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Original Article

A Lytic Bacteriophage PS2 with Potential for Controlling *Salmonella* infections in Chickens and Ducks

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Abstract: *Salmonella* is one of the most common and dangerous pathogens, causing significant economic losses to the poultry industry worldwide. It is also a leading cause of foodborne diseases in both humans and animals. To control *Salmonella* infections, antibiotics are frequently used. In Vietnam, antibiotics are administered not only for disease prevention and treatment but also as growth promoters in livestock. This practice has contributed to the emergence and spread of antibiotic-resistant bacterial strains. Additionally, antibiotic residues in poultry products, such as eggs and meat, pose direct and indirect risks to human. Recently, phage therapy has been explored as a promising alternative to antibiotics for the prevention and treatment of bacterial infections, particularly those caused by antibiotic-resistant strains. In this study, we describe the isolation, selection, and biological characterization of a bacteriophage strain with strong lytic activity against *Salmonella enterica* subsp. *enterica*, a causative agent of diarrhea in chickens and ducks in Hai Duong province, Vietnam. The isolated phage strain, PS2, exhibited specific lytic activity against *Salmonella* and was capable of lysing all 30 tested *Salmonella* strains. Morphologically, PS2 has Myovirus-like morphology with an icosahedral head of 80.02 ± 3 nm in diameter and a long straight tail of 90.6 ± 8 nm in length. PS2 demonstrated an optimal multiplicity of infection (MOI) of 0.01, a latent phase of approximately 25 minutes, and a burst size of about 225 plaque-forming units (PFU) per cell. Moreover, PS2 maintained stable biological activity across a temperature range of -20 °C to 60 °C and remained stable within a pH range of 5 to 12. Taken together, these results suggest that PS2 is a promising candidate for the development of phage therapy to combat *Salmonella* infections in poultry in Vietnam.

Keywords: Bacteriophages, diarrhea syndrome, poultry industry, *Salmonella*, Salmonellosis.

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The poultry industry in Vietnam is a vital sector, contributing to social security, food security, and increased income for farmers [1]. However, the livestock industry in the country is facing complex disease challenges, including infections caused by *Salmonella*. *Salmonella* is one of the most common and dangerous pathogens affecting poultry worldwide [2]. It is a genus of rod-shaped, Gram-negative facultatively anaerobic and non-spore forming bacteria of the family Enterobacteriaceae [3]. Among the two identified *Salmonella* species, *Salmonella bongori* and *Salmonella enterica*, *S. enterica* is the primary cause of intestinal diseases in poultry, often leading to high morbidity and mortality rates. In particular, *S. gallinarum* and *S. pullorum* are the two most harmful serovars, causing fowl typhoid with systemic infections in all age groups with pathological symptoms such as an enlarged liver, anemia, and intestinal hemorrhage [4], ultimately leading to significant economic losses [5]. Over time, it has evolved to survive in diverse environments and multiple hosts, making long-term control of its spread challenging. Remarkably, poultry and poultry-related products have been identified as significant sources of *Salmonella* transmission to humans [6].

In recent years, antibiotic resistance has become an increasingly serious issue in the global poultry industry, including in Vietnam [5, 7]. The expansion of poultry production, larger livestock operations, and the improper use of antibiotics have contributed to the rise of antibiotic-resistant bacteria and an increased reliance on these drugs. A report on antibiotic use in chicken farming in Vietnam estimates that the amount of antibacterial agents used per kilogram of meat is 1.6 times higher than in European countries [8].

Numerous studies have shown that *Salmonella* circulating in chickens and ducks exhibits a high rate of antibiotic resistance. According to a survey by Mahmud et al., *Salmonella* isolated from poultry farms in Bangladesh demonstrated extremely high resistance rates to antibiotics, including ampicillin (100%), amoxicillin (99%), and tetracycline (98%) [9]. Other studies have also reported that *Salmonella* isolated from poultry samples showed high resistance to various antibiotics, including colistin, ciprofloxacin, doxycycline, kanamycin, streptomycin, sulfamethoxazole, and tetracycline, with resistance rates ranging from 42.1% to 97.6% [10-14]. In Vietnam, Binh et al., [15] isolated *Salmonella* strains from 3,700 ducks in northern provinces and found that all isolated strains exhibited strong resistance to antibiotics such as kanamycin, colistin, and neomycin. From 2017 to 2020, Nguyen et al., [16] isolated *Salmonella* strains from livestock farms in the Mekong Delta and found that these strains were resistant to multiple antibiotics, including chloramphenicol (62.98%), tetracycline (55.80%), and ampicillin (54.14%). Similarly, a survey by Xuan et al., [17] found that all 20 *Salmonella* strains isolated from chicken farms (fecal and environmental samples) in Vinh Long province were resistant to between two and ten commonly used antibiotics. Resistance rates were particularly high for ampicillin (100%), cefuroxime, streptomycin, and tetracycline (90%), as well as doxycycline (85%), with moderate resistance observed for trimethoprim/sulfamethoxazole (60%).

In recent years, phage therapy has been increasingly studied as a potential alternative to antibiotics for controlling pathogenic bacteria in humans and animals, including poultry, particularly antibiotic-resistant strains [18, 19]. Phage exhibits strong antibacterial activity, and high specificity for its host bacteria, and is considered safe for both humans and animals [20-22]. Previous studies have demonstrated the potential of phage therapy for controlling *Salmonella* infections in poultry. Administering phages significantly reduced *Salmonella* contamination in the gastrointestinal tracts of chickens, thereby preventing the spread of the pathogen through feces [23]. Similarly, Żbikowska et al., [24] found that when phages were administered to chicks prior to *Salmonella*

infection, no bacteria were detected after 15 days of treatment. Several bacteriophage-based products have been developed and commercialized to combat *Salmonella* infections in poultry production, and clinical studies have shown that these products effectively prevent *Salmonella* infections.

In this study, we aimed to isolate and characterize the biological properties of a lytic phage specifically targeting *Salmonella enterica* subsp. *enterica* strains associated with diarrhea syndrome in chickens and ducks from Hai Duong province, Vietnam.

2. Materials and Methods

2.1. Sampling

Wastewater samples were collected from chicken and duck farms in Hai Duong province with signs of *Salmonella* infection (diarrhea, feather loss), kept on ice, delivered to the Laboratory of Molecular Microbiology, Institute of Biotechnology (IBT), Vietnam Academy of Science and Technology (VAST), and stored in a refrigerator until use.

2.2. Bacterial Strains

Thirty *Salmonella enterica* subsp. *enterica* strains isolated from the intestines of chickens and ducks with diarrhea syndrome in Hai Duong in 2024, two *E.coli* strains, two *Vibrio parahemolyticus* strains and two *Bacillus cereus* strains were from the bacterial collection of the Laboratory of Molecular Microbiology, IBT, VAST. Bacterial strains were identified using the MALDI-TOFF mass spectrometry and 16S rRNA sequencing. In addition, *Salmonella* strains were also determined for the presence of the toxic gene *invA* by PCR method [25].

2.3. Phage Enrichment and Isolation

Ten mL of wastewater was added to 5 mL of 2X Peptone Water (Merck, Germany) supplemented with a mixture of host bacteria and the mixture was incubated at 37 °C with shaking at 120 rpm for 3 hours to enrich phages. The inoculum was then centrifuged at 10.000 rpm for 10 minutes at 4 °C and the supernatant was collected and filtered through a 0.22 µm filter membrane (Sartorius, Germany). The presence of phages is determined through the formation of clear spots (plaques) on a double-layer agar medium according to the double agar spot assay method [26]. Briefly, 10 µL of each phage enrichment solution was dropped onto the surface of two-layer agar containing host bacteria. The double-layer agar plate includes a lower layer (layer 1) of Luria-Bertani (LB) agar 2% and an upper layer prepared by adding 100 µL of bacteria (10^9 CFU/mL) to 15 mL of LB agar 0.7% medium at 50 ºC - 55 ºC, mixed well and poured onto agar layer 1, let the plate dry before use. Isolated phages with high lytic activity were grown on corresponding indicator bacteria, purified and determined the phage titer using the double-layer agar method [26].

2.4. Phage Stock Preparation

Phages were added to the bacterial inoculum (OD600 = 0.5– 0.6), incubated at 37 ºC with shaking at 150 rpm for 3 hours. 10% (v/v) chloroform solution was added to the inoculum, mixed well with a voltexer, then centrifuged the mixture at 12.000 rpm for 20 minutes. The upper phase was collected and filtered through a 0.22 µm filter membrane to completely remove bacteria. Phage was then precipitated using a chemical method using 5M NaCl and PEG 8000, the mixture was incubated at 4 ºC for 3 hours. The mixture was centrifuged at 12.000 rpm for 15 minutes at 4 °C, and the resulting pellet was collected and resuspended in SM buffer (sodium chloride, magnesium sulfate). The phage density was determined using the double-layer agar method [26]. The prepared phage stock was then used for subsequent experiments.

2.5. Transmission Electron Microscopy (TEM)

The morphology of phage was observed using the TEM JEM 1010 (Jeol, Japan) according to the manufacturer's instructions at an accelerating voltage of 80 kV. The phage

morphology was identified based on Committee on Taxonomy of Viruses (ICTV) guidelines.

2.6. Host Range

The host spectrum of phage was determined by the double- agar spot assay as described above with 30 strains of *Salmonella* and other bacteria including *E. coli*, *B. cereus*, and *V. parahemolyticus*, to determine the species specificity of the phage. The experiment was repeated three times.

2.7. Multiplicity of Infection (MOI)

The optimal multiplicity of infection (MOI) was determined following the method described by Lukman et al. [27]. *Salmonella* cultures were grown overnight in LB medium and then incubated for 1.5–2 hours at 37 °C until the optical density at 600 nm (OD600) reached 0.5–0.6. Phages and bacterial cultures were then mixed to achieve different phage-to-bacteria ratios (MOI) of 0.001, 0.01, 0.1, 1, 10, and 100. The mixtures were incubated at 37 °C with shaking for 3 hours. After incubation, bacterial cells were disrupted by adding 10% chloroform, followed by centrifugation of the supernatant and filtration through a 0.22 µm membrane filter. The phage titer for each MOI was then measured using the double-layer agar method. The experiment was performed in triplicate, and the MOI that produced the highest phage concentration was selected as optimal for further experiments.

2.8. One-step Curve

The growth curve of phage was determined using a previously described method [28]. Briefly, the phage was mixed with indicator bacteria in the mid-log phase at the optimal MOI and incubated with shaking at 37 °C for 15 minutes. The mixture was then centrifuged at 12,000 rpm for 1 minute, and the bacterial pellet was resuspended in 20 mL of fresh LB medium. The suspension was subsequently shaken at 37 °C and 200 rpm for 90 minutes. Every 5 minutes, a 300 µL sample was taken, diluted to an appropriate concentration, and the phage titer was determined using the double-layer agar method. The experiment was performed in triplicate. The latent period was determined directly from the growth curve, and the burst size was calculated using the following formula [29]:

Burst size = Number of phage particles released / Number of infected cells (PFU/cell)

2.9. pH stability

Phage lysate was added to SM buffer solutions with pH values of 2, 3, 5, 7, 9, 11, and 12, ensuring a final phage concentration of 10^9 PFU/mL. The mixtures were incubated at 37 °C with shaking for 3 hours. At 1, 2, and 3-hour intervals, 100 µL of the phage suspension was sampled, diluted appropriately, and the phage titer was determined using the double-layer agar method as previously described.

2.10. Temperature stability

Phage lysate (10^9 PFU/mL) was incubated at various temperatures, including -20 °C, 4 °C, 25 °C, 37 °C, 40 °C, 50 °C, 60 °C, 70 °C, and 80 °C, for 3 hours. At 1, 2, and 3-hour intervals, 100 µL of the phage suspension was collected, diluted appropriately, and the phage titer was determined using the double-layer agar method as previously described.

2.11. Data Analysis

Data in this study were analyzed using IBM SPSS Statistics version 20. Differences in phage density between study groups were assessed using one-way ANOVA followed by Tukey's post-hoc test. Results are presented as mean ± SD, with statistical significance set at $p < 0.05$.

3. Results

3.1. Phage Isolation

In this study, bacteriophages were isolated using the drop method on double-layer agar plates. Our results demonstrated the successful isolation of 11 distinct phage strains (PS1–PS11), each exhibiting varying degrees of lytic activity against their respective hosts (Figure 1A). Notably, strain PS2 displayed strong lytic activity against multiple indicator bacterial

strains, achieving a phage density of 2.5×10^8 PFU/mL. PS2 was subsequently purified, enriched, and used for further experiments.

3.2. Phage Morphology

TEM analysis revealed that phage PS2 has an icosahedral head with a diameter of 80.02 ± 3 nm and a long, straight tail measuring 90.6 ± 8 nm (Figure 1B). According to the International Committee on Taxonomy of Viruses (ICTV) guidelines, phage PS2 exhibits a Myovirus-like morphology and belongs to the class Caudoviricetes.

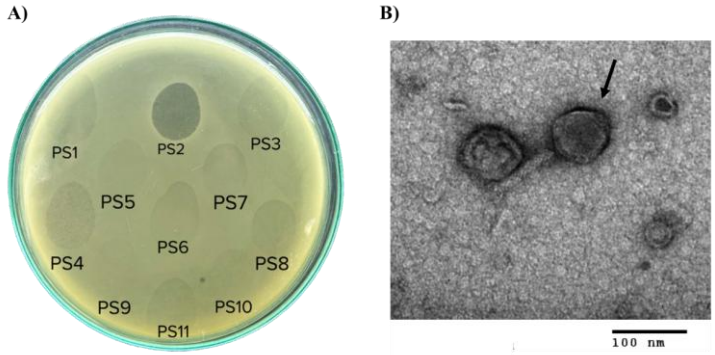

Figure 1. A) Lytic activity of phage isolates (PS1- PS11) against *Salmonella enterica* subsp. *Enterica* strain 30. B) Transmission electron microscopy image of phage PS2 morphology. PS2 exhibits icosahedral capsids and long straight tails, showing the Myovirus-like morphology.

3.3. Host Range Determination

The host range of PS2 was determined based on its lytic activity against 30 strains of *S. enterica*, two strains of *E. coli*, two strains of *B. cereus*, and two strains of *V. parahaemolyticus*. The results indicated that PS2 was capable of lysing all tested *S. enterica* strains to varying degrees (Table 1, Figure 2). Among them, PS2 exhibited strong lytic activity against *Sal_1*, *Sal_2*, *Sal_5*, *Sal_8*, *Sal_9*, *Sal_15*, *Sal_27*, *Sal_30*, *Sal_33*, *Sal_35*, *Sal_43*, and *Sal_44*. However, its lytic activity against *Sal_28* and *Sal_40* was relatively weak. Additionally, PS2 did not exhibit lytic activity against *E. coli*, *B. cereus*, or *V. parahaemolyticus*. These findings suggest that PS2 specifically targets *S. enterica* strains.

Table 1. Results of host range determination

Phage	Bacterial strains										
	Sal_1	Sal_2	Sal_3	Sal_4	Sal_5	Sal_6	Sal_7	Sal_8	Sal_9	Sal_15	Sal_29
PS2	++++	++++	+++	+++	++++	+++	+++	++++	++++	++++	+++
	Sal_16	Sal_17	Sal_20	Sal_21	Sal_22	Sal_23	Sal_24	Sal_25	Sal_27	Sal_28	Sal_30
	++	+++	+++	++	+++	++	+++	+++	++++	+	++++
	Sal_33	Sal_34	Sal_35	Sal_40	Sal_41	Sal_42	Sal_43	Sal_44	*E. coli_1*	*E. coli_2*	*B. cereus_1*
	++++	+++	++++	+	++	+++	++++	++++	-	-	-
	B. cereus_2		*V. parahemolyticus_1*			*V. parahemolyticus_2*					
	-		-			-					

Note: ++++, +++, ++, and + indicate a decreasing order in plaque clarity and diameter; '-' indicates that no phage plaque was observed.

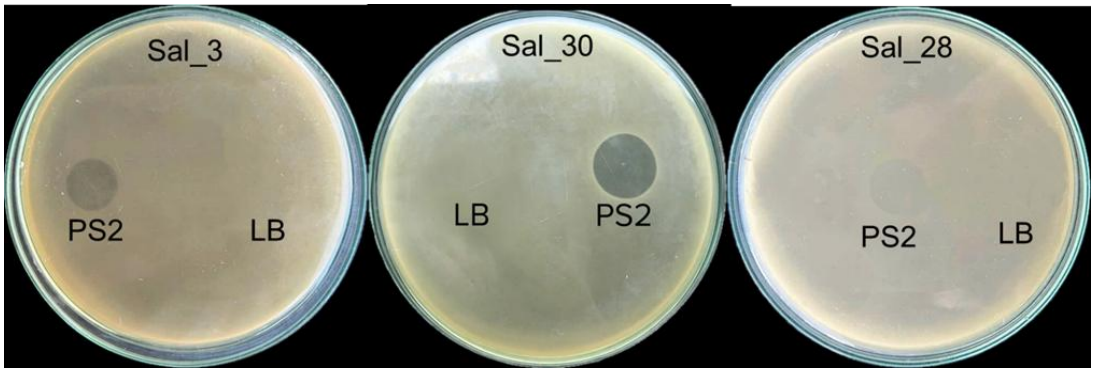

Figure 2. The phage PS2 drop test results on indicator bacteria *Sal_3*, *Sal_30*, and *Sal_28*, with LB medium as the negative control.

3.4. Optimal MOI Determination

When evaluating phage density at different infection rates (MOIs), the results (Figure 3) showed that at MOI of 0.01, the PS2 achieved the highest density of 9.59 ± 0.34 log (PFU/mL) ($p < 0.05$). The phage densities at MOIs of 0.001 and 0.1 were not significantly different ($p > 0.05$) (8.85 ± 0.53 log and 8.9 ± 0.61 log (PFU/mL), respectively) (Figure 3). Additionally, phage density tended to decrease as MOI increased ($p < 0.05$), with the lowest density observed at MOI of 100 (4.36 ± 0.5 log (PFU/mL)). These findings indicate that MOI of 0.01 was optimal for PS2 and was used in subsequent experiments.

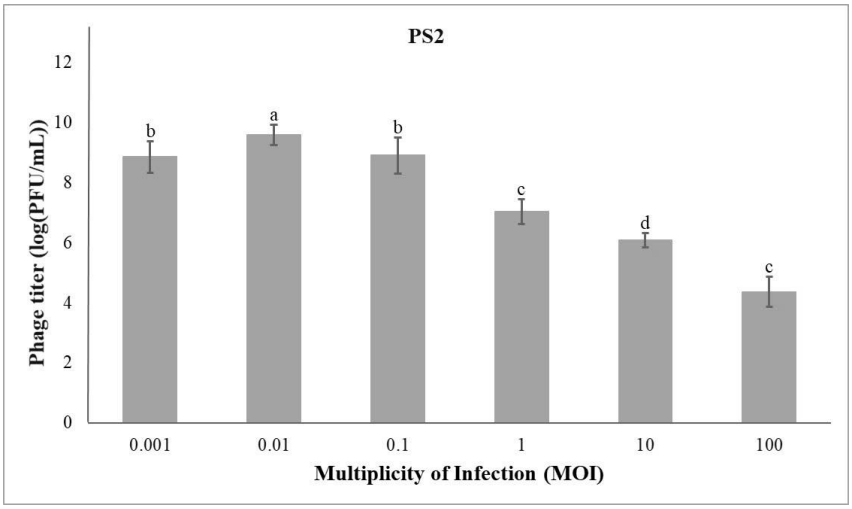

Figure 3. The infection rate of phage PS2 at different MOIs. MOI of 0.01 was optimal for phage PS2. Different letters (a, b, c and d) indicate statistically significant differences ($p < 0.05$).

3.5. The Growth Curve Determination

The growth curve of PS2 was evaluated by infecting its host bacteria at an MOI of 0.01. As shown in Figure 4, the latent phase of PS2 lasted approximately 25 minutes, during which the phage invaded and replicated within the host cell. Between 25 and 45 minutes, the density of PS2 increased sharply. After 45 minutes, the phage density rose by approximately 3.4 log (PFU/mL) before reaching equilibrium at around 8.2 log (PFU/mL) (Figure 4). The burst size of PS2 was estimated to be approximately 225 phage particles per infected host cell.

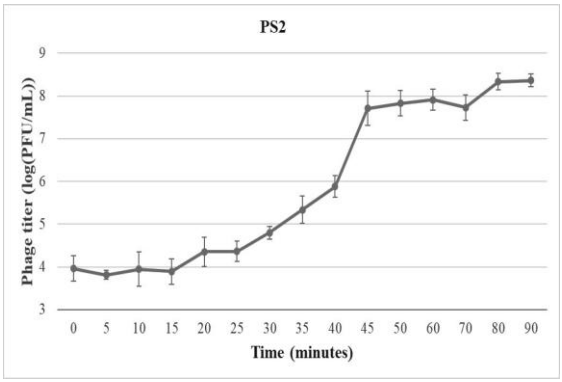

Figure 4. Growth curve of PS2. After 45 minutes, the phage density rose by approximately 3.4 log (PFU/mL), and reached equilibrium at around 8.2 log (PFU/mL).

3.6. Effect of Temperature on the Activity of Phage PS2

The activity of PS2 was evaluated under various temperature conditions, including -20 ºC, 4 ºC, 25 ºC, 37 ºC, 40 ºC, 50 ºC, 60 ºC, 70 ºC, and 80 ºC. The results (Figure 5) showed that PS2 remained stable at temperatures ranging from -20 ºC to 60 ºC throughout the 3-hour experiment ($p > 0.05$). However, at 70 ºC, PS2 density significantly decreased ($p < 0.01$) compared to the control (37 ºC), with reductions of 3 log, 4.2 log, and 5.2 log (PFU/mL) after 1, 2, and 3 hours of incubation, respectively. PS2 was completely inactivated after just 1 hour of incubation at 80 ºC.

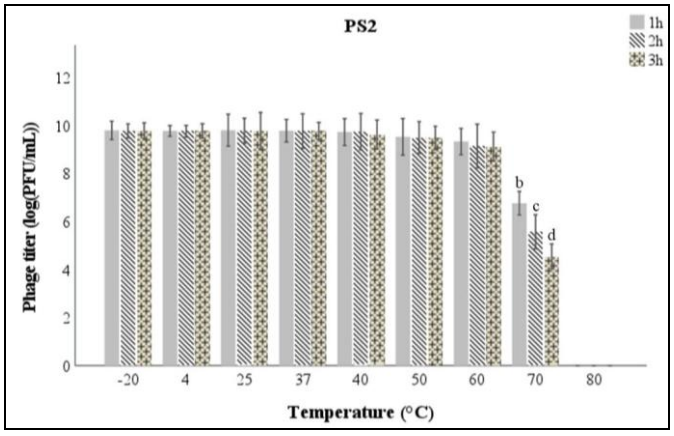

Figure 5. The phage PS2 density under different temperature conditions after 1, 2, and 3 hours of incubation. PS2 was completely inactivated after just 1 hour of incubation at 80 ºC. Different letters (a, b, c and d) indicate statistically significant differences ($p < 0.05$).

3.7. Effect of pH on the Activity of Phage PS2

pH is a critical factor influencing the survival and stability of phages. In this study, we assessed the stability of phage PS2 under various pH conditions (pH 2, 3, 5, 7, 9, 11, and 12) over 3 hours. The results (Figure 6) showed that PS2 remained stable across a broad pH range (5 to 12) throughout the experiment ($p > 0.05$). However, at pH 3, PS2 density slightly declined, decreasing by approximately 0.3 log after 1 hour ($p > 0.05$) and significantly dropping by 0.8 log and 2 log (PFU/mL) after 2 and 3 hours, respectively ($p < 0.05$). At pH 2, PS2 was completely inactivated within 1 hour.

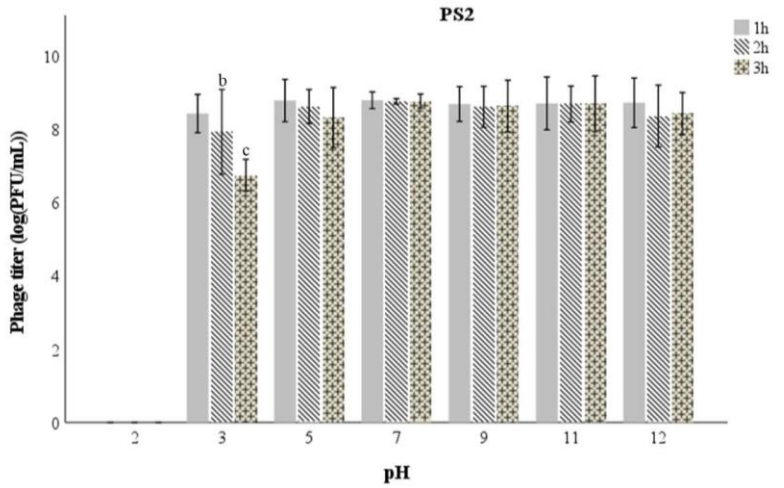

Figure 6. The phage PS2 density after 1, 2, and 3 hours of incubation under different pH. Different letters (a, b, c and d) indicate statistically significant differences ($p < 0.05$).

4. Discussion

Antibiotic-resistant *Salmonella* is becoming an increasingly serious challenge for the global poultry industry [11-13]. Phage therapy is considered a promising alternative for controlling bacterial pathogens responsible for poultry diseases, particularly those caused by antibiotic-resistant strains of *Salmonella* [30, 31]. In this study, using the double-layer agar method, we isolated and identified the bacteriophage PS2 from poultry farm wastewater samples in Hai Duong province, Vietnam. Morphologically, phage PS2 exhibits a Myovirus-like structure and belongs to the class Caudoviricetes, with an icosahedral head measuring 80.02 ± 3 nm in diameter and a long, straight tail of 90.6 ± 8 nm in length. Its morphological characteristics are similar to those of previously reported *Salmonella* phages, such as vB_Si_35FD and vB_Si_DR94 [32], phage SB18 [33], and phage AUFM_Sc1 [34].

Phages are known for their high host specificity, typically infecting only a particular bacterial species or even a specific strain within the same species [35]. Our findings indicate that PS2 demonstrated strong and specific lytic activity against *S. enterica* subsp. *enterica* and exhibited no lytic activity against other tested bacterial species, including *E. coli*, *V. parahaemolyticus*, and *B. cereus.* Notably, PS2 was able to lyse all tested *S. enterica* strains (30/30) isolated from chickens and ducks with diarrhea syndrome. Similarly, Zhang et al., [36] isolated phage Pu20 from wastewater in Wuhan, China, which effectively lysed 21 out of 26 tested *Salmonella* strains, including nine drug-resistant strains, while showing no lytic activity against *E. coli*, *Listeria monocytogenes*, or *Staphylococcus aureus.* In another study, Wang et al., [37] isolated phage P6 from duck farm wastewater and fecal samples in Shandong, China. This phage specifically lysed *Salmonella enteritidis* and *Salmonella typhimurium* but did not affect *E. coli*, *Proteus mirabilis*, or *S. aureus*. Additionally, Khan et al. [38] isolated phage L223 from poultry market wastewater in Dhaka, Bangladesh, which was capable of infecting *S. typhimurium* and *S. enteritidis* but showed no lytic activity against *E. coli* ATCC25922, *Pseudomonas aeruginosa*, *Vibrio cholerae*, *Shigella flexneri*, *Acinetobacter baumannii*, or *S. aureus*. A phage with a broad host range, capable of targeting multiple bacterial strains, can be comparable to a broad-spectrum antibiotic. Given its specificity in lysing *S. enterica* subsp. *enterica* strains, PS2 holds promise as a potential candidate for the development a phage therapy to control *Samonella* infections in poultry.

The multiplicity of infection (MOI) refers to the ratio of phages to bacterial cells in an infected environment [39]. The optimal MOI is the ratio that maximizes infection efficiency and phage progeny production [37]. This value can vary depending on factors such as the number of infecting phages, the rate and duration of adhesion to host cells, and host density. Determining the optimal MOI is crucial for enhancing the effectiveness of phage therapy, particularly in large-scale phage production [37]. Our results indicated that PS2 had an optimal MOI of 0.01. This is consistent with phage vB_SalP_LDW16, which was isolated from wastewater and fecal samples from a broiler farm in Shandong, China [40], as well as phage AUFM_Sc3, derived from chicken and goose feces in Kahramankazan District, Ankara Province, Türkiye [34]. In contrast, phage P6, isolated from duck farm wastewater and fecal samples in Shandong, China, exhibited a lower optimal MOI of 10^{-4} [37], while phage AUFM_Sc1 had a higher optimal MOI of 0.1 [34].

Phage growth curves illustrate the population dynamics of phages over time, highlighting distinct growth phases. Determining the growth curve is essential for identifying key characteristics such as the latent period and burst size. The latent period is the time between a phage entering a bacterial cell and the release of newly produced phages. A shorter latent period is generally associated with greater bacterial killing efficiency [41]. The burst size refers to the number of new phage particles released per infected host cell and is closely related to the latent period. Our results showed that PS2 had a latent period of approximately 25 minutes. Between 25 and 45 minutes, the phage density in the medium increased rapidly, reaching equilibrium after 45 minutes. The burst size of PS2 was estimated at 225 phage particles per infected host cell. In a similar study, Cao et al., [40] reported that *Salmonella* phage vB_SalP_LDW16 had a much shorter latent period of only 10 minutes, followed by a rapid increase in phage density from 10 to 60 minutes. This phage reached a plateau after 70 minutes, with an average burst size of approximately 110 PFU per infected cell. Additionally, Zhang et al., [42] examined the growth characteristics of two *Salmonella* phages, S4lw and D5lw, isolated from wastewater samples collected at the University of California, Davis wastewater treatment plant. Using *S. typhimurium* ATCC14028 as the host bacterium, they found that the complete lysis cycles of phages S4lw and D5lw were 65 and 60 minutes, respectively. Phage S4lw had a latent period of 12 minutes and a burst size of 41 PFU/infected cell, whereas phage D5lw exhibited a shorter latent period of just 4 minutes and a burst size of 37 PFU/infected cell. Notably, *Salmonella* phage L223, isolated from a poultry market wastewater sample, had a latent period of 30 minutes and an exceptionally high burst size of 515 PFU per infected cell [38]. These findings highlight the diversity of phage latent periods and burst sizes, which can be influenced by various factors, including MOI, the growth phase of the host bacterium, and environmental conditions that affect phage-host interactions. Understanding these growth characteristics is crucial for optimizing phage enrichment processes and improving recovery efficiency for the production of phage-based therapeutics.

Phage activity is influenced by various environmental physicochemical factors, such as temperature, pH, and ion concentration [43]. Optimal environmental conditions are essential for efficient phage replication and lysis. Among these factors, temperature plays a crucial role, not only affecting phage survival but also influencing lytic activity [44]. A suitable temperature range extends phage survival, enhances infection rates, and impacts mechanical properties. At low temperatures, biochemical reactions in both bacterial cells and phages slow down or even halt, leading to stagnation in phage adsorption, penetration, and replication. When the temperature falls below the optimal range, only a small amount of phage genetic material enters host cells, and fewer phages participate in replication [45]. However, low temperatures typically do not kill

phages but instead temporarily inhibit their activity [46]. Conversely, excessively high temperatures can denature the proteins forming the phage capsid and essential enzymes, rendering the phage incapable of adhering to and penetrating bacterial cells. Moreover, high temperatures can disrupt the cell membranes of both phages and bacteria, leading to cell death [47]. In this study, phage PS2 remained stable over a relatively broad temperature range, from -20 °C to 60 °C. However, phage density declined at temperatures above 60 °C and was completely inactivated at 80 °C. The temperature stability of PS2 was comparable to that of *Salmonella* phage L223 [38], phage S55 [48], phage vB_SalP_LDW16 [40], and phage P6 [37]. Investigating the thermal stability of phages provides valuable insights into optimizing enrichment, preservation, and storage conditions, as well as improving phage preparation methods.

pH is another important environmental factor that influences phage survival and activity. Most phages that target human and avian pathogens are adapted to neutral or near-neutral pH conditions, aligning with those of their hosts [44, 49]. However, significant pH changes, particularly when it drops below 4.5, can severely limit their ability to replicate [47]. Additionally, phage activity is often affected at pH levels above 9 or below 5 [50]. Our results showed that phage PS2 remained stable within a pH range of 5 to 12 but was inactivated at highly acidic conditions (pH 2). PS2 exhibited greater pH tolerance compared to some previously reported phages. For instance, phage vB_SalP_LDW16 maintained its activity between pH 6 and 12, but its titer decreased significantly at pH 2–5 [40]. In addition, phage L223 remained stable between pH 4 and 10 after one hour of exposure, but its density declined significantly under strongly acidic (pH 2–3) and strongly basic (pH 11) conditions [38]. Similarly, two phage strains, PSPu-95 and PSPu-4-116, which lyse *S. pullorum* isolated from chicken samples in Jiangsu, China, remained active within a narrower pH range (pH 6–9). Their activity significantly declined in more acidic or alkaline conditions after just one hour of exposure [51]. Another example is phage ST4, isolated from a wastewater treatment facility in Gwachon, Korea, which infected *S. gallinarum* KVCC BA00722. This phage exhibited the most stable activity within a very narrow pH range of 5–8 [52] Our findings demonstrate that phage PS2 maintains stable activity over a broad pH range (pH 5–12). Assessing phage stability under different pH conditions provides valuable insights for phage therapy applications in different environments. For example, phages that can tolerate highly acidic conditions (pH 2–3) may be suitable for oral administration, while those with stability in a neutral pH range (pH 6.5–7.3) could be used for injection, immersion, or surface spraying in barns [53].

5. Conclusion

This study successfully isolated and characterized phage PS2, which exhibits a Myovirus-like morphology and strong lytic activity against *Salmonella enterica* subsp. *enterica* strains. Phage PS2 has an optimal MOI of 0.01, a latent phase of approximately 25 minutes, and a burst size of 225 phage particles per cell. It remains stable across a broad temperature range (-20 °C to 60 °C) and a wide pH range (pH 5–12), demonstrating its adaptability to various environmental conditions. These findings suggest that phage PS2 is a promising candidate for phage therapy to combat *Salmonella* infections in poultry. Its potential application could help reduce antibiotic use in livestock farming, contributing to sustainable poultry production in Vietnam.

Acknowledgements

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