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PHAGE THERAPY AS A PROMISING SOLUTION FOR FOOD SAFETY: ISOLATION AND BIOLOGICAL CHARACTERIZATION OF BACTERIOPHAGE P2 FOR CONTROLLING *Salmonella enterica* INFECTIONS

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ABSTRACT

Food poisoning and foodborne illnesses not only directly impact human health and quality of life but also result in significant economic losses and healthcare costs. Among the factors causing food poisoning, bacteria are the most common, including *Salmonella enterica*, which belongs to the Enterobacteriaceae family. Salmonellosis, which is introduced by *S. enterica* infection, can lead to symptoms such as fever, diarrhea, vomiting, and, in severe cases, even death. Remarkably, *S. enterica* has developed a high level of antibiotic resistance. Hence, the imperative pursuit of a reliable approach capable of averting *S. enterica* infections has emerged as an important goal essential for ensuring public health safety. This study aims to identify bacteriophages capable of effectively controlling *S. enterica*. Ten phage strains were isolated, among which phage P2, classified under the Siphoviridae family, exhibited strong lytic activity and a broad host range. Phage P2 had an optimal multiplicity of infection (MOI) of 0.01, reaching a titer of about 8.4×10^9 PFU/mL. It displayed a latent period of 15 minutes and a burst size of 63 PFU/cell. Notably, phage P2 demonstrated tolerance to a wide range of conditions, including temperatures from 4 ^oC to 50 ^oC, pH levels from 2 to 12, and salt concentrations of 0.1 M–7 M. The *in vitro* lytic activity assay showed that phage P2 significantly reduced bacterial counts by 3.08 log CFU/mL compared to the control group after one hour of incubation at 37 ^oC. These findings highlight the potential of phage P2 as a promising biocontrol agent for food preservation.

Keywords: bacteriophage, food poisoning, food preservation, phage therapy, *Salmonella enterica* infection.

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INTRODUCTION

Salmonella enterica is one of the most common foodborne pathogens, predominates in mammals, and accounts for nearly 99% of *Salmonella* infections observed in humans and warm-blooded animals (Coburn et al., 2007). This pathogen introduced salmonellosis, which is defined by symptoms like diarrhea, vomiting, and high fever due to the consumption of contaminated livestock and food products (Sattar et al., 2022). Recently, *S. enterica* accounts for the second most prevalent and verified etiological agent with approximately 900 outbreaks, and 24000 hospitalizations reported from 2009 to 2015 in the United States, and 63340 hospitalizations from 2014–2028 in South Korea (Lee et al., 2022). Up to date, over 2600 serovars of *S. enterica* have been characterized, such as Typhimurium, Enteritidis, Typhi, Paratyphi, Bareilly, etc.

A variety of approaches have been employed to control the proliferation of *S. enterica* in food matrices, including physical methods (irradiation, autoclave sterilization, and ozone treatment), chemical treatments (chlorine disinfectants and trisodium phosphate), and biological strategies (plant extracts), (Shang et al., 2021) the decontamination of *S. enterica* continues to pose challenges due to inherent limitations in conventional methodologies (Huang et al., 2018; Islam et al., 2019; Shang et al., 2021). Considering the limited effectiveness of conventional approaches to *Salmonella* spp., antibiotics were once hailed as a potent tool for controlling *S. enterica* prevalence across agricultural and industrial sectors (Sritha & Bhat, 2018). However, their integration into food faced opposition due to environmental concerns and broad antimicrobial effects (Huang et al., 2018). In fact, the increasing prevalence of multi-drug resistant *Salmonella* and resistance to clinically important antimicrobial drugs like fluoroquinolones and third-generation cephalosporins are global concerns (Talukder et al., 2023). Currently, a meta-analysis of *Salmonella* isolated from human, animal, and environment samples in South Asia shows that the temporal distribution of the overall antimicrobial resistance (%) against *Salmonella* was increased from 53 to 77% within 10 years with mostly resistance to nalidixic acid (74.25%) and tetracycline (37.64%) (Talukder et al., 2023). Therefore, it is urgent to develop alternative methods to control pathogenic *S. enterica* strains.

More recently, bacteriophages (phages), viruses that specifically infect and lyse bacteria, have gained increasing attention as a promising biocontrol tool for food safety (El-Dougdoug et al., 2019). Phage therapy offers several advantages over traditional antimicrobial approaches, including high specificity, self-replication at the site of infection, and minimal impact on beneficial microbiota (El-Dougdoug et al., 2019; Islam et al., 2019; Shang et al., 2021). Unlike antibiotics, which often promote resistance through broad-spectrum activity, bacteriophages selectively target specific bacterial strains, reducing the likelihood of disrupting microbial ecosystems (Abbas et al., 2022).

In this study, we aimed to isolate and characterize a phage with strong lytic activity against *S. enterica*. The interested phage was further investigated for its host range, lytic properties, environmental stability under different conditions, and effectiveness in reducing bacterial populations. Our findings provide insights into the potential application of phage therapy as a sustainable and effective strategy for enhancing food safety.

MATERIALS AND METHODS

Sampling

Mud and water samples were collected from sewage near the Cau Dien Market in Bac Tu Liem district, Ha Noi (21.0419^{o} N, 105.7599^{o} E), a location where poultry is slaughtered and processed with water.

Bacterial strains

Six *S. enterica* strains (SK1, SK2, SK3, SK4, SK5, SK6) isolated from commercial

meat samples (chicken meat, pork, and beef) and other bacterial species, including *Escherichia coli*, *Vibrio parahaemolyticus*, and *Bacillus cereus*, were provided by the Laboratory of Molecular Microbiology, Institute of Biology, Vietnam Academy of Science and Technology.

Phage isolation

Phages were isolated from the collected sewage samples with *S. enterica* as a host using the method published by Islam, Hu et al. (2020) with slight modifications (Islam et al., 2019). In short, the sewage sample was incubated with 10 mL of *S. enterica* inoculum (OD_{600} = 0.5–0.6) and agitated at 160 rpm per minute, and 37 ^{o}C for 24 hours. After incubation, the mixture was centrifuged for 10 minutes at 10,000 × *g*, at 4 ^{o}C. Cell pellets and large particulates were discarded while the supernatants were filtered and acquired through a 0.45 µm filter (Sartorius, Germany). Phage presences were observed by plaque formation through a double-agar plate using a double-agar spot assay (Carey-Smith et al., 2006). A 10 µL aliquot of the phage suspension was introduced instantly to the soft agar overlay, including the host bacteria, and incubated at 37 ^{o}C overnight to detect plaque formation. The phage was extracted and purified by consecutive single-plaque isolations and enriched afterward. Phage titer was measured by serial dilution (10^{-1} to 10^{-7}) in SM buffer (100 mM NaCl, 8 mM $MgSO_4.7H_2O$, 50 mM Tris-HCl pH 7.5, and 0.01% gelatin). The diluted phage solution was combined with the bacterial culture, including 5 mL of LB soft agar (0.7%), and then applied to an LB agar (2%) plate for overnight incubation at 37 ^{o}C. The purified phage was maintained in SM buffer at -4 ^{o}C for further analysis.

Host range determination

To execute the host range, 100 µL of exponential-phase test bacterial culture was mixed with 4 mL of molten LB agar (0.7% w/v). This mixture was then spread evenly onto the surface of LB agar plates and allowed to dry for 5 minutes. Once the overlay agar solidified, 5 µL of each phage solution was spotted onto the bacterial lawns and left to dry. Subsequently, the plates were incubated at 37 ^{o}C for 20 to 24 hours. After incubation, any bacterial property displaying clear spots or plaques was considered phage-sensitive (Islam et al., 2019).

Transmission electron microscopy (TEM)

The morphology of the phage was obtained by Transmission Electron Microscopy (TEM) using TEM JEM 1010 (Jeol, Japan) at an accelerating voltage of 80 kV according to the manufacturer's instructions. The phage was classified according to the Virus Taxonomy of classification and nomenclature of viruses.

Optimizing multiplicity of infection (MOI)

To determine the optimal MOI value, the bacterial culture was exposed to different dilutions of phage suspensions, resulting in MOI values of approximately 0.0001, 0.001, 0.01, 0.1, and 1. Phage titers were performed using the agar overlay method after diluting the filtrate in SM buffer (100 mM NaCl, 8 mM $MgSO_4.7H_2O$, 50 mM Tris-HCl pH 7.5, and 0.01% gelatin) (Wong et al., 2014). Phage titers were determined using isolated *S. enterica* SK2 as a host on double-layer agar plates (Huang et al., 2018). The experiment was done in triplicate.

One-step growth curve

One-step growth curve experiments were employed to assess the phage P2 burst sizes and latent periods. The concentration of the host strain (10^8 CFU/mL) was mixed with phage at an MOI of 0.01. The mixture was then incubated at 37 ^{o}C for 15 minutes for absorption and then centrifuged at 10,000 rpm for 5 minutes to remove free phages. The pellets were mixed in 10 mL of LB broth and incubated at 37 ^{o}C with shaking for 60 minutes. Samples were obtained every 5 minutes for the first 30 minutes and subsequently, every 10 minutes throughout the later period to determine the phage titer.

The double-layer agar method determined the count of plaques after 24 hours of incubation at 37 ^{o}C with *S. enterica* strain SK2 as the host. The relative burst size was calculated as the ratio of the number of phages generated during the rise period to the number of infected cells (El-Dougdoug et al., 2019). The experiment was done in triplicate.

Temperature stability

Phage lysates (10^9 PFU/mL) were subjected to a range of temperatures in a water bath, varying from 30 ^{o}C to 80 ^{o}C for 60 minutes (Kim et al., 2018). Then, 100 µL of each solution was withdrawn to determine the density using the double-layer agar method. The experiment was done in triplicate.

pH stability

Phage lysates (10^9 PFU/mL) were mixed in various tubes containing SM buffer (100 mM NaCl, 8 mM $MgSO_4.7H_2O$, 50 mM Tris-HCl pH 7.5, and 0.01% gelatin). adjusted to different pH values ranging from 2 to 12. These mixtures were then incubated for 60 minutes at 37 ^{o}C. Phage titers were determined using *S. enterica* strain SK2 as a host on double-layer agar plates (Huang et al., 2018). The experiment was done in triplicate.

UV stability

To assess the survivability of phage under UV light, phage suspensions (10^9 PFU/mL) were exposed to ultraviolet (UV) radiation (wavelength 254 nm), with a distance of 0.6 m from the sample to the UV source inside the microbiological incubator for 10, 20, 30, 40, 50 and 60 minutes (Wang et al., 2017). The phage samples were assayed using double-layer agar. The experiment was done in triplicate.

Salt tolerance

Phage suspensions (10^9 PFU/mL) were exposed to NaCl concentrations of 0.1 M, 0.5 M, 1 M, 2 M, 3 M, 5 M, and 7 M for 60 minutes at 37 ^{o}C (Wang et al., 2017). Subsequently, the phage titer was determined using the double-layer agar method. The experiment was done in triplicate.

***In vitro* lytic activity**

Phage lytic activity was assessed in an LB agar plate by measuring the *S. enterica* strain SK2 titer every hour, using a MOI of 0.01 when treatment with phage following a previously described method (Islam et al., 2019). The control experiment involved bacterial suspensions without phages. All solutions were incubated at 37 ^{o}C on an orbital shaker at 160 rpm for 12 hours. For *S. enterica* assessment, every hour, proper dilutions of each suspension were plated onto LB agar at 37 ^{o}C for 24 hours (Wong et al., 2014).

Data analysis

Data were analyzed using IBM SPSS Statistics version 20. The bacterial or phage titer differences were assessed using one-way ANOVA and the Turkey HSD post-hoc test. Results were presented as mean values ± SD. The statistical significance level was set at $p < 0.05$ (Tamura et al., 2021).

RESULTS

Isolation and host range determination of phage P2

Six serotypes of *S. enterica*, named SK1, SK2, SK3, SK4, SK5, and SK6, along with 3 other bacterial species, including *E. coli*, *V. parahaemolyticus*, and *B. cereus*, were used for isolation and determination of the host range of the isolated phages. A total of 10 phages were isolated from sewage, and all of them showed lytic activities against at least 1 out of 6 tested *S. enterica* strains. Among these isolated phages, two phages lyzed 3 out of the 6 bacteria tested, while the remaining phages only lyzed 2 host strains. Remarkably, 1 phage was found to have lytic activity against all 6 *S. enterica* strains tested (Table 1). This phage was purified, chosen for further study, and named phage P2 (Fig. 1). Remarkably, phage P2 showed lytic activities against 6 *S. enterica* strains tested but did not lyse other species (Table 1). The data emphasizes that P2 is host species-specific.

Table 1. Host range result of phage P2. (+): Clear plaque when treated with phage P2; (-): No plaque was found

Bac / Phage	*Salmonella enterica* strains						*Escherichia coli*	*Vibrio parahaemolyticus*	*Bacillus cereus*
	SK1	SK2	SK3	SK4	SK5	SK6			
P1	+	+	-	+	-	+	-	-	-
P2	**+**	**+**	**+**	**+**	**+**	**+**	-	-	-
P3	-	-	+	-	+	-	-	-	-
P4	-	+	+	+	+	-	-	-	-
P5	+	-	-	-	+	+	-	-	-
P6	-	+	-	+	+	+	-	-	-
P7	-	-	+	-	+	+	-	-	-
P8	+	+	+	-	+	+	-	-	-
P9	-	-	-	+	+	+	-	-	-
P10	-	+	+	+	+	-	-	-	-

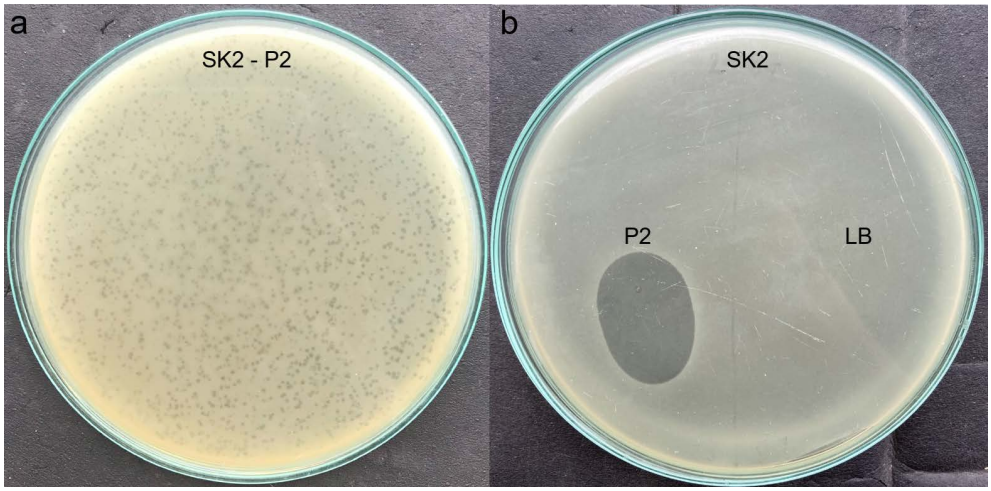

Figure 1. Phage P2 in LB double-agar plates with *Salmonella enterica* SK2 as host. (a) Plaque formation of phage P2, (b) Spot test. LB as control

Morphology of phage P2

TEM observed the morphology of phage P2. As seen in Figure 2, phage P2 has an isometric head of 71.83 ± 3.38 nm in diameter and a long non-contractile tail of 121.18 ± 33.96 nm in magnification of 100 nm. Based on this structure, phage P2 is observed to be a siphovirus (previously a Siphoviridae family) according to the International Committee on Virus Classification (ICTV) guidelines.

Optimal multiplicity of infection (MOI)

As shown in Figure 3a, phage titers were significantly different at each MOI value, indicating that the phage titer of P2 was affected dramatically by MOI. As a result, a MOI value of 0.01 was chosen for further study of phage P2, as the titer reached the highest titer of about 11.94$\log_{10}$ PFU/mL ($p < 0.05$) (Fig. 3a).

One-step growth curve

The infection dynamics of phage P2 were examined on the host SK2 in 60 minutes. The high burst in 15 minutes reveals that the latent and rise periods were around 15 to 20 minutes. The average burst size was estimated at 63 PFU/cell (Fig. 3b).

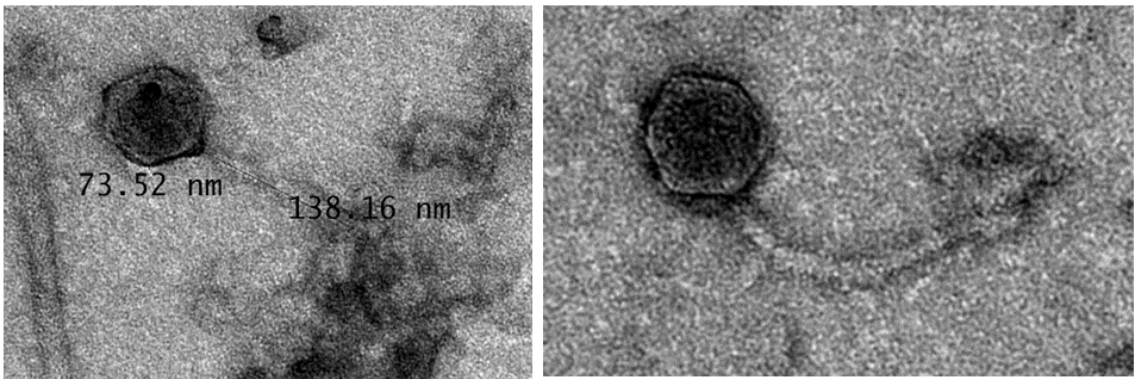

Figure 2. Morphology of phage P2 visualized by TEM

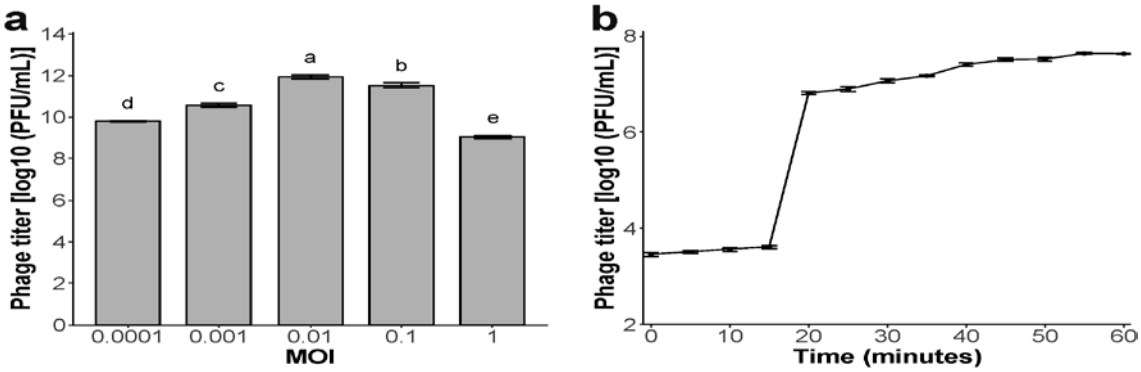

Figure 3. (a) Phage titer measure at different MOI values. Data are expressed as mean ± SD. Superscript letters (a, b, c) denote significant differences (Tukey's RiR test, $p < 0.05$). (b) The one-step growth curve of phage P2

Temperature stability of phage P2

The stability of phage P2 was evaluated under different temperature conditions. Phage titers remained stable between 30 ^{o}C and 50 ^{o}C with a slight decrease of approximately 0.2log$_{10}$ PFU/mL. However, exposure to 70 ^{o}C significantly declined phage titer to 3.7log$_{10}$ PFU/mL ($p < 0.05$). (Fig. 4a).

pH stability of phage P2

The stability of phage P2 was assessed across a wide pH range. After 60 minutes of incubation, phage P2 maintained a high titer (> 7log$_{10}$ PFU/mL) between pH 2 and 11, demonstrating strong tolerance to acidic and moderately alkaline conditions. The highest titer was observed at approximately 9.1log$_{10}$ PFU/mL at pH 5 and slightly decreased from pH 7 to 11. However, at pH 12, no detectable phage particles remained, indicating that extreme alkalinity completely inactivated the phage. These results suggest that phage P2 is highly stable in acidic and neutral environments and remains effective under moderate alkaline conditions, making it a robust candidate for diverse applications (Fig. 4b).

UV and salt stability of phage P2

Phage P2 exhibited strong resistance to ultraviolet (UV) light exposure, as no significant reduction in titer was observed across different exposure durations for roughly 8.6log$_{10}$ PFU/mL (Fig. 4c). Regarding salt stability, phage P2 showed a slight change in titer between 0.1 M and 0.5 M, followed by consistent stability across a wide range of salt concentrations for approximately 8.5log$_{10}$ PFU/mL (0.5 M to 7 M) for 60 minutes (Fig. 4d).

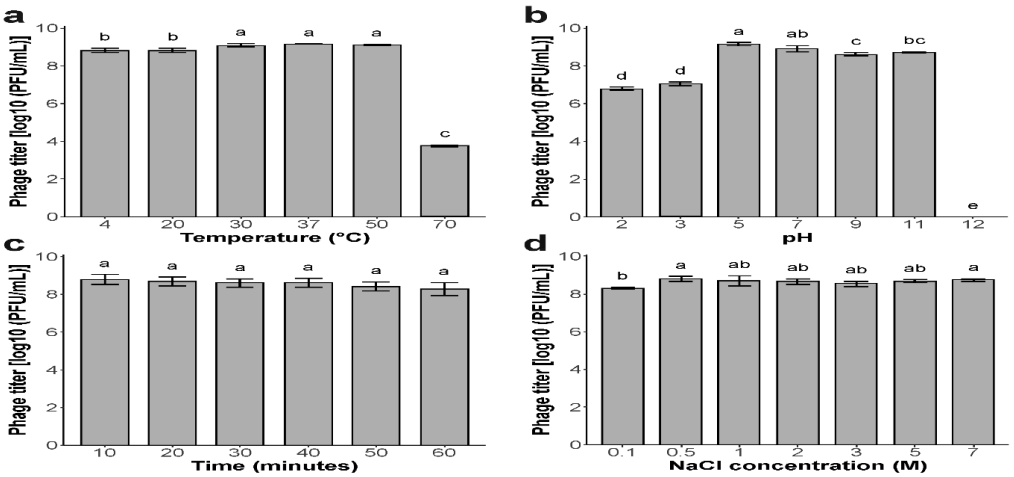

Figure 4. (a) pH stability, phage was incubated at different pHs for 60 minutes. (b) Temperature stability. Phages were incubated at different temperatures for 60 minutes. (c) UV stability test. Phage was exposed to UV, incubated, and counted titer at different time points. (d) Salt stability in different NaCl concentrations

In vitro lytic activity of phage P2

The growth of the host SK2 was measured under the inhibition of phage P2 to determine the lytic capacity against the host strain. The MOI chosen was 0.01 based on the optimal MOI result and incubated for 12 hours.

As shown in Figure 5, phage proliferation could consistently be inhibited by P2 for 12 hours. Without the presence of phage, as a control, SK2 began to increase dramatically within 2 hours, up to 3.38log$_{10}$ CFU/mL, as showed to be in log growth and start the stationary state afterward. With phage P2 treatment, the inhibition appeared clearly in the log growth state of SK2; the bacteria titer decreased by 3.08log$_{10}$ CFU/mL within 1 hour and began to stay low for 1–12 hours.

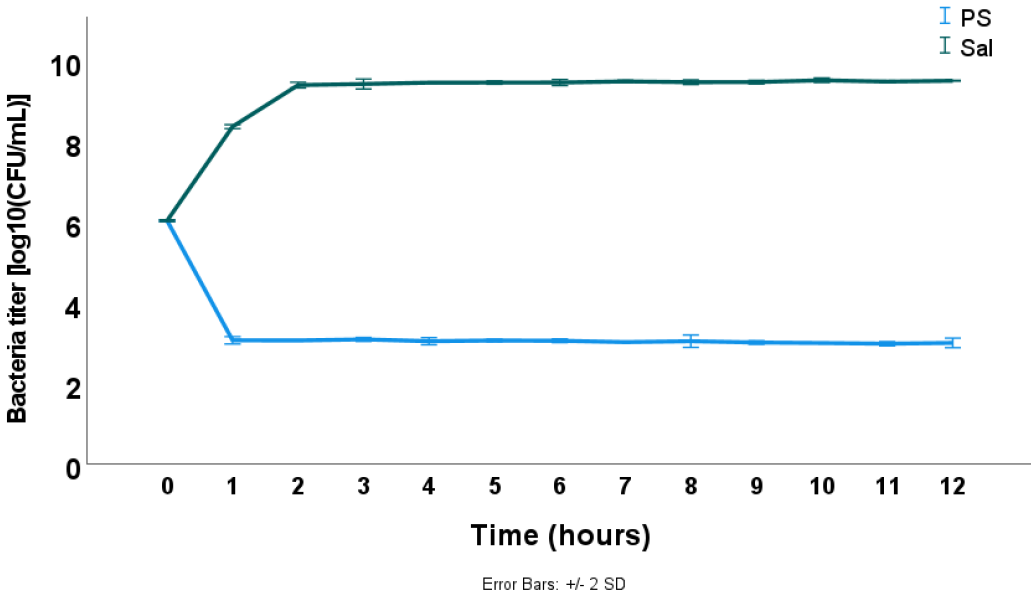

Figure 5. In vitro lytic activity of phage P2 against *Salmonella enterica* SK2

DISCUSSION

The increasing prevalence of foodborne salmonellosis, the rise of multidrug-resistant *S. enterica* strains, and the slow progress in developing new antibiotics have necessitated the search for alternative control measures (Khan et al., 2024). Lytic bacteriophages have emerged as promising antimicrobial agents in the food industry due to their host specificity, ease of application, and cost-effectiveness (Zhang et al., 2024). In this study, we successfully isolated and characterized a lytic *Salmonella* phage, P2, from poultry environmental sewage in Northern Vietnam.

Consistent with previous studies, *Salmonella* phages were successfully isolated from poultry sewage and food consumption environments, where the bacteria are predominantly found (Kumar et al., 2022; Shang et al., 2021). We isolated 10 phages using *S. enterica* strains as hosts, with phage P2 demonstrating high lytic activity and specificity against *S. enterica* isolates without lysing non-target bacteria. This aligns with a study in China, where 42 phages were isolated from water, and 17% formed clear plaques with at least two serotypes, while the others remained highly specific (Islam et al., 2019). The strict host specificity of phage P2 suggests its potential for targeted biocontrol applications without impacting non-pathogenic microflora (Holtappels et al., 2023; Huang et al., 2018).

Transmission electron microscopy (TEM) analysis revealed that phage P2 has a hexagonal nucleocapsid structure with a long, narrow tail, classifying it within the Siphoviridae family (Knapik, 2013). This morphology is typical of phages with a relatively stable infection process, capable of efficient bacterial lysis. The structural properties of phage P2 indicate a robust ability to attach to and infect *S. enterica* cells, supporting its suitability for practical applications.

The optimal multiplicity of infection (MOI) is critical for maximizing bacterial inactivation (Silva et al., 2014). Among the five tested MOI values (0.0001–1), the highest phage titer was observed at a MOI of 0.01, suggesting that lower phage concentrations still target and infect bacteria. Similar findings were reported in studies from China, where optimal MOI values for different *Salmonella* phages ranged from 0.01 to 0.1 (Islam et al., 2019; Liu et al., 2020; Tang et al., 2019). Choosing an appropriate MOI is essential for phage therapy, as too high an MOI can lead to premature bacterial resistance, whereas too low an MOI may result in insufficient bacterial suppression. Furthermore, phages with low MOI values are less likely to induce strong selective pressure on bacterial populations, thereby reducing the risk of bacterial resistance development. The ability of phage P2 to maintain its lytic activity at a low MOI (0.01) makes it an attractive candidate for food safety applications where minimal phage introduction is desirable, ensuring efficient bacterial control without significant alterations to the microbial environment (Islam et al., 2019; Tang et al., 2019).

The one-step growth curve analysis determined that phage P2 has a latent period of 15 minutes and an average burst size of 63 PFU/cell. While these values indicate efficient replication, they differ from those reported for other phages in previous studies. In addition, a previous study (Shang et al., 2021) observed a similar latent period of 15 minutes but a much higher burst size of 211 PFU/cell, whereas Li and partners reported a shorter latent period of 10 minutes with a burst size of 10^4 PFU/cell (Li et al., 2020). A shorter latent period generally allows for quicker bacterial infection cycles, meaning the phage can propagate more rapidly within a bacterial population. However, a very short latent period could lead to rapid depletion of host bacteria, limiting sustained bacterial control over time. The burst size, representing the number of new phage particles released per infected bacterial cell, is another key determinant of phage effectiveness. A higher burst size typically results in a greater phage population in a shorter time, enhancing bacterial clearance. However, an extremely high burst size may also lead to rapid host

depletion, limiting the phage's long-term sustainability in dynamic environments such as food matrices (El-Dougdoug et al., 2019). Phage P2 burst size, though lower than other reported phages, may still be advantageous in certain applications. A moderate burst size allows controlled bacterial reduction, minimizing the risk of excessive bacterial lysis, which could trigger bacterial resistance mechanisms. Additionally, phages with moderate burst sizes are often better suited for gradual, sustained bacterial control in food preservation, where maintaining a stable microbial balance is essential.

Additionally, thermal stability tests showed no significant changes in phage P2 titer between 30 ^{o}C and 50 ^{o}C, with minor reductions at lower temperatures (4–20 ^{o}C) and a significant decline at 70 ^{o}C. Previous studies have also reported phage viability at 30–50 ^{o}C but reduced activity above 60 ^{o}C (Kim et al., 2020; Li et al., 2020). These results suggest that phage P2 can be effectively applied in food matrices, particularly in environments such as meat, fruits, and dairy products with varying pH and temperature conditions (Huang et al., 2018; Shang et al., 2021).

Further more, phage P2 exhibited stability across a pH range of 5–11, with significant reductions at pH levels below 3 and complete inactivation at pH 12. Similar findings were reported in previous studies, where phages remained stable between pH 4–12 but were inactivated in strong acidic or alkaline conditions (Islam et al., 2019; Shang et al., 2021). Food matrices such as meat and fruit often exhibit pH values within this stable range, making phage P2 an effective biocontrol agent.

Phage P2 demonstrated high UV resistance, with no significant changes in titer upon exposure to UV-C light. This contrasts with previous reports where phages were significantly reduced or completely inactivated after 15 minutes of UV exposure (Kim et al., 2018). Given that UV light is widely used for food and water disinfection (Scarascia et al., 2021), the high UV stability of phage P2 enhances its potential application in combination with existing decontamination methods (Khan & Rahman, 2022). This resistance suggests that phage P2 can persist longer in UV-treated environments, potentially offering prolonged protection against *S. enterica* contamination.

It has been known that salt concentration plays a crucial role in food preservation, particularly in cheese production, where outbreaks of S. enterica contamination are reported (Modi et al., 2001). Although cheese consumption in Vietnam is lower than in Western countries, it remains popular among young people (Nguyen et al., 1983). Our NaCl stability test indicated that phage P2 remained viable at concentrations ranging from 0.5 M to 7 M, with a slight reduction at 0.1 M. This aligns with previous findings showing no significant reduction in phage titer after 1 hour of exposure to high NaCl concentrations (Wang et al., 2017). This suggests that phage P2 could be effectively applied in high-salt environments, including cheese and processed meat products, where bacterial contamination is a concern.

Finally, phage P2 exhibited strong lytic activity against *S. enterica* strain SK2, inhibiting bacterial growth for 12 hours at an MOI of 0.01. A previous study reported similar results, with single-phage treatments suppressing bacterial growth for 12–15 hours, while phage cocktails extended inhibition up to 20 hours (Islam et al., 2019). These findings highlight the potential of phage P2 as an effective biocontrol agent for *S. enterica* in food safety applications.

As antibiotic resistance continues to rise, phage therapy has emerged as a promising strategy against multidrug-resistant *S. enterica* strains (Teklemariam et al., 2022). Implementing a multifactorial approach, from farm to fork, can significantly reduce *Salmonella* prevalence in the food chain (Bardina et al., 2012). Research indicates that phages such as SaFB14, isolated from a drug-resistant *S. enterica* strain, exhibit a broad host range and strong *in vivo* activity, demonstrating potential for infection control in poultry farms

(Tang et al., 2019). However, bacterial resistance mechanisms can limit phage effectiveness by altering or eliminating phage-binding receptors (Laure & Ahn, 2022). Broad-host-range phages, similar to broad-spectrum antibiotics, can target multiple bacterial species, making them valuable for managing co-infections (Chung et al., 2023). To mitigate the emergence of phage-resistant bacteria, a phage cocktail targeting multiple *S. enterica* strains, including resistant ones, offers a viable solution (Chung et al., 2023; Khan & Rahman, 2022).

The ability to sustain inhibition over an extended period and the multiple strains targeted suggests that phage P2 could be incorporated into food preservation strategies, promising the development of phage therapy, which provides a natural and efficient means of controlling bacterial contamination. Future research should explore its efficacy in real-world food production settings and its integration into phage-based biocontrol strategies.

CONCLUSION

This study presents the environmental isolation of the lytic *S. enterica* phage P2, which demonstrates effectiveness against various *S. enterica* isolates. Phage P2 exhibits strong stability under different conditions, including temperature, pH, UV exposure, and salt, while maintaining rapid lytic activity against its host. These findings suggest that phage P2 could serve as a promising biocontrol agent for *S. enterica* in food processing environments.

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