

Broad-Host-Range Phage GSP006 Effectively Controls Horizontal Transmission of Salmonella Pullorum in Poultry Feed and Drinking Water

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Abstract

Background

Salmonella enterica subsp *enterica* serovar Pullorum (*Salmonella* Pullorum) is the major pathogen that is harmful to the poultry industry in developing countries. This bacterium is susceptible to acute systemic disease and severe gastrointestinal disease in chicks and is highly lethal. As a natural alternative to conventional antimicrobial agents, phage therapy is increasingly recognized as highly effective and promising for the control of multidrug-resistant bacterial infections, including salmonellosis caused by *Salmonella*.

Results

In the current study, a broad-host-range phage, GSP006, targeting *Salmonella* Pullorum was isolated from poultry farm wastewater. It exhibits lytic activity against *Salmonella* Pullorum, *Salmonella* Enteritidis and *Salmonella* Typhimurium and other serotypes of *Salmonella* in host range tests. Genomic analysis revealed that GSP006 possesses a double-stranded DNA (dsDNA) genome of 42,165 bp with a G + C content of 50%. Phylogenetic analysis based on the large subunit of terminase confirmed that GSP006 belongs to the genus Jerseyvirus. Biological characterization showed that phage GSP006 was stable to heat (70°C for 1 h) and pH (pH 3 and pH 11 for 1 h). In addition, the phage had a short latent period of about 20 min, followed by the lysis phase. In vitro experiments, phage GSP006 was able to inhibit the bacterium for more than 6 h at 37°C under different infection multiplicities. In the bacteriostatic test of poultry feed and drinking water, phage GSP006 (Multiplicity of Infection, MOI = 100, 1000, 10000) was able to inhibit the growth of *Salmonella* Pullorum at 37°C. These results suggest that phage GSP006 is expected to be an antidote to the horizontal transmission of *Salmonella* Pullorum.

Conclusions

The broad-host-range phage GSP006 exhibits excellent environmental stability and demonstrates strong inhibitory effects on the growth of *Salmonella* Pullorum in poultry feed and drinking water. It holds promise as an effective means of inhibiting the horizontal transmission of *Salmonella* Pullorum.

Introduction

Salmonella is a common foodborne pathogen belonging to the family *Enterobacteriaceae*, which is widely distributed in nature, especially in poultry, livestock and their products [1, 2]. In the poultry industry, *Salmonella* Pullorum is susceptible to acute systemic disease and severe gastrointestinal disease in chicks and is highly lethal, making it one of the most serious pathogenic bacteria in the poultry industry [3]. This pathogen can be transmitted vertically to chicks through incubated eggs or horizontally through direct contact with infected/dead chicks, their feces, or contaminated environments

[4]. Although *Salmonella* Pullorum rarely causes severe clinical signs in adult chickens [5] and has been successfully controlled in developed countries, it remains a major threat in developing regions where intensive poultry production systems are still emerging [4].

The overuse of antibiotics not only reduces efficacy but also pollutes the environment and increases public health risks [6]. In recent years, many countries have banned the use of antibiotics as growth promoters in animal feed [7, 8]. Along with increasing public awareness of sustainable development has increased the demand for eco-friendly biological alternatives. This has brought phage therapy back into the public eye as a hot topic [9]. Phages are viruses that infect and kill bacteria. They are the most ubiquitous organisms on Earth [10, 11]. As natural antimicrobial agents, phages have many advantages over antibiotics, such as self-replication, overcoming drug resistance, target specificity, some phages have broad spectrum and synergistic properties in phage cocktails or in combination with other antibiotics, and they are easy to isolate and propagate [12]. In recent years, there has been a dramatic increase in phage therapy research, especially in the U.S. There is already a precedent for the U.S. Food and Drug Administration (FDA) to approve phage therapy for clinical trials [13], and the European Union and other countries have relaxed their regulations on phage therapy [14]. Previous studies have demonstrated that phages can block horizontal transmission of *Salmonella* gallinarum [15]. In addition, numerous studies have demonstrated the potential value of phages in antimicrobial therapy. [16, 17].

In this study, a broad-host-range phage GSP006 capable of infecting *Salmonella* Pullorum was isolated and characterized. We report the biological characteristics and genome identification of phage GSP006. To investigate the effect of phage GSP006 on controlling the horizontal transmission of *Salmonella* Pullorum, we added the phage to artificially contaminated poultry feed and drinking water and observed a significant reduction in the number of bacteria. In addition the host profile of GSP006 shows that it can also lyse *Salmonella* of different serotypes. These results may help to develop new strategies to protect chicks from horizontally transmitted *Salmonella* Pullorum infections.

Materials and methods

Bacterial strains and phage in this study

Phage GSP006 was isolated from farm wastewater obtained from Daqing, China. The *Salmonella* Pullorum SaP001 was used as the trapping host for phage isolation. All bacterial strains used in the phage host range assay are listed in Tables S1 and S2. Strains were cultured in LB broth at 37°C for 16 h with constant shaking, and bacteria were stored frozen at -80°C with 25% glycerol until subsequent analysis.

Phage isolation and purification

Phages were isolated using a double-layer plate method as described previously [18]. 5 mL of wastewater sample was centrifuged at 6,000×*g* for 5 min at 4°C, followed by filtration of the supernatant through 0.22 µm filters. Add 3 mL of filtrate and 2 mL of host culture to 3 mL of LB medium and incubate

at 37°C for 6 h. Cultures were centrifuged at 1,0000×*g* for 5 min at 4°C and the supernatant was filtered through a 0.22 µm filter. Mix 100 µL of filtrate and 100 µL of the indicator strain with 5 mL of melted LB soft agar (0.7% agar) and cover with LB agar plate (1.5% agar). After 6 h of incubation at 37°C, a clear plaque was picked out from the LB bilayer with a needle tip and resuspended in SM buffer (100 mM NaCl, 10 mM MgSO₄, 50 mM Tris-HCl, pH 7.5). The samples were then serially diluted in SM buffer and purified three times using the double-layer agar method.

Determination of host range and efficiency of plating

The host range of phage GSP006 was determined by the efficiency of plating (EOP) as described previously [19]. In brief, freshly propagated phage GSP006 (10⁹ PFU/mL) was serially diluted 10-fold (10⁻³ to 10⁻⁹) in SM buffer. Aliquots (10 µL) of each dilution were added dropwise to bacterial lawns of test strains. Following overnight incubation at 37°C and EOP was calculated based on the number of plaques formed (EOP, phage titer of test bacteria/phage titer of host bacteria).

Morphology analysis by Transmission Electron Microscopy

Transmission electron microscopy (TEM) was photographed using the method proposed by Wang, X et al [20]. Briefly, a drop of phage lysate was dropped onto a copper grid containing carbon (Carbon Type-B 200 mesh; Beijing Zhongjingkeyi Technology Co., Ltd., Beijing, China) and staining was performed with 2% (wt/vol) phosphotungstic acid (pH 6.5) as described previously. Samples were observed and photographed using a transmission electron microscope (H-7650, Hitachi, Tokyo, Japan) with an acceleration voltage of 100 kV.

Determination of optimal Multiplicity of Infection (MOI)

The MOI was tested following previously described procedures with some modifications [21].

Salmonella Pullorum SaP001 (10⁷ CFU/mL) culture medium was added with phage lysate to achieve different MOIs (MOI = 100, 10, 1, 0.1, 0.01, 0.001, 0.0001) and then cultured at 37°C with shaking for 4 h. The mixture was centrifuged at 10,000×*g* for 1 min at 4°C. The phage titers were determined by the double-layer agar plate method. The MOI with the highest titer was considered the optimal MOI of the phage.

One-step growth curve

One-step growth curves were performed as previously described with modifications [22]. Briefly, SaP001 was infected with phage at an MOI of 0.1 and incubated at 37°C for 15 min. The mixture was then centrifuged at 10,000×*g* for 1 min at 4°C. The precipitate was washed three times with LB broth to remove phage not adsorbed on bacteria, and finally the precipitate was resuspended with an equal volume of LB broth. The resuspended mixture was immediately incubated in a shaker at 37°C with oscillation at 160 rpm. 200 µL of sample was collected every 10 min (up to 150 min) and centrifuged at

10,000×g for 1 min at 4°C. Determination of phage titer at different times by the double-layer agar method.

Phage thermal and pH stability assays

The thermal and pH stability of phages were determined according to the above methods with slight modification [23]. For thermal stability experiments, 1 mL of phage lysate (10^9 PFU/mL) was incubated at different temperatures (4°C, 25°C, 37°C, 40°C, 50°C, 60°C, 70°C and 80°C) for 1 h. For pH stability tests, 100 µL of phage lysates were incubated with different pH (2–14) in SM buffer and incubated for 1 h at 37°C. After thermal and pH stability tests, phage titer were determined by the double-layer agar plate method.

Assessment of the Lytic Activity Level of phage at different MOIs

The lytic activity of phage GSP006 against *Salmonella* Pullorum was evaluated in vitro at different MOIs. Briefly, 100 µL of *Salmonella* Pullorum SaP001 cultured to logarithmic growth phase was mixed with 100 µL of phage GSP006 in 96-well plates according to different MOIs (MOI = 10, 1, 0.1, 0.01, 0.001, 0.0001), and incubate at 37°C for 12 h with shaking in the Feyond-A300 Multi-function enzyme immunoassay analyser (ALLSHENG, Hangzhou, China). The OD₆₀₀ value was monitored every 30 min for 12 h. Positive control is culture without phage, negative control is phage added to LB broth [24].

Genome analysis and phylogenetic analysis

The DNA of phage GSP006 was extracted by viral genome extraction kit (Omega B IO-Tek Inc., Doraville, GA, United States) after DNase I and RNase A were used to remove the nucleotide contamination of the concentrated phage. The whole genome of phage GSP006 was sequenced using the Illumina MiSeq system at Novogene Bioinformatics Technology Co. Ltd. The data obtained after sequencing were assembled using SPAdes v3.15.2 [25]. The phage genome sequences were annotated using RAST (<http://rast.nmpdr.org/>) and manually verified using BLASTp (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). The circular genome map of GSP006 was constructed and visualized using the Proksee server (<https://proksee.ca/>) [26]. Genome comparison of phage GSP006 was performed using easyfig software [27]. The classification of phage GSP006 was determined by downloading the terminal enzyme large subunit sequences of different phages from the NCBI database according to the classification report of the International Committee on Taxonomy of Viruses (ICTV). The phylogenetic tree was constructed in MEGA 11 based on the sequence of the large subunit of terminase of phage GSP006 and analyzed by the neighborhood joining method [28]. The presence of potential virulence and antibiotic resistance genes in the phage genome was examined using CARD (<https://card.mcmaster.ca/analyze/rgi>) [29]. The annotated phage genome was used to manually verify whether there were lysogen-associated proteins in the phage genome.

Bactericidal effect of phage in poultry feed and drinking water

Poultry feed (Laying-hen Feed 524, Zhengda Feed Co., Ltd, Daqing, China) and drinking water (tap water) were disinfected by autoclaving. Contaminate the surface of poultry feed with *Salmonella* Pullorum SaP001 (10^6 CFU/mL), or add to drinking water (for poultry feed, wait for it to dry at room temperature to promote bacterial attachment to the feed surface). Subsequently, samples were inoculated with phage at concentrations of 10^8 , 10^9 , and 10^{10} PFU/mL, corresponding to MOIs of 100, 1000, and 10000, respectively. Samples were incubated at room temperature. The control group was untreated with phage. After 2, 4, 6, 12 and 24 h of storage, dilutions were spread on LB plates and incubated overnight at 37°C in order to count the number of viable bacteria.

Data analysis

Each experiment was repeated three times, and data are presented as mean $\pm$ SD. Statistical analysis was performed using GraphPad Prism 9.0 (GraphPad Software, San Diego, CA, USA). $P \leq 0.05$ was considered statistically significant. (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.0001$)

Results

Determination of host range

The host range of phage GSP006 was determined using 57 *Salmonella* strains (Fig. 1). Phage GSP006 was capable of infecting 45 of 57 strains of *Salmonella*. These include *Salmonella* Pullorum, *Salmonella* Enteritidis and *Salmonella* Typhimurium and other serotypes of *Salmonella*. Among other *Salmonella* that can be infected, the lysis efficiency of phage GSP006 also varied. According to the EOP experiments, phage GSP006 demonstrated high infectivity against *Salmonella* Pullorum and *Salmonella* Enteritidis.

Morphological analysis of phage

Plaque morphology of phage GSP006 on LB-agar after three times purification as shown in Fig. 2A. Transmission electron microscopy (TEM) showed that phage GSP006 had an icosahedral head (64 ± 0.9 nm) and a contractile tail (328 ± 1.4 nm) as shown in Fig. 2B.

Determination of optimal Multiplicity of Infection

The highest titer of phage indicates that the efficiency of phage lysis of bacteria is the highest, and the ratio of phage to bacteria number at this time is called the optimal MOI. According to the results shown in Fig. 3A, the phage GSP006 showed the highest lysis efficiency against the host bacteria when the MOI of 0.1. As a result, the optimal MOI of phage GSP006 was 0.1.

One-step growth curve

The latent phase and the amount of lysis of the virus can be obtained from the one-step growth curve. According to the results shown in Fig. 3B, the one-step growth curve revealed that the phage had a short latent phase of 20 min and followed by the lytic phase.

Thermal and pH Stability.

In the temperature stability test, phage GSP006 has long-lasting activity in the temperature range of 4°C to 60°C. Phage titers decreased significantly at 70°C, but phage were not inactivated. At temperatures as high as 80°C phages lose their activity (Fig. 3C). In the pH stability test, phage GSP006 remained stable activity in the pH values of 4–10. The phage titer decreased significantly, although it did not inactivate at pH 3 and 11 (Fig. 3D).

Assessment of the Lytic Activity Level of phage

Subsequently, we tested whether phage GSP006 could inhibit the growth of *Salmonella* Pullorum SaP001 in dynamic medium. When *Salmonella* Pullorum SaP001 was infected with phage GSP006 at different MOIs (10, 1, 0.1, 0.01, 0.001, and 0.0001), the results showed that compared with the positive control value, *Salmonella* Pullorum SaP001 could be effectively inhibited by phage GSP006 for 6.5 h. The OD₆₀₀ nm value was always lower than the positive control value and with the increase of MOI value, the OD value of *Salmonella* Pullorum SaP001 was lower than the positive control value, the inhibitory effect was more obvious (Fig. 4). The results showed that phage GSP006 could effectively inhibit the dynamic growth of *Salmonella* Pullorum SaP001 for a certain period of time.

Genomic Characterization and Taxonomy of GSP006

GSP006 has a dsDNA genome comprising 42,165 bp with 50% G + C content. In total, 59 ORFs were predicted in the whole phage genome (GenBank accession no. PV890987.1) (Fig. 5A). Phage GSP006 showed high homology with *Salmonella* phage CKT1 (GenBank accession no. OK143508.1) by BLASTn comparison from NCBI, as 91.64% (87% coverage) (Fig. 5C). There were no genes associated with lysogenicity, virulence, or antibiotic resistance in the phage genome. The terminase large subunit of phage GSP006 was used for phylogenetic analysis. The analysis found that phage GSP006 belonged to *Jerseyvirus* genus (Fig. 5B). These results indicate that phage GSP006 is a new virulent phage belonging to the genus *Jerseyvirus*, and can be safely used for biocontrol or therapeutic applications from the genomic point of view.

Phage GSP006 reduced Salmonella Pullorum colonization in poultry feed and drinking water

In order to evaluate the ability of phage GSP006 to control the horizontal transmission of *Salmonella* in the poultry rearing environment, the effect of phage GSP006 on the control of *Salmonella* Pullorum SaP001 in artificial infections of poultry feed and drinking water was investigated. The addition of *Salmonella* Pullorum SaP001 and phage GSP006 (MOI of 10000) to poultry feed and drinking water for 24 h at 25°C resulted in a decrease in bacterial titers of 1.9 log CFU/g in poultry feed (Fig. 6A) and 2 log CFU/mL in drinking water (Fig. 6B). Bacterial growth was significantly reduced when poultry feed and drinking water were treated with phage with an MOI of 1000, with a decrease of 0.9 log CFU/g in poultry feed (Fig. 6A) and in drinking water by 2 log CFU/mL (Fig 6B) after 24 h at 25°C. Similarly, when poultry feed and drinking water were treated with phages at an MOI of 100, the bacterial titer was reduced by 0.2

log CFU/g in poultry feed (Fig 6A) and by 1.6 log CFU/mL in drinking water (Fig. 6B) after 24 h at 25°C. This suggests that phage GSP006 has a good antimicrobial effect and can be used to reduce the transmission of *Salmonella Pullorum* in poultry feed and drinking water in the poultry rearing environment.

Discussion

The history of phage therapy can be traced back to the early 20th century, when Frederick Twort and Félix d'Hérelle respectively discovered phages and proposed the possibility of their therapeutic applications [30]. Despite the impressive achievements of phage therapy in the 1920s and 1930s, the advent of antibiotics led to the decline of phage therapy in mainstream medicine. Given the escalating global crisis of antibiotic resistance, phage therapy is enjoying a global renaissance and has shown unique advantages in the fight against drug-resistant bacteria [31–33].

In general, the high specificity and environmentally friendly of phages make them ideal candidates for antimicrobial agents [34]. The concept of phage for the control of bacterial infections has gained wide public acceptance [35]. Although many studies have reported the application of phages for controlling bacterial transmission, their relatively narrow host range has severely hindered their further application [36, 37]. Therefore, it is valuable to isolate and develop new phages with broad host range [38]. In this study, we isolated a broad-host-range *Salmonella* phage from the wastewater of a poultry farm and named it GSP006. Spot tests demonstrated that GSP006 can infect *Salmonella* of different serotypes. Genome analysis showed that phage GSP006 was a member of the genus *Jerseyvirus* and the GSP006 was a virulent phage and did not contain any antibiotic resistance, virulence, or lysogeny-associated genes, indicating that this phage could be a candidate phage in practical applications.

Reducing the cost of phage product purification and preparation processes can greatly improve their clinical feasibility [39]. The MOI value is the phage to host bacteria, and the optimal MOI value produces the highest phage titer. Therefore, the optimal MOI between phage and host bacteria must be determined to reduce the cost of phage application. The optimal MOI for phage GSP006 is 0.1 (Fig. 3A), indicating that phage GSP006 has the potential to be applied in large doses. According to previous studies, Phages with short latency can lyse more bacterial cells in a given time [40]. The latency period of phage GSP006 is only 20 min (Fig. 3B), this suggests that it has the potential for rapid application of bacteriostatic action. The biological characterization of phages are a key factor in the applications they carry out [41]. The pH and temperature of the environment affect phage activity and stability. Therefore these parameters must be measured to determine the environmental settings for phage application. Phage GSP006 can remain active for more than 1 h at temperatures ranging from 4 to 60°C, and still survive even at 70°C (Fig. 3C). Compared with previously reported phages [42, 43], phage GSP006 showed higher tolerance to harsh environments. Poultry feed and drinking water end up in the intestines where the pH is acidic. If the phage is unable to survive in the low pH environment of the stomach, the phage will not be able to effectively inhibit bacterial growth after feed and drinking water enter the stomach. Therefore it is necessary to determine the pH stability of the phage. Phage GSP006 remains

active in the pH range of 3-11 for more than 1 h (Fig. 3D). This has a similar pH stability compared to other previously reported phages [44, 45]. Temperature sensitivity and pH sensitivity tests showed that phage GSP006 can tolerate extreme temperature and pH, which are important evaluation indexes and potential properties for phage applications.

As eco-friendly natural antimicrobial agents, having a broad-host-range is one of the important criteria for the selection and application of phage. Phage GSP006 lyses seven serotypes of *Salmonella*, including *Salmonella* Pullorum, *Salmonella* Enteritidis, *Salmonella* Typhimurium, *Salmonella* Stanley, *Salmonella* Chester, *Salmonella* Infantis and *Salmonella* Eastbourne. A previous study has shown that *Jerseyvirus* phage has a relatively broad-host-range and relatively good bacteriostatic efficacy [46]. Phage GSP006 has 91% sequence identity to phage CKT1, and it is clear that phage GSP006 is in this class of *Jerseyvirus* (Fig. 5C). The bacterial inhibition assay in this study showed that phage GSP006 consistently inhibited the growth of *Salmonella* at different MOIs for at least 6 h (Fig. 4).

Genome sequencing analysis revealed showed that phage GSP006 has DNA replication/modification, structural components, packaging module, and host lysis, which are similar to other *Salmonella* phages [47]. Since different phages were isolated in different geographic locations, the high degree of homology between them may depend on the evolutionary relationship with a common host. The genome of phage GSP006 is most similar to that of phage PJNS016, but the characteristics of the latter have not yet been reported. Phage GSP006 has a similar host range to the reported phage CKT1 [46], which may be due to having similar tail proteins. However, these two phages also have some different gene regions (Fig. 5C), suggesting that phage GSP006 is a new strain of *Salmonella* phage. Annotation of the genome of phage GSP006 identified a number of proteins with antimicrobial activity (ORF 37, 38, 39, 53) (Table S3). These proteins are key proteins involved in the process of bacterial lysis by phages [48]. There is evidence that phages are likely to be potential reservoirs for bacteria to produce antibiotic resistance genes and acquire virulence genes [36]. Therefore, the selection of phages that can be used for applications presupposes the exclusion of phages that carry deleterious genes in their genomic sequences. In this study, no relevant genes were detected in phage GSP006, indicating that this phage can be safely applied.

Previous studies have demonstrated that phages can inhibit bacteria in the gut or treat *Salmonella* infections [46, 49]. Poultry can be infected with *Salmonella* in a variety of ways, including feed, water, equipment, insects, and feeders [50]. Therefore, if phages are added at the source (*e.g.*, poultry feed or drinking water) wouldn't it be possible to reduce the *Salmonella* load in the environment, thereby reducing the risk of horizontal transmission of *Salmonella*, as well as further reducing *Salmonella* colonization of the gut to reduce the risk of infection. Therefore, we sprayed phage GSP006 into the feed and drinking water of birds artificially infected with *Salmonella* Pullorum. The results show that the concentration of *Salmonella* Pullorum in feed or water sprayed with phage GSP006 was significantly lower than that in unsprayed GSP006 (Fig. 6A, B). Phage GSP006 could not completely destroy *Salmonella* Pullorum, probably because the level of *Salmonella* contamination in the experiment was

much higher than that in nature. The above results show that that phage GSP006 is able to reduce the risk of *Salmonella* Pullorum transmission from the source.

Conclusion

In this study, we isolated a broad-host-range *Salmonella* phage GSP006 and further characterized GSP006 using *Salmonella* Pullorum SaP001 as the host bacterium. Biological characterization and genomic analysis showed that phage GSP006 has excellent tolerance range, lysis ability and biological safety. In addition, phage GSP006 showed significant effects in reducing *Salmonella* Pullorum in poultry feed and drinking water. Although phage GSP006 as an eco-friendly biocontrol agent is expected to be an antibiotic alternative antimicrobial agent, there is still a lot of work to be done to realize the application of GSP006 in preventing and controlling *Salmonella* infections in poultry.

Declarations

Abbreviations

Not applicable.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

PSY and ZHY: Conceptualization, data curation, formal analysis, writing-original draft, validation, investigation, methodology, software. LXC, WJL, PSY and KXY: Investigation, methodology, formal analysis, and data curation, software. GDY and SJ: Resources, supervision, project administration, writing-review, and editing.

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Figures

Bacterial strain	Strain no.	Host range	Bacterial strain	Strain no.	Host range
<i>Salmonella Pullorum</i>	SaP001	+++	<i>Salmonella</i> Anatis	Sa1001	-
	SaP002	++	<i>Salmonella</i> Motudi	Sa1002	-
	SaP003	+++	<i>Salmonella</i> Eastbourne	Sa1003	+
	SaP004	+++	<i>Salmonella</i> Stanley	Sa1004	+
	SaP005	++	<i>Salmonella</i> Chester	Sa1005	++
	SaP006	+++	<i>Salmonella</i> Newland	Sa1006	-
	SaP007	++	<i>Salmonella</i> Infantis	Sa1007	+
	SaP008	+++	<i>Salmonella</i> Dublin	CICC 21497	-
<i>Salmonella Typhimurium</i>	SaT001	++	<i>Salmonella</i> Choleraesuis	ATCC 10708	-
	SaT002	++	<i>Salmonella</i> Para-typhi C	CMCC 50118	-
	SaT003	++	<i>Escherichia coli</i>	ATCC 35150 O157: H7	-
	SaT004	+++		DEL 933 O157: H7	-
	SaT005	+++		ATCC 43895	-
	SaT006	+++		ATCC 43889	-
	SaT007	-		EC001 O157: H7	-
	SaT008	+		EH001 O157:H7	-
	SaT009	+		EH002 O157:H7	-
	SaT010	-		EH003 O157:H7	-
	SaT011	-		EH004 O157:H7	-
	SaT012	-		EH005 O157:H7	-
	SaT013	+		EH006 O157:H7	-
	SaT014	+		EH007 O157:H7	-
	SaT015	+		EH008 O157:H7	-
	SaT016	+		EH009 O157:H7	-
	SaT017	+		MG 1655	-
	SaT018	+		CICC 10667	-
	SaT019	+		ATCC 25922	-
	SaT020	+	<i>Staphylococcus aureus</i>	ATCC 25923	-
	SaT021	+	<i>Klebsiella pneumoniae</i>	ATCC 700603	-
	SaT022	-	<i>Proteus mirabilis</i>	ATCC 49005	-
	SaT023	-	<i>Pseudomonas aeruginosa</i>	ATCC 27853	-
	ATCC 14028	+++	<i>Listeria monocytogenes</i>	ATCC 13932	-
	CMCC 50115	+++	<i>Listeria monocytogenes</i>	ATCC 19117	-
	CVCC 541	+++			
	CVCC 542	++			
<i>Salmonella Enteritidis</i>	SE006	+++			
	SaE001	+			
	SaE002	+++			
	SaE003	+++			
	SaE004	++			
	SaE005	+++			
	SaE006	+++			
	SaE007	++			
	SaE008	+++			
	SaE009	+++			
	SaE010	+++			
	ATCC 13076	+			

+++ : 0.1 ≤ EOP ≤ 1.5

++ : 0.001 ≤ EOP ≤ 0.1

+: EOP < 0.001

- : Not susceptible to phages

Figure 1

Host range analysis of phage GSP006. Host range was determined by the efficiency of plating (EOP). ++, 0.1 ≤ EOP ≤ 1.5; +, 0.01 ≤ EOP ≤ 0.1, +, EOP < 0.001, -, Not susceptible to phages. ATCC, American Type Culture Collection; CICC, China Industrial Culture Collection; CMCC, National Center for Medical Culture Collections.

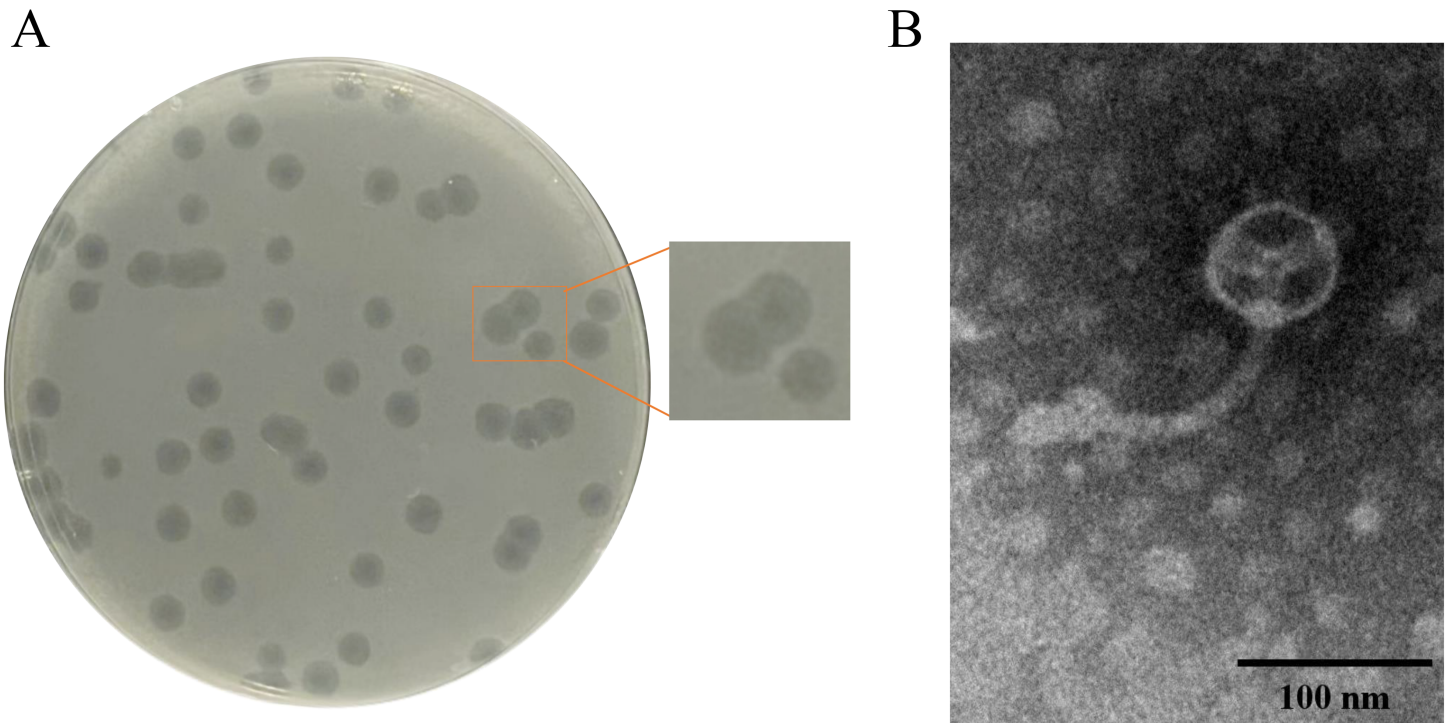


Figure 2

Morphology of phage GSP006. (A) The plaques formed by phage GSP006 on the lawns of *Salmonella Pullorum*. (B) TEM of phage GSP006. Scale bar, 100 nm.

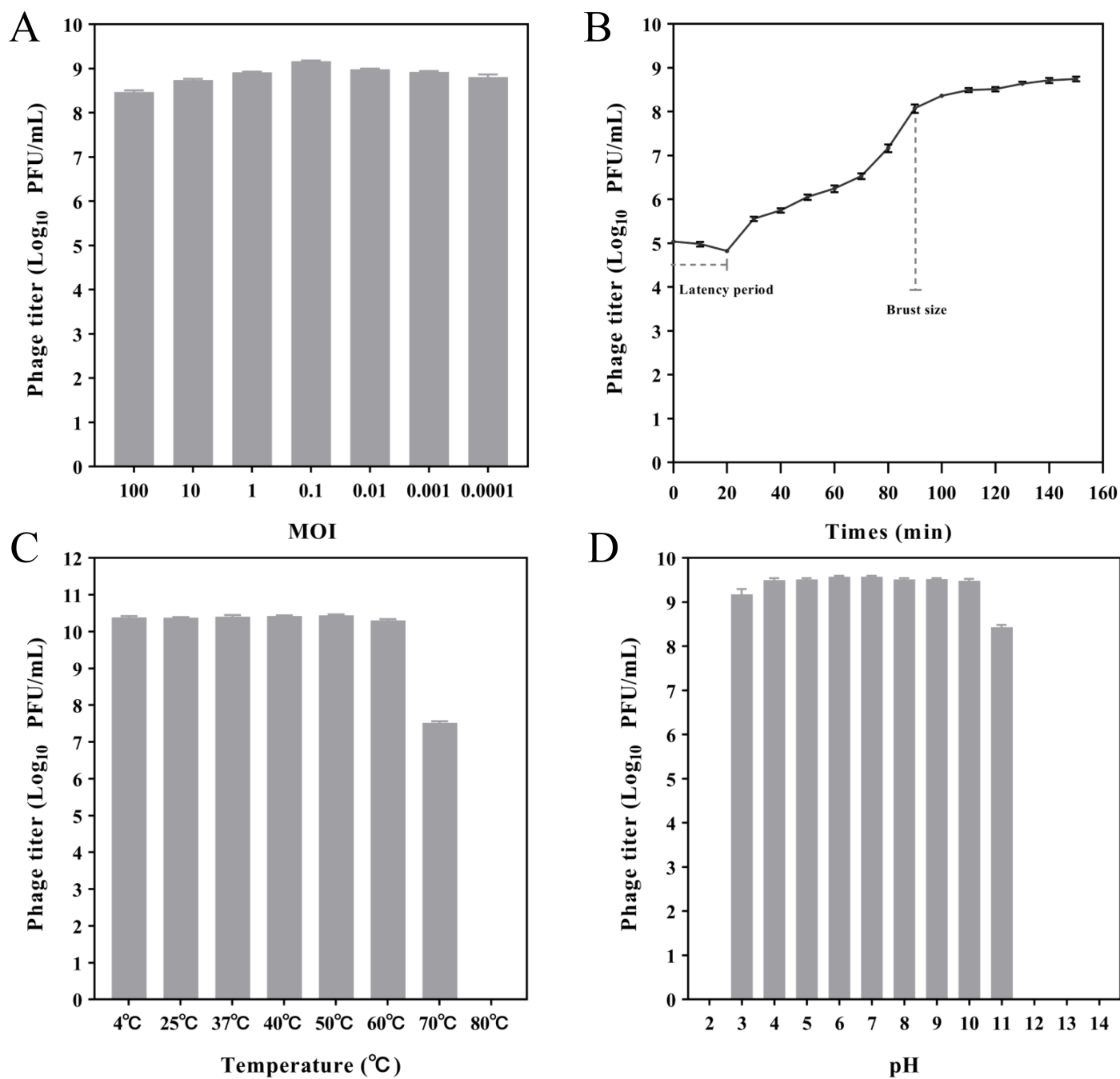


Figure 3

Biological characteristics of the phage GSP006 (A) MOI of the phage GSP006. Phage titers were determined at different MOI values. (B) One-step growth curve of phage GSP006. (C) Thermal stability of phage GSP006. The titers of bacteria were measured at different temperatures. (D) The pH stability of phage GSP006. The titer of phage was determined under different pH conditions.

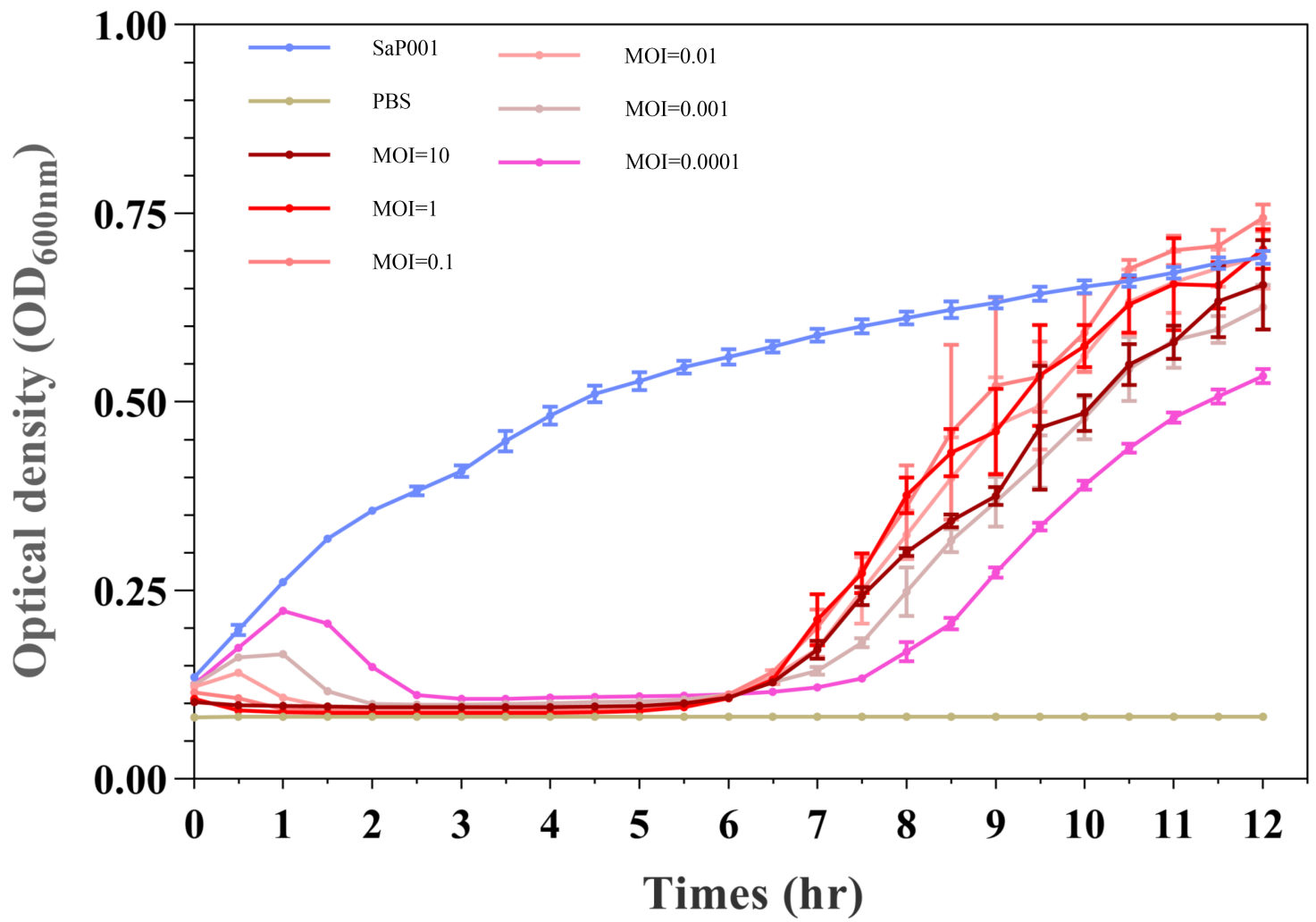


Figure 4

Reduction in *Salmonella Pullorum* SaP001 growth by phage GSP006 at different MOIs.

Application of phage GSP006 for biological control of *Salmonella* Pullorum SaP001. (A) Effect of phage on the viability of *Salmonella* Pullorum in poultry feed. (B) Effect of phage on the viability of *Salmonella* Pullorum in drinking water. The number of viable cells after phage infection of bacteria at different times was determined. **, $P \leq 0.1$, ***, $P \leq 0.01$.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [GSP006SupplementaryMaterial.docx](#)
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