\mu\text{g}/\text{ml}$), Incubation (37 °C, 12 h). The cfu of each sample was measured through plate counting method. Following, the recovered culture washed twice with PBS buffer; and fixed with 2.5% pre-cooling glutaraldehyde at room temperature for 3 h in dark place. Wash twice with PBS buffer, then dehydrated in an increasing ethyl alcohol gradient (15%, 30%, 50%, 70%, 100% v/v) for 10 min for each step. Afterward, dry overnight and gilt, the results obtained through scanning electron microscope with an accelerating voltage of 20 kV. *S. aureus* seed solution was inoculated in BHI for the proportion of 4‰ of overnight culture. Then 200 times dilution with BHI and added to 96-well plate (200 $\mu\text{L}/\text{hole}$), each sample has three multiple holes. One group, added phage, streptomycin, and mixtures of streptomycin and phage, respectively, nothing added as control (The addition amount of phage was MOI = 0.1, the final concentration of streptomycin was 10 $\mu\text{g}/\text{ml}$), incubation (37 °C, 24 h). The other group, firstly, host culture for 12 hours, after that, added phage, streptomycin, and mixtures of streptomycin and phage, respectively, nothing added as control (The addition amount of phage was MOI = 0.1, the final concentration of streptomycin was 10 $\mu\text{g}/\text{ml}$), incubation (37 °C, 12 h). The bacterial population density (OD_{600 nm}) was measured using a ELIASA (Thermo Scientific, EUA) and discarded bacteria solution. The wells washed twice with PBS to remove unattached bacteria, repeated three times, added of 99% methanol and fix for 15 min, then discard methanol and dry at room temperature, following added 2% crystal violet and stain for 8 min. Rinse the culture plate with running water until the water is colorless.

After drying, measured the absorption light at 570 nm wavelength with a microplate reader. The experiment was repeated for three times.

Results

Characteristics and morphology of isolated phages

Virulent *S. aureus* phage WX was isolated from pig farm trash can in Wuxi China. The plaque of phage WX was appeared 1 mm in diameter after overnight incubation at 37°C (Fig. 1.A). Negatively stained of purified *S. aureus* phage WX was observed with an electron microscope. Transmission electron microscopy (TEM) revealed phage WX virion with an icosahedral head of 80 ± 2 nm in diameter, and a non contractile tail of 200 ± 5 nm long (Fig. 1.C). The morphology of phage WX indicated it was belonged to *Siphoviridae* family. One step growth curve of the phage WX was obtained by inoculation on *S. aureus* according to MOI of 0.1 in 37°C (Fig. 1.B). The latent period for the phage WX was 60 min. The titer of phage WX was reached peaks very quickly in 5 h and appear going down after 20 hours later. The burst size of phage WX was approximately 300 times.

Optimum temperature, pH and MOI of isolated phages

Phage WX has the highest activity after treatment for 1 h at 42°C, then there was a noticeable decline at 60 °C and complete inactivation until 90 °C (Fig. 2). The result show that phage WX has low temperature adaptability and which consistent with the optimum survival temperature of it's host. Phages WX has the most plaque at pH = 6 ~ 7, further, plaque at pH = 10 ~ 11 and pH = 3 ~ 4 were significantly decreased (Fig. 2). These results indicated that the phage WX intolerant to alkali and acids. Multiplicity of infection (MOI) refers the ratio of the number of phages to cells. The optimum MOI of phage WX was 0.01, among them, the plaque of WX was decreased significantly after 0.1 and reach minimum at MOI = 100 (Fig. 2).

Characteristic and analysis of genome

The genome size of phage WX was 141, 342 bp. We have identified of 204 protein-coding genes (open reading frames (ORFs) for complete genome (Table S1). WX DNA was digested by *EcoR* I and *Hind* III but can't be digested by *NotI* and *XhoI* I. Genome analysis revealed that phage WX was a virulent phage (Fig. 1.D, Fig. 3).

Phage lytic spectrum and antibiotic resistance of *S. aureus*

The *S. aureus* strains were isolated from clinical patients in the laboratory of first people's hospital of Yunnan province. Unfortunately, they have a broad spectrum of resistance (Table 1), but fortunately, most of them can be removed by the phage WX (Table 2). They were all possess resistant to Penicillin,

kanamycin sulfate, Erythromycin and tetracycline, but sensitive to Streptomycin, Gentamicin, Ciprofloxacin, Levofloxacin, Rifampicin. The lytic of *S. aureus* phage WX was able to infect four *S. aureus* strains which were isolated from the First People Hospital of Yunnan Province, China (Table 2). This analysis underlined the wide host range of the isolated phage WX.

Table 1
Antibiotic resistance of *S. aureus* isolates used in this study

<i>Staphylococcus aureus</i>												
Antibiotic	A	B	C	D	E	F	G	H	I	J	MSSA	Sau
Penicillin	R	R	R	R	R	R	R	R	R	R	R	R
Streptomycin	S	S	S	S	S	S	S	S	S	S	S	S
kanamycin sulfate	R	R	R	R	R	R	R	R	R	R	R	R
gentamicin	S	S	S	S	S	S	S	S	S	S	S	S
Ciprofloxacin	S	S	S	S	S	S	S	S	S	S	S	S
levofloxacin	S	S	S	S	S	S	S	S	S	S	S	S
rifampicin	S	S	S	S	S	S	S	S	S	S	S	S
vancomycin	S	S	S	S	S	S	S	S	S	S	S	S
Erythromycin	S	R	S	R	R	R	R	R	S	S	R	S
teicoplanin	S	S	S	S	S	S	S	S	S	S	S	S
tetracycline	S	S	S	S	S	S	R	R	R	R	R	R

Table 2
Host range analysis of phage WX.

Strain	WX
<i>Staphylococcus aureus</i> -A	-
<i>Staphylococcus aureus</i> -B	-
<i>Staphylococcus aureus</i> -C	-
<i>Staphylococcus aureus</i> -D	-
<i>Staphylococcus aureus</i> -E	-
<i>Staphylococcus aureus</i> -F	-
<i>Staphylococcus aureus</i> -G	-
<i>Staphylococcus aureus</i> -H	□
<i>Staphylococcus aureus</i> -I	-
<i>Staphylococcus aureus</i> -J	□
<i>Staphylococcus aureus</i> -MSSA	□
<i>Staphylococcus aureus</i> -Sau	□

Compare the effects of phages and streptomycin on host biofilm

SEM was used to assess *S. aureus* biofilm formation on round coverslip that was affected by phage WX (MOI = 0.1) and streptomycin (10 µg/mL). Under the condition of *S. aureus* inoculation at rate of 4‰, added phage WX (MOI = 0.1) and streptomycin (10 µg/mL) in immediately and culture for 24 hours, streptomycin have better sterilization effect than phage WX whether in the case of scanning electron micrograph, OD₆₀₀ of bacterial culture solution, or microplate reader OD₅₇₀ of *S. aureus* biofilm (Fig. 4, 5,6). Nevertheless, under the condition of *S. aureus* inoculation at rate of 4‰ and culture for 12 hours, then WX (MOI = 0.1) and streptomycin (10 µg/mL) were added and cultured for 12 hours, phage WX have better sterilization effect than streptomycin whether in the case of scanning electron micrograph, OD₆₀₀ of bacterial culture solution, or microplate reader OD₅₇₀ of *S. aureus* biofilm (Fig. 4, 5,6). In addition, under the condition of *S. aureus* inoculation at a rate of 4‰ and culture for 12 hours, then WX (MOI = 0.1) and streptomycin (10 µg/mL) were added and cultured for 12 hours, collaboration of phage and antibiotics have better sterilization effect than alone of phage WX or streptomycin whether in the case of scanning electron micrograph, OD₆₀₀ of bacterial culture solution, or microplate reader OD₅₇₀ of *S. aureus* biofilm (Fig. 4,5,6). The result of host colony-forming unit indicate collaboration of phage WX and streptomycin have better sterilization effect than alone of phage WX or streptomycin (Fig. 6). Meanwhile, under the

condition of *S. aureus* inoculation at a rate of 4‰ and culture for 12 hours, then WX (MOI = 0.1) and Streptomycin (10 ug/mL) were added and cultured for 12 hours, phage WX have better sterilization effect than streptomycin (Fig. 6).

Discussion

The *S. aureus* used in this study was isolated from a clinical patient in the first people's hospital of Yunnan province, China, Unfortunately, they were all possess the ability to resistant penicillin, kanamycin sulfate, erythromycin and tetracycline. The emerging of multidrug-resistant (MDR) strains urgent needing new measures to inhibit pathogens. With the rise of antibiotic abuse, multiple resistant bacteria and superbacteria hence a public health hazard. Phage, are dawn of this increasing drug resistance. The isolated *S. aureus* phage WX was ideal substitute for antibiotics for they strong endolysin performance. What's more, phage have better sterilization effect than antibiotics in a high concentration of bacteria, and that, collaboration of phages and antibiotics have better sterilization effect than alone of phages or antibiotics in a low concentration medium of bacteria (Fig. 4,5,6).

The isolated *S. aureus* phage WX was belong to *Siphoviridae* family and the genome sizes for 141,342 bp. Corresponding, the genome sizes of *S. aureus* phage fRuSau02 for 148,464 bp and DRA88 for 141,907 bp [33, 34]. The genome characteristics of phage WX reveal that has a special small endolysin with good ability lytic (Fig. 3).

Compared to antibiotics and phages, phage endolysin has many advantages as an antibacterial agent for clinic patients and combating food spoilage [35]. Phage endolysin was one-use enzymes that with a short half-life [36]. Guo found that *S. aureus* phage endolysin contains a catalytic domain of Ch-type lysozyme at the N-terminus, and this functional area is able to cleave the 6-O-acetylated peptidoglycans which present in the cell wall of *S. aureus*. Comparative, the use of a endolysin as an alternative for antibiotics and phages to inhibit pathogenic. At the same time, there are some challenges for long-term control in the clinic and food industry for the strong vitality of *S. aureus* [37, 38].

The ability of microorganism to form biofilm on different food surfaces increases the risk of cross-contamination, particularly in poultry products, which was a serious problem for food industries, clinic and public health [39–41]. *S. aureus* biofilms in catheter sites and wounds present important problems to patients, drug resistance, morbidity and mortality [33]. Although the significant problems in pathogen control caused by biofilms, exploiting effective eliminate of biofilms is still challenging [42]. Until now, there is no ideal technology of biofilm control, hence, the new control strategies for biofilm are constantly recommended [43]. In this study, we demonstrated that the phage WX has better properties than antibiotics to reduced biofilm formation of *S. aureus* (Fig. 4,5,6).

The result shown that phage WX and Streptomycin can infect *S. aureus* biofilm and has the potential to reduce tested *S. aureus* strains. Antibiotics have better anti-biofilm effect than phages in a low concentration medium of bacteria (Fig. 4,5,6). Nonetheless, phages have better anti-biofilm effect than antibiotics in a high concentration of bacteria (Fig. 4,5,6). The data of this study provided the strong

evidence that the application of phage could reduce the growth and biofilm of *S. aureus* that are important to maintain public health.

In this study, the novel *S. aureus* phage WX, which has good bactericidal and anti-biofilm property on condition of that phage WX endolysin just contains 57 amino acids. This study provides a solid theoretical basis for the study of *Staphylococcus aureus* phage endolysin.

Conclusion

In conclusion, firstly, we have isolated and characterized of a lytic *Staphylococcus aureus* phage and acquired its biological properties, then, we have found that combination use of phages and antibiotics possess significantly better anti-biofilm and bactericidal properties on host bacteria than either antibiotics or phages alone. The data of this study provided the strong evidence that the application of phage could reduce the growth and biofilm of *Staphylococcus aureus* that are important to maintain public health.

Abbreviations

S: sensitive; R: resistive; cfu: colony-forming unit; pfu: plaque forming unit; MOI: Multiplicity of Infection

Declarations

Authors' contributions

Conceived and designed the experiments: LMJ. Performed the experiments: LMJ. Analyzed the data: LMJ. Contributed reagents/materials/analysis tools: RZ, LMJ. Wrote the paper: LMJ. Both authors read and approved the final manuscript.

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Ethics Statement

Ethical approval was obtained from the Institutional Ethics Committee (The First People's Hospital of Yunnan Province, Kunming, Yunnan, China). The study protocol was in accordance with the Declaration of Helsinki for Human Research of 1974 (last modified in 2000). Written informed consent was received from each patient before sample collection.

Availability of data and material

Please contact author for data requests.

Consent for publication

Not applicable.

Conflicts of Interest

The authors declare no conflict of interest.

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Figures

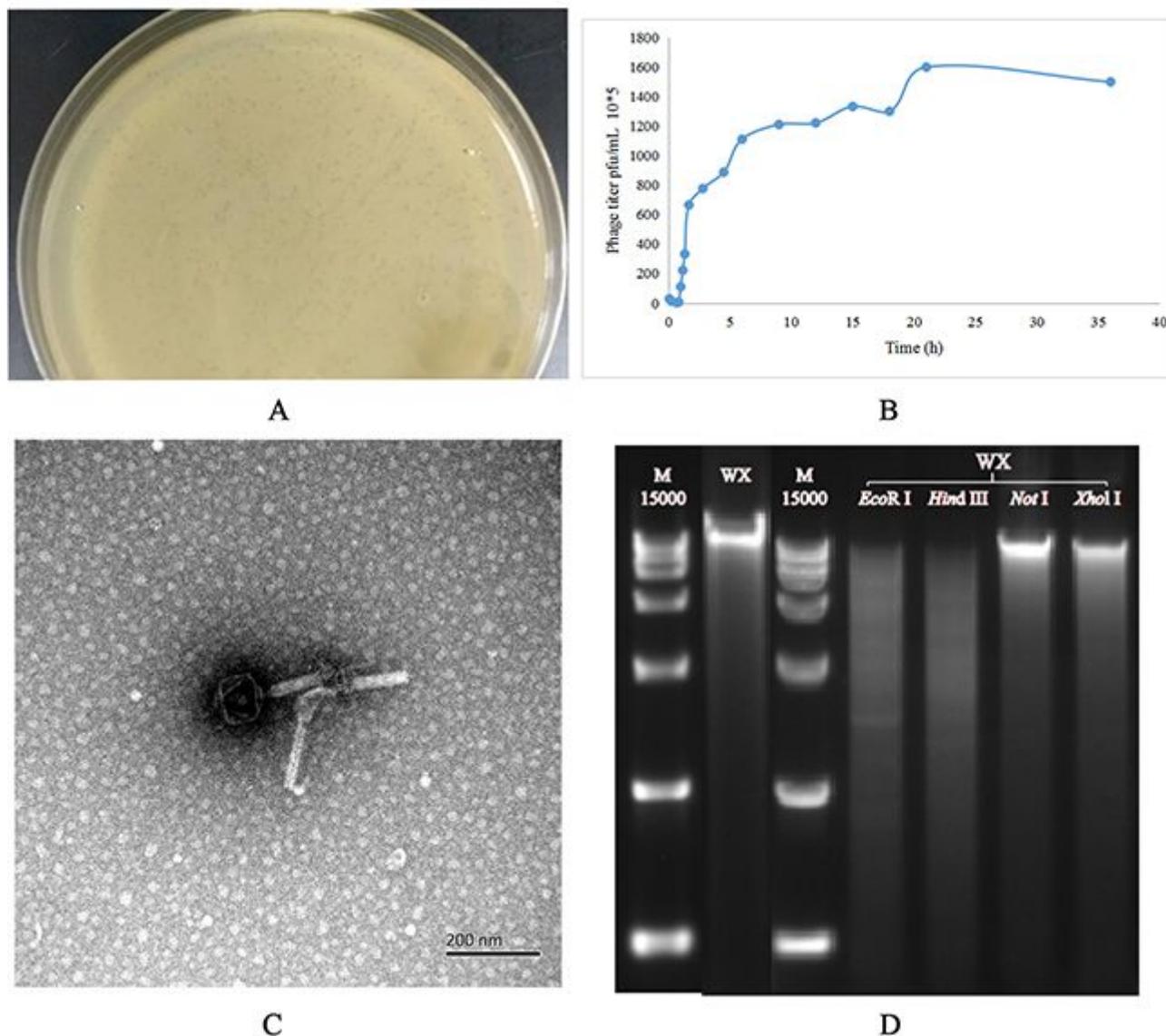


Figure 1

A. Plaques formed by *Staphylococcus aureus* phage WX, the host strains of *S. aureus* after an overnight incubation at 37°C. B. Population dynamics of phage WX inoculate in *S. aureus*. C. Morphological features of *S. aureus* phage WX by transmission electron microscopy (TEM). D. Restriction enzyme

digests of phage WX. Phage WX DNA was digested by EcoRI (lane 4) and Hind III (lane 5), whereas it can't be digested by NotI (lane 6) and XhoI (lane 7).

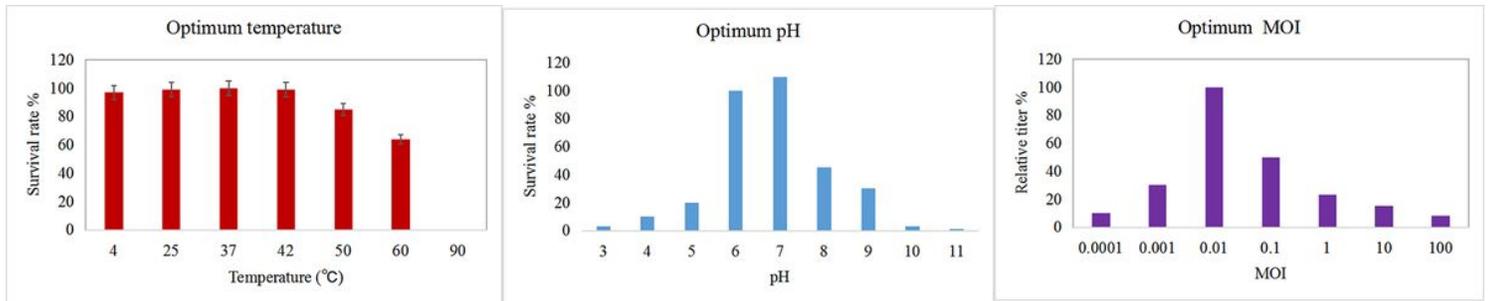


Figure 2

Optimum temperature, pH and MOI of isolated phage WX.

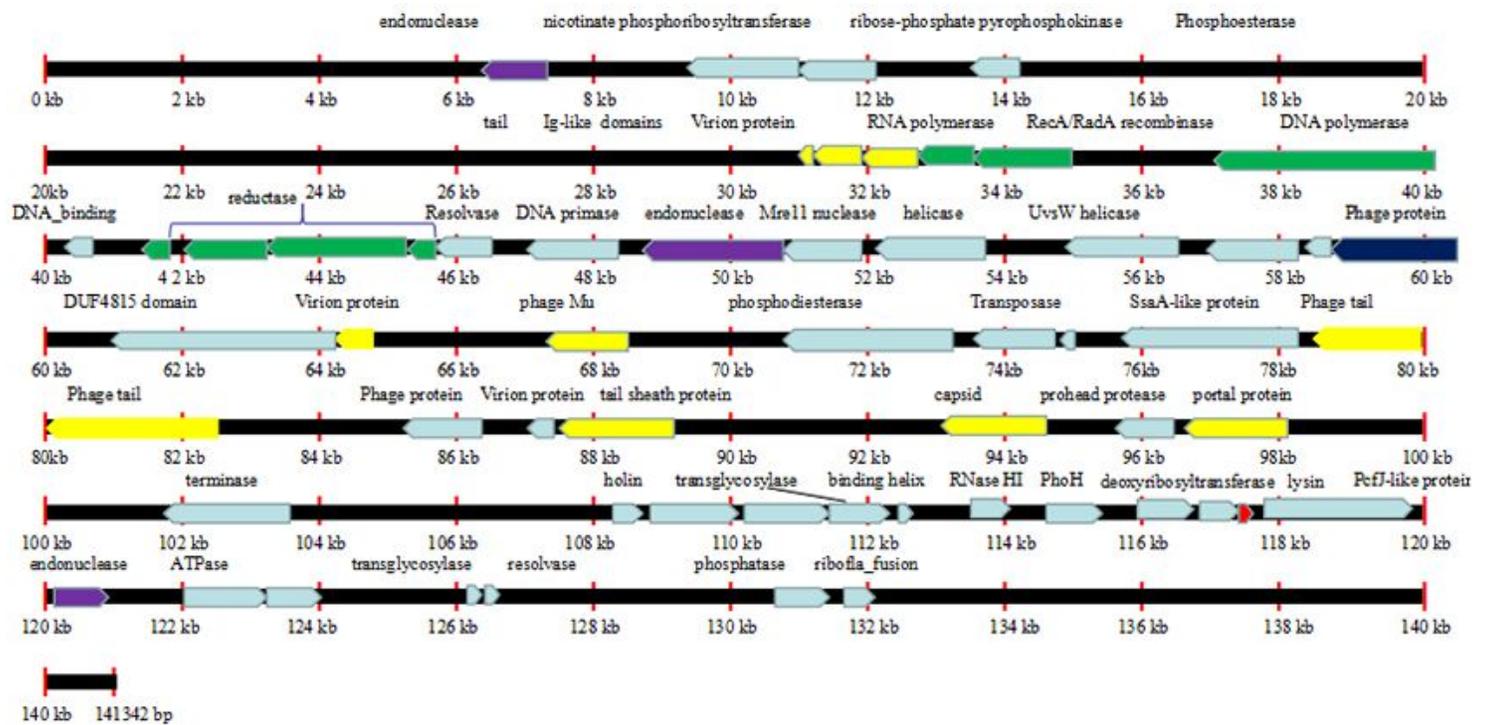


Figure 3

Line map of the phage WX genome. In the WX track, genes colored red instructions for lysozyme, genes colored yellow instructions for tail and genes colored purple instructions for endonuclease. The arrows represent the ORFs and point the direction of transcription.

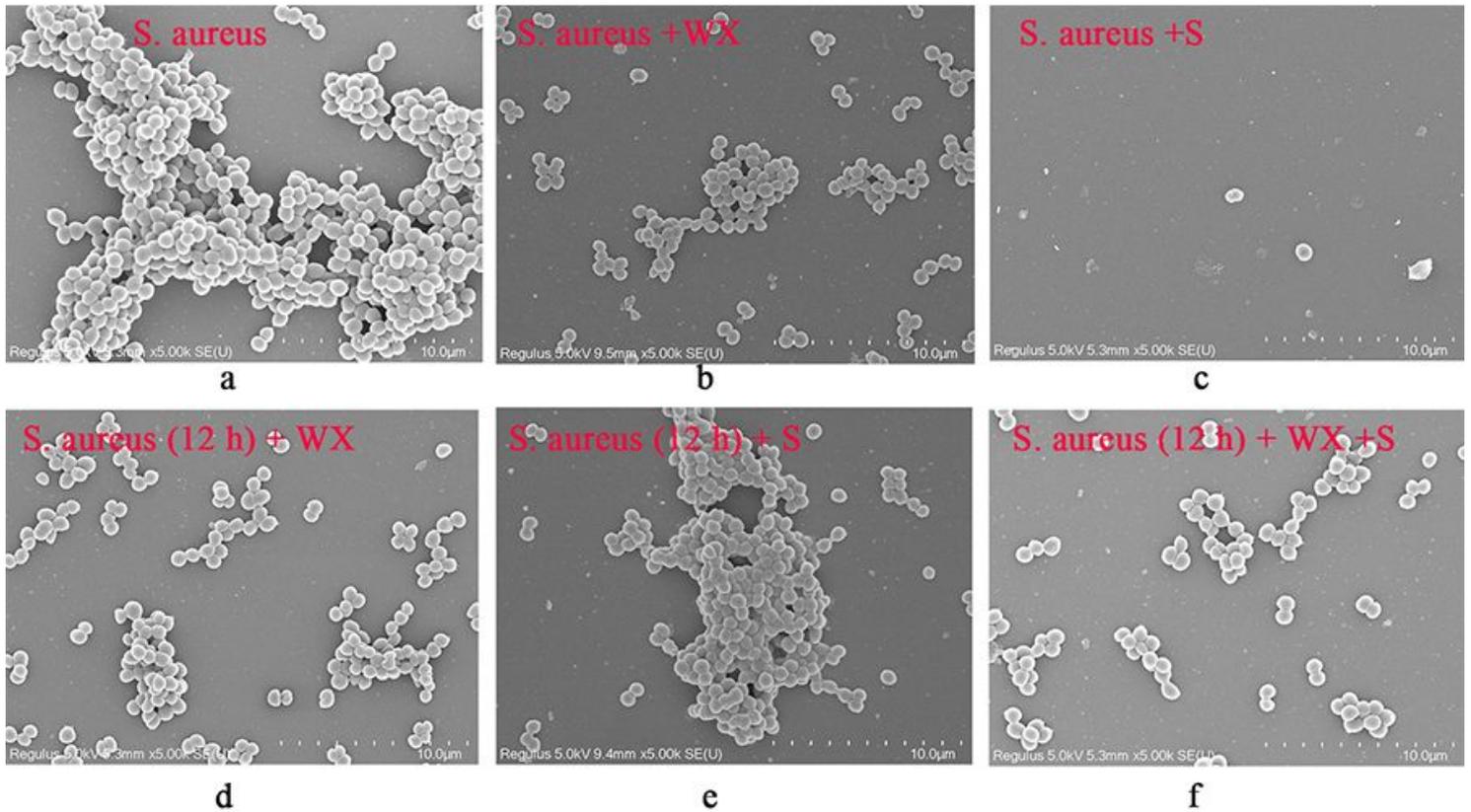


Figure 4

Scanning electron micrograph (SEM) of *S. aureus* colonization before and after phage WX (MOI=0.1) and Streptomycin (10 µg/mL) application to biofilms formed on round coverslip. (A) *S. aureus* inoculation at a rate of 4‰ and culture for 24 hours, (B) A added phage WX (MOI=0.1), (C) A added Streptomycin (10 µg/mL). (D) *S. aureus* inoculation at a rate of 4‰ and culture for 12 hours, then phage WX (MOI=0.1) was added and cultured for 12 hours, (E) *S. aureus* inoculation at a rate of 4‰ and culture for 12 hours, then Streptomycin (10 µg/mL) was added and cultured for 12 hours, (F) *S. aureus* inoculation at a rate of 4‰ and culture for 12 hours, then phage WX (MOI=0.1) and Streptomycin (10 µg/mL) were added and cultured for 12 hours. (5,000× magnification)

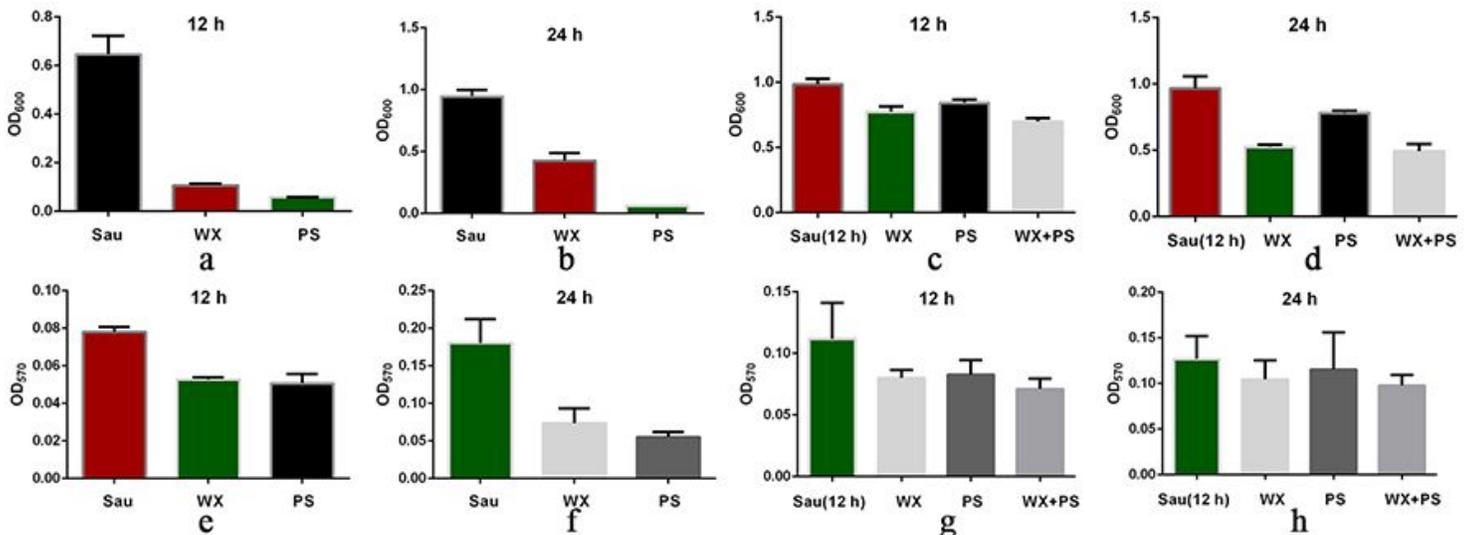


Figure 5

Effects of phage WX and streptomycin (10 ug/mL) on biofilms. (A,B) Effects of phage WX and Streptomycin (10 ug/mL) on *S. aureus* (inoculation at a rate of 4‰) growth that culture for 12 h and 24 h (OD600). (E, F) Effects of phage WX and Streptomycin (10 ug/mL) on *S. aureus* (inoculation at a rate of 4‰) biofilm that culture for 12 h and 24 h (OD570). (C, D) Effects of phage WX and Streptomycin (10 ug/mL) on *S. aureus* (inoculation at a rate of 4‰) growth, first culture for 12 hours, then phage WX and Streptomycin (10 ug/mL) were added and cultured for 12 h and 24 h (OD600). (G,H) Effects of phage WX and Streptomycin (10 ug/mL) on *S. aureus* (inoculation at a rate of 4‰) biofilm, first culture for 12 hours, then phage WX and Streptomycin (10 ug/mL) were added and cultured for 12 h and 24 h (OD570,).

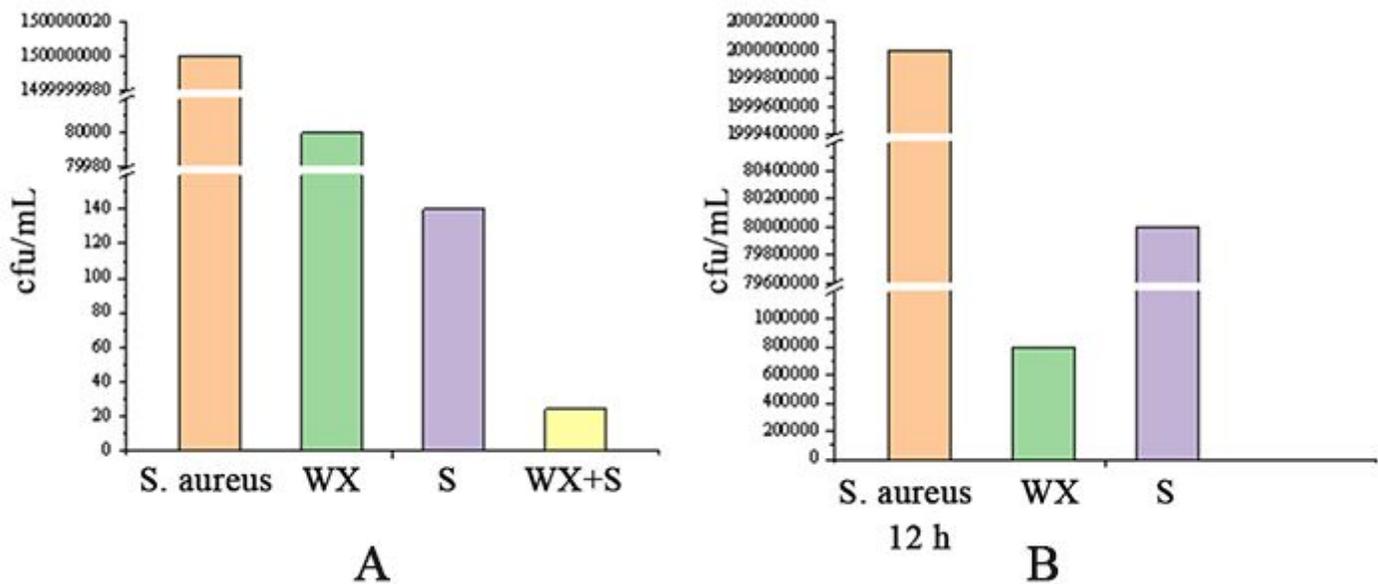


Figure 6

Effects of phage WX and Streptomycin (10 ug/mL) on colony-forming unit of *S. aureus*. (A) Effects of phage WX and Streptomycin (10 ug/mL) on *S. aureus* (inoculation at a rate of 4‰) that culture for 24 h. (B) Effects of phage WX and Streptomycin (10 ug/mL) on *S. aureus* (inoculation at a rate of 4‰), first culture for 12 hours, then phage WX and Streptomycin (10 ug/mL) were added and cultured for 12 h.

Supplementary Files

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