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ARTICLE

Isolation and characterization of FS01, a lytic bacteriophage infecting *Salmonella typhimurium*

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Resumen

Aislamiento y caracterización de FS01, un bacteriófago lítico que infecta a Salmonella typhimurium

La contaminación por *Salmonella* representa un problema sanitario y económico. Este estudio aisló y caracterizó un bacteriófago lítico, FS01, activo contra *S. typhimurium*. FS01 mostró una MOI de 1, tasa de adsorción de $1{,}9x10^{-9}$ mL/min, y una curva de crecimiento con eclipse de 10 minutos, latencia de 15 minutos y tamaño de explosión de 10 viriones por célula. Posee cápside icosaédrica (64,4 nm) y cola flexible (182,2 nm), lo que sugiere pertenecer a la familia Siphoviridae, con estrecho rango de hospedero; es estable térmicamente entre 40–60 ºC por 30 minutos, viable entre pH 6–9, sensible a cloroformo y luz UV. FS01 inhibió el crecimiento bacteriano desde las 3 horas. FS01 presenta propiedades de un agente biocontrolador prometedor para descontaminar alimentos y superficies.

Palabras clave: Biocontrol; Aguas residuales; Cloroformo; Tasa de adsorción, Siphoviridae

Abstract

Salmonella contamination represents a health and economic problem. This study isolated and characterized a lytic bacteriophage, FS01, active against *S. typhimurium*. FS01 showed a MOI of 1, an adsorption rate of $1.9x10^{-9}$ mL/min, and a growth curve with an eclipse period of 10 minutes, a latency period of 15 minutes, and a burst size of 10 virions per cell. It has an icosahedral capsid (64.4 nm) and a flexible tail (182.2 nm), suggestive of Siphoviridae family affiliation, with a narrow host range. It is thermally stable between 40–60 ºC for 30 minutes, viable within a pH range of 6–9, and sensitive to chloroform and UV light. FS01 inhibited bacterial growth starting at 3 hours. FS01 exhibits properties of a promising biocontrol agent for decontaminating food and surfaces.

Key words: Biocontrol; Wastewater; Chloroform; Adsorption rate; Siphoviridae.

Introduction

Bacteriophages are the most abundant biological entities on the planet, reaching an estimated 10^{31} virions in the terrestrial biosphere. Although there is debate about its accuracy, this figure remains valid according to recent studies. (Mushegian 2020, Breitbart *et al.* 2018). In particular, the high abundance and ability of tailed phages to kill host bacteria have made them a key object of study, leading to advancements in the understanding of their ecological function. Furthermore, their genetic plasticity, reflected in the diversity of their nucleic acids, genes, and proteins, has unveiled complex evolutionary relationships (Dion *et al.* 2020). In the oceans alone, it is estimated that 10^{28}-10^{29} bacteriophage infections occur daily, which plays a crucial role in shaping the composition and evolution of bacterial communities (Chevallereau *et al.* 2022, Luo *et al.* 2022). A promising alternative is biological control with bacteriophages, a natural technique that uses lytic phages to eliminate or reduce specific pathogens in food. This method has shown effectiveness in various foods, and its commercial use is increasing, being recognized as a safe and natural option to eliminate pathogenic bacteria (Moye *et al.* 2018).

Salmonella enterica subspecies *enterica*, serovar Typhimurium (=*Salmonella typhimurium*) is a gram-negative bacterium belonging to the Enterobacteriaceae family and is one of the most common facultative intracellular foodborne pathogens. Poultry meat and eggs are the primary vehicles for the transmission of salmonellosis (Ni *et al.* 2018). Chickens infected with *S. typhimurium* are often asymptomatic; however, they can disseminate the pathogen into the environment and may reach slaughterhouses already contaminated, raising concerns about direct or indirect cross-contamination through tools and equipment during processing (Islam *et al.* 2019). The development of preventive strategies to control bacterial pathogens remains an ongoing challenge due to the limited efficacy of traditional methods. Among alternative approaches, bacteriophages have gained increasing attention as a promising biocontrol tool. Although their application presents challenges regarding safety and regulatory approval, their potential in combating infections and enhancing food safety remains significant (Harada *et al.* 2018; Singh 2018).

Bacteriophages, as natural antibacterial agents, utilize specialized tail structures, such as fibers and spikes, to recognize and target receptors on the surface of bacterial cells. Each phage has a host range determined by the affinity of these structures for bacterial receptors, which include proteins such as the O antigen of lipopolysaccharides, OmpC, BtuB, FhuA, and flagella, as seen with *Salmonella* phages. (Nobrega *et al.* 2018, Taslem 2022); they use the host cell machinery to propagate their progeny, are ubiquitously distributed in the environment, occurring wherever bacteria are present. Their abundance and ease of enrichment from environmental sources using the host bacterium make them the most prevalent replication entities. For biocontrol applications, the use of virulent (lytic) bacteriophages is essential, as they induce bacterial lysis, thereby effectively regulating pathogen populations (Mutlu & Şahin 2019, O'Sullivan *et al.* 2019). In this study, a lytic bacteriophage infecting *Salmonella typhimurium* was isolated and characterized from wastewater samples collected at the "La Taboada" wastewater treatment plant in Lima, Peru. Its morphology was determined, a one-step growth curve was established, its host range was evaluated, its sensitivity to physicochemical factors was assessed, and its ability to lyse cultures in broth with *S. typhimurium* was analyzed. Additionally, the behavior of phage FS01 was evaluated against two *Salmonella* serovars, its performance under specific environmental conditions was assessed, and its potential as a biocontrol tool for managing Salmonella infections was explored.

Materials and methods

Isolation and purification of phages

Bacteriophage FS01 was isolated from wastewater at the "La Taboada" wastewater treatment plant, located in Lima, Peru; 300 mL were filtered through Whatman grade 1 paper to remove solid residues. The filtrate was then centrifuged at 4400 rpm for 15 minutes. The supernatant was filtered through 0.45 µm nitrocellulose membranes (Millipore™), resulting in a filtrate free of bacteria. The drop test (Segundo-Arizmendi *et al.* 2017) was then performed for the qualitative detection of bacteriophages. Twenty microliters of filtrate were inoculated on a layer of *S. typhimurium* ATCC 14028, incubated at 37 ºC for 24 hours.

The sample was purified using the double-layer plaque assay (Yang M. *et al.* 2020): a series of serial decimal dilutions of the suspension was prepared in saline solution (0.85%). Then, 900 µL of each dilution and 900 µL of *S. typhimurium* ATCC 14028 were inoculated, incubated for 15 minutes, and 5 mL of soft Brain Heart Infusion (BHI) agar was added. All this was poured onto plaques with BHI agar and incubated at 37 ºC for 24 hours. Once the lysis plaques appeared, one of them was selected and transferred to a 1.5 mL microcentrifuge tube containing sterile saline solution, 100 µL of chloroform was added and vortexed for 1 minute. Subsequently it was centrifuged at 5000 rpm for 10 minutes and filtered through 0.45 µm nitrocellulose membranes. The above operation was repeated, where a single plaque was repeatedly purified 5 times to obtain the purified phage (Kim *et al.* 2022).

Determination of the optimal multiplicity of infection (MOI) of bacteriophage

The determination of the optimal MOI (Multiplicity of Infection) of bacteriophage was performed as recommended by Zhang *et al.* (2018), with some modifications. 1 mL of *S. typhimurium* ATCC 14028 (10^8 CFU/mL), and 1 mL of several dilutions of the viral suspension (10^9, 10^8, 10^7 y 10^6 PFU/mL), were added to a tube with 8 mL of BHI, (MOI = 10, 1, 0.1 and 0.01 respectively). It was incubated at 37 ºC for 4 hours. Then, each culture was centrifuged at 4400 rpm for 15 minutes and filtered through a 0.45 µm membrane filter. The bacteriophage titer in each tube was determined by the double-layer agar assay. Each test was performed in triplicate, and the optimal MOI considered was the one that generated the highest titer at the end of each test.

Host range determination

The host range of FS01 was determined using the drop test, following the methodology described by Segundo-Arizmendi *et al.* (2017). First, all indicator strains were adjusted to the same concentration (10^8 CFU/mL). Then 100 µl of each indicator strain was spread onto a BHI agar plate and allowed to dry for 10 minutes. Subsequently, 20 µL of the phage suspension ($2x10^8$ PFU/mL) were applied to the plate, which was then incubated at 37 ºC for 24 hours.

The indicator strains used in this study were: *Salmonella choleraesuis* ATCC 10708, *Escherichia coli* ATCC 25922, *Enterobacter aerogenes* ATCC 13048, *Proteus vulgaris* ATCC 6380, *Proteus mirabilis* ATCC 29906, *Enterococcus faecium* ATCC 35667, *Enterococcus faecalis* ATCC 29212, *Shigella dysenteriae* ATCC 13313, *Serratia marcescens* ATCC 43862, *Klebsiella pneumonia* ATCC 13883, *Pseudomonas aeruginosa* ATCC 9027, *Pseudomonas aeruginosa* ATCC 28573 and *Bacillus cereus* ATCC 11778.

Determination of the adsorption rate

The adsorption rate of FS01 was performed as recommended by Rahaman *et al.* (2014) and Pereira *et al.* (2016), with some modifications. A 1 mL sample of *S. typhimurium* strain ATCC 14028 (10^8 CFU/mL) was combined with 1 mL of the FS01 phage suspension at 10^8 PFU/mL (MOI = 1), which was then incubated at 37 ºC. Samples of 1 mL were taken at intervals of 0, 5, 10, 15, 20, 25, and 30 minutes, centrifuged at 5000 rpm for 10 minutes. The supernatants were filtered using 0.45 µm Millipore membranes. The bacteriophage titer in each tube was determined by the double-layer agar assay.

One-step growth curve

The one-step growth curve of bacteriophage FS01 was conducted following the procedure outlined by Shang *et al.* (2021), with some modifications. A 1 mL sample of the logarithmic growth-phase *S. typhimurium* strain ATCC 14028 (10^8 CFU/mL) was combined with 1 mL of the FS01 bacteriophage suspension at 10^8 PFU/mL (MOI = 1). The mixture was incubated at 37 ºC for 20 minutes. Subsequently, the tube was centrifuged at 5000 rpm for 10 minutes, the supernatant was discarded, and the pellet was resuspended in 1 mL of BHI (Brain Heart Infusion) broth. This procedure was performed in triplicate. It was then resuspended with 1 mL of BHI broth and vortexed for 2 minutes. 100 µL of this culture was transferred to a flask containing 100 mL of BHI broth. Two 1 mL samples were taken every 5 minutes from baseline (T_0 = 0 minutes) to 90 minutes. To determine the latent period, the first 1 mL sample was serially diluted 5 times in saline solution (0.85%) to then determine the titer of each sample by the double-layer agar assay. The second sample was used to determine the time of the eclipse period. For this, 200 µL of chloroform was added to the

microcentrifuge tube containing the sample, which was vortexed for 1 minute and then centrifuged at 5000 rpm for 10 minutes. The aqueous phase was transferred to a new sterile microcentrifuge tube from which the titer was determined by the double-layer agar assay.

Bacteriophage morphology

The morphological analysis of FS01 was performed using transmission electron microscopy, following the sample preparation guidelines provided by Jebri *et al.* (2016). Briefly, a drop of the high-titer FS01 suspension was placed on a copper grid, stained with 2% (w/v) uracil acetate, and examined using an FEI Tecnai 10 transmission electron microscope at an accelerating voltage of 100 kV and a magnification of 135,000X. Morphological characteristics were used following the criteria set by Adriaenssens & Brister (2017).

Determination of thermal stability

To assess the effect of temperature on the stability of phage FS01, the methodology recommended by Manohar *et al.* (2018) was followed with modifications. An aliquot of 1 mL phage suspension ($2x10^8$ PFU/mL) was incubated in a microcentrifuge tube and exposed to temperatures of 40, 50, 60, 70 and 80 ºC in a water bath for 5, 15, 30, 45 and 60 minutes at each temperature. Each assay was performed in triplicate. The titer of each bacteriophage suspension at different temperatures was determined by the double-layer agar assay.

Determination of sensitivity at different pH

To assess the effect of pH variation on the stability of phage FS01, the experiment was conducted following the methodology described by Wang *et al.* (2017). A 1 mL aliquot of the bacteriophage suspension ($2x10^8$ PFU/mL) was dispensed into tubes containing 9 mL of saline solution, adjusted to values ranging from 2 to 13. Each sample was incubated at 37 ºC for 60 minutes and tested in triplicate. The titer of each bacteriophage suspension at different pH values was determined by the double-layer agar assay.

Determination of chloroform sensitivity.

The determination of chloroform sensitivity was performed following the methodology described by Chénard *et al.* (2015), with modifications. A 500 µl aliquot of the bacteriophage was dispensed into a microcentrifuge tube, to which an equal volume of chemically pure chloroform was added. The mixture was maintained under constant agitation at room temperature for 60 minutes. Subsequently, it was centrifuged at 5000 rpm for 10 minutes The aqueous phase was then transferred to a microcentrifuge tube and incubated at room temperature for 6 hours to allow evaporation of any remaining chloroform. A control was included following the same procedure, but replacing chloroform with saline solution (0.85%). Each assay was performed in triplicate and the titer was determined using the double-layer agar assay.

Determination of sensitivity to UV light

The sensitivity of bacteriophage FS01 was determined following the methodology described by Megha *et al.* (2017), with certain modifications. An 1 mL aliquot of the bacteriophage suspension ($2x10^8$ PFU/mL) w|as dispensed into a Petri dish and exposed to UV light at 20 cm for 15 seconds. Additionally, the same experiment was performed at time intervals of 30, 45 and 60 seconds. The assay was performed in triplicate, and the titer was determined by the double-layer agar assay.

Determination of bacterial growth inhibition

To evaluate the growth reduction efficiency of *S. typhimurium* ATCC 14028, the methodology described by Duc *et al.* (2018) was used, after a few modifications. A 1 mL aliquot of the host strain (10^8 CFU/mL) and 1 mL of FS01 (10^8 PFU/mL) were inoculated into 100 mL of BHI broth. A control was prepared using the same bacterial concentration, replacing the phage suspension with 1 mL of saline solution (0.85%). Both mixtures were incubated at 37 ºC for 12 hours. Samples (1 mL) were collected at the beginning (0 minutes) and at 1-hour intervals. Decimal dilutions were then prepared in saline solution (0.85%). Subsequently, 1 mL of the last 3 dilutions was mixed with liquefied BHI agar at 45 ºC in Petri dishes, allowed to solidify, and incubated at 37 ºC for 24 hours. Only plates containing between 30 and 300 colonies were considered. The assay was performed in triplicate.

Statistical analysis

All experiments were conducted in triplicate. Results are expressed as mean values ± standard deviation or standard error or 95% confidence inter-

val. When applicable, Student's t-test was used. A p-value < 0.05 was considered statistically significant. Statistical analyses were performed using Microsoft® Excel® and PAST 4.03 software (Hammer 2001).

Results

Phage isolation and purification

A bacteriophage was isolated using the drop test method with *S. typhimurium* ATCC 14028 as the indicator strain. Lysis zones were observed (positive result in Fig. 1A). Subsequently, the bacteriophage, designated FS01, was purified through the double-layer agar assay, producing uniform and transparent lysis plaques after three rounds of purification. These plaques were circular, with diameters ranging from 0.5 to 1 mm (Fig. 1B-C).

Determination of bacteriophage optimal multiplicity of infection (MOI)

In triplicate assays testing four different MOIs, *S. typhimurium* reached a concentration of 10^8 CFU/mL. The highest phage production was observed at a MOI of 1, which was considered the

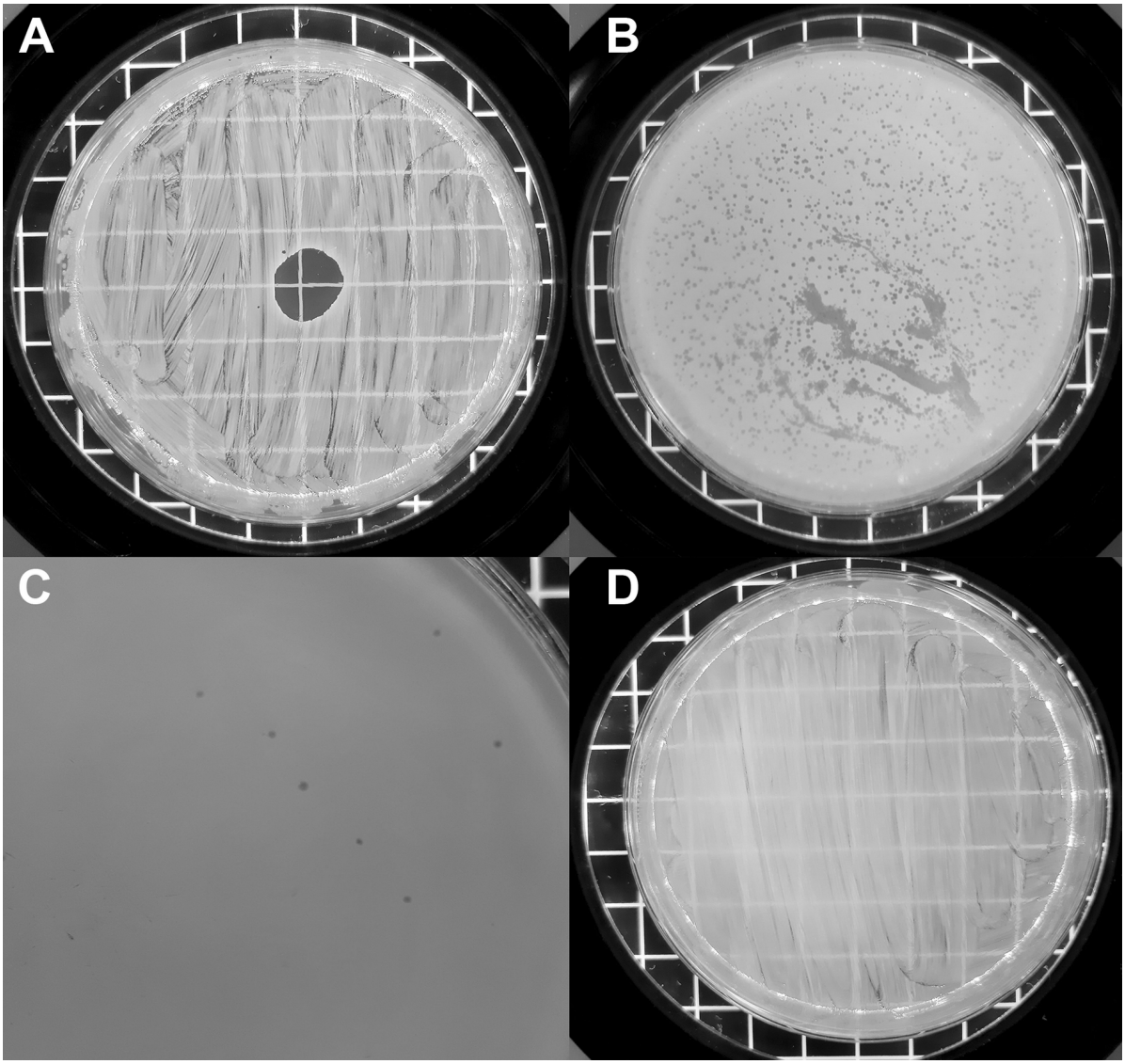


Figura 1. A: Aislamiento inicial de fagos mediante la prueba del goteo en zona en agar con *Salmonella* typhimurium ATCC 14028. Se observa la zona de lisis como resultado positivo; **B:** Placas individuales del fago FS01 que infectan a *S. typhimurium* ATCC 14028; **C:** Comportamiento de lisis bacteriana tras la exposición de *S. typhimurium* ATCC 14028 a FS01, produciendo placas de entre 0,5 y 1 mm de diámetro; **D:** El mismo ensayo con *Salmonella choleraesuis* ATCC 10708, con un resultado negativo para las 13 cepas restantes analizadas.

Figure 1. A: Initial isolation of phages by the agar spot test using *Salmonella typhimurium* ATCC 14028. It can be observed the lysis zone as a positive result. B: Single plaques of FS01 phage infecting *S. typhimurium* ATCC 14028. C: Bacterial lysis behavior after exposing *S. typhimurium* ATCC 14028 to FS01, producing plaque sizes ranging from 0.5 to 1 mm in diameter. D: Agar Spot test using *Salmonella choleraesuis* ATCC 10708, with a negative result as for the remaining 13 tested strains.

MOI	Repl. 1	Repl. 2	Repl. 3	Mean	Standard deviation
0.01	2.07E+07	1.93E+07	2.31E+07	2.10E+07	1.57E+06
0.1	5.32E+08	5.49E+08	5.38E+08	5.40E+08	7.04E+06
1	1.19E+09	1.10E+09	1.02E+09	1.10E+09	6.94E+07
10	1.61E+08	1.47E+08	1.43E+08	1.50E+08	7.72E+06

Tabla 1. Multiplicidad de infección (MOI) para FS01 contra *Salmonella typhimurium*.
Table 1. Multiplicity of infection (MOI) for FS01 against *Salmonella typhimurium*.

Bacterial strain	Lysis
Salmonella typhimurium ATCC 14028	+
Salmonella choleraesuis ATCC 10708	-
Escherichia coli ATCC 25922	-
Enterobacter aerogenes ATCC 13048	-
Proteus vulgaris ATCC 6380	-
Proteus mirabilis ATCC 29906	-
Enterococcus faecium ATCC 35667	-
Enterococcus faecalis ATCC 29212	-
Shigella dysenteriae ATCC 13313	-
Serratia marcescens ATCC 43862	-
Klebsiella pneumonia ATCC 13883	-
Pseudomonas aeruginosa ATCC 9027	-
Pseudomonas aeruginosa ATCC 28573	-
Bacillus cereus ATCC 11778	-

Tabla 2. Determinación del rango de hospedadores del bacteriófago FS01 con diferentes cepas bacterianas
Table 2. Determination of the host range of bacteriophage FS01 with different bacterial strains.

optimal MOI, reaching a titer of $1.1x10^9$ PFU/mL. In contrast, at MOI of 10, the titer was $1.5x10^8$ PFU/mL, despite starting with 10 times more virus than at a MOI of 1. Meanwhile, at MOIs of 0.1 and 0.01, an increase in concentration was observed, reaching titers of $5.5x10^8$ and $2.1x10^7$, respectively (Table 1).

Host range determination

The bacteriophage exhibits a narrow host range. A total of 14 ATCC strains were tested (Table 2), among which lysis zones were only observed in *S. typhimurium* ATCC 14028 in the remaining 13 strains no lysis zones were detected using the spot test, indicating that these strains are not susceptible to bacteriophage FS01 (Fig. 1D).

Determination of the adsorption rate

The adsorption rate of bacteriophage FS01 was $1.9x10^{-9}$ mL/min. The percentage of unadsorbed phage was 19.85% at 10 minutes, decreasing to 2.86% at 20 minutes (Fig. 2A).

One-step growth curve

Figure 2B presents the one-step growth curve of bacteriophage FS01. The eclipse period lasted 10 minutes, the latency period extended to 15 minutes, and the burst size was calculated as 10 virions per infected cell.

Bacteriophage morphology

Transmission electron microscopy analysis revealed that phage FS01 possesses an icosahedral head with a diameter of 64.4 nm and a long, flexible tail measuring 182.2 nm in length. These morphological characteristics suggest that FS01 belongs within morphotype B1 of the Siphoviridae family in the Caudovirales order (Fig. 2C).

Stability of the isolated phage at various temperatures

FS01 remains stable at 40 ºC and 50 ºC for the first 15 minutes, showing a one-logarithm reduction after 30 to 60 minutes at these temperatures. At 60 ºC it undergoes a one-logarithm reduction and remains stable for up to 60 minutes. A significant decline in bacteriophage concentration is observed at 70 ºC, with complete loss of viability at 80 ºC (Fig. 2D).

Stability of the isolated phage at various pH values

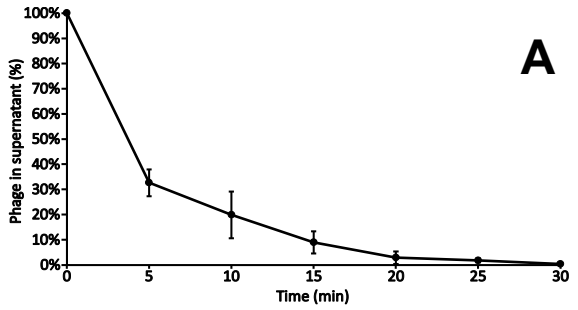
Bacteriophage FS01 showed tolerance to basic pH conditions, with only a one-log reduction in titer. A comparable stability was observed at pH 5. In contrast, phage viability decreased significantly at acidic pH values, with reductions of six, four, and three orders of magnitude at pH 2, 3, and 4, respectively (Fig. 2E).

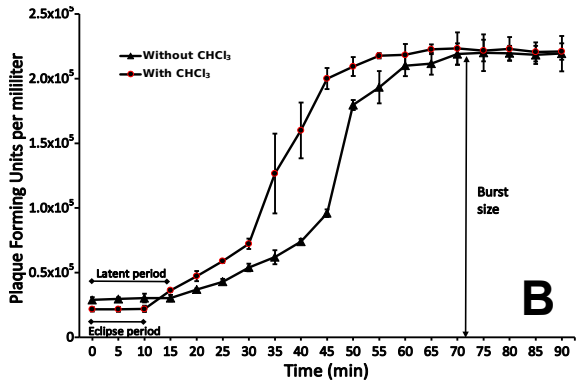
Determination of chloroform sensitivity

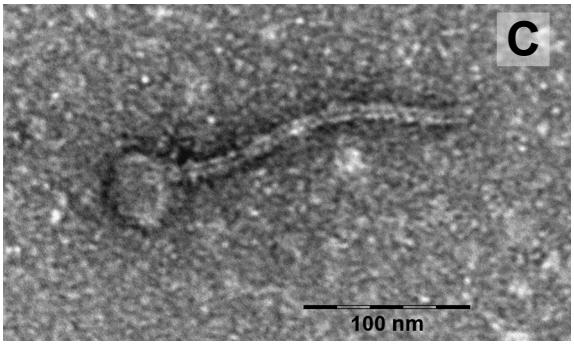
The titer of FS01 decreased by 0.86 logarithmic units after 60 min of exposure to chloroform, from 9.82 to 8.96 (average of three trials, $p < 0.05$).

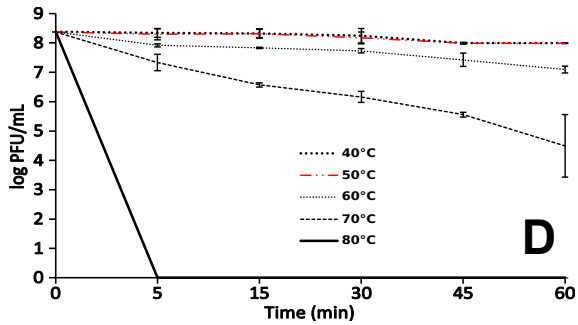
Sensitivity to UV radiation

Bacteriophage FS01 was highly sensitive to UV radiation. Titer decreased by approximately one log unit every 15 seconds of exposure, reaching









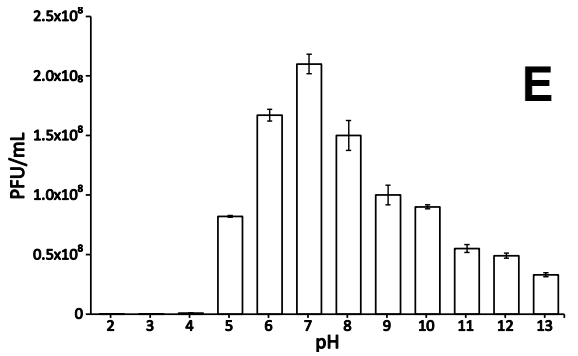


Figura 2. A: Porcentaje de partículas no adsorbidas de FS01 a los intervalos mostrados; **B:** Curva de crecimiento de un paso del bacteriófago FS01 de la cepa *Salmonella typhimurium* ATCC 14028 durante 90 minutos; **C:** Micrografía electrónica del bacteriófago FS01. Barra= 100 nm. **D:** Estabilidad térmica del bacteriófago FS01 a diferentes temperaturas y tiempos. El FS01 es muy estable a 40-50 ºC. **E:** Estabilidad del FS01 a varios pH, durante 60 minutos en cada ensayo. FS01 muestra la mayor estabilidad a pH 7. Todos los datos corresponden a $\bar{x}$ ± DE; n=3; intervalos de confianza al 95%.

Figure 2: A: Percentage of FS01 unadsorbed particles at shown intervals; **B:** One-step growth curve of bacteriophage FS01 on *Salmonella typhimurium* strain ATCC 14028 for 90 minutes; **C:** Electron micrograph of bacteriophage FS01. Bar= 100 nm; **D:** Thermal stability of bacteriophage FS01 at different temperatures and times. FS01 is very stable at 40-50 ºC; **E:** Stability of FS01 to several pH, for 60 minutes each assay. FS01 shows higher stability at pH 7. All data correspond to $\bar{x}$ ± SD; n=3; 95% confidence intervals.

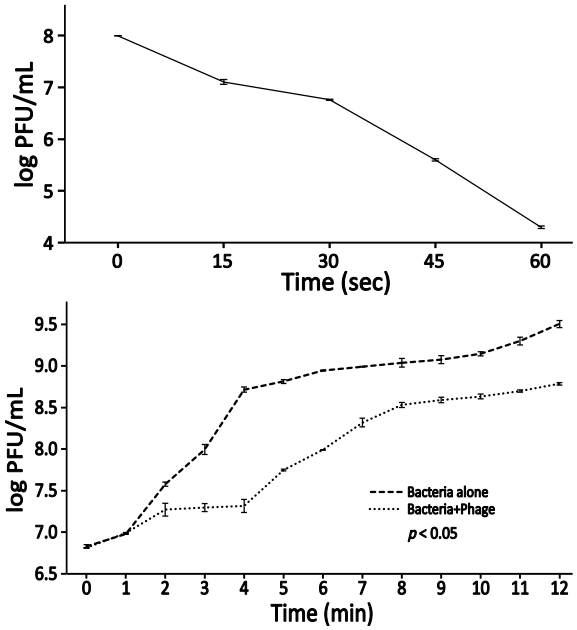


Figura 3. A: Sensibilidad del bacteriófago FS01 a la luz UV; **B:** Inhibición de *Salmonella typhimurium* ATCC 14028 por efecto de FS01, con diferencia significativa entre ellas (p<0.05). Los datos corresponden a $\bar{x}$ ± DE; n=3; intervalos de confianza al 95%.

Figure 3. A: Sensitivity of bacteriophage FS01 to UV light; **B:** FS01-mediated inhibition of *Salmonella typhimurium* ATCC 14028, with a significant difference between them (p<0.05). Data correspond to x ± SD; n=3; 95% confidence intervals.

$2x10^4$ PFU/mL at 60 seconds, compared to the initial titer of $2x10^8$ PFU/mL (Fig. 3A).

Determination of bacterial growth inhibition

The inhibition of Salmonella Typhimurium ATCC 14028 growth by bacteriophage FS01 is shown in Figure 3B. A reduction in bacterial titer begins at hour 3 and continues until hour 12, compared to the control (without bacteriophage FS01). This reduction was statistically significant ($p < 0.05$).

Discussion

In this study, a lytic bacteriophage was isolated against *S. typhimurium* ATCC 14028 from wastewater, confirming that treatment plants are viable sources of phages useful in biocontrol strategies (Yildirim *et al*. 2018). This finding reinforces the potential of phage-based approaches as sustainable alternatives for bacterial control in the food industry.

Phage FS01 produced small lysis plaques (0.5–1 mm), similar to those reported for other phages such as LPST89 (Islam *et al*. 2020a), which may be related to its infection dynamics or the physiological state of the host. This contrasts with phages like fmb-p1, which produces larger plaques (Wang *et al*. 2017).

The optimal multiplicity of infection (MOI) was determined to be 1, achieving a two-log increase in viral concentration. Lower MOIs were less effective, while a MOI of 10 induced "lysis from without" (Jung *et al*. 2017), a behavior also observed in LPSE1 (Huang *et al.* 2018b). These results underscore the importance of optimizing phage dosage to maximize biocontrol efficacy.

FS01 exhibited an adsorption rate of $1.9x10^{-9}$ mL/min, with over 97% of viral particles bound within 20 minutes. This performance is comparable to STP-1 (Jung *et al.* 2023), although lower than faster-acting strains (Tao *et al.* 2021). This kinetic profile supports its viability as a biological control agent, even though a small non-adsorbed fraction persisted, likely due to natural variability within the viral population (Abedon, 2023).

One-step growth assay revealed an eclipse period of 10 minutes and a latent period of 15 minutes (Choi *et al.* 2020, Yan *et al.* 2020, Petsong *et al.* 2019), with a burst size of 10 particles per cell. Although lower values have been reported for other phages, such as TYM101 (Abhisingha *et al.* 2020) or PS5 (Duc *et al.* 2020), its rapid replication cycle compensates for this limitation, favoring practical applications when host physiological conditions are considered (García-Salazar & Porras-Nicho, 2018).

FS01 displayed a narrow host range, infecting only *S. typhimurium*, with no lytic activity against other tested serovars or bacterial genera. This specificity may help minimize collateral effects on beneficial microbiota; however, further evaluation using a broader set of serotypes is required. This contrasts with phages such as LPST10 and LPST23, which can infect multiple *Salmonella* serotypes (Huang *et al.* 2018a).

Morphologically, FS01 appears to belong to the family Siphoviridae, characterized by flexible tails and double-stranded DNA (Harper *et al.* 2021), similar to previously described phages such as fmb-p1 (Wang *et al.* 2017), supporting its taxonomic classification.

In terms of environmental stability, FS01 exhibited thermal resistance between 40 ºC and 60 ºC for 30 minutes, losing viability at higher temperatures due to protein denaturation and genetic damage (García-Salazar & Porras-Nicho, 2018, Thung *et al.* 2019). Regarding pH tolerance, it remained stable between 6 and 9, tolerated moderate alkaline conditions (pH 10–13) with only a one-log reduction, but was highly sensitive to acidic pH, showing a six-log reduction at pH 2 consistent with findings reported for other phages (Jung *et al.* 2017, Cao *et al.* 2022).

Furthermore, FS01 was significantly affected by chloroform (40% reduction, $p < 0.05$), suggesting interaction with lipid components of the viral particle (Hyman 2019, Oluwarinde *et al.* 2024). It was extremely sensitive to UV radiation, exhibiting a four-log reduction in viability within one minute, contrasting with resistant phages like T7 (Wdowiak *et al.* 2023).

Finally, FS01 significantly inhibited the growth of *S. typhimurium* within 3 hours post-infection (~1 log reduction), with the effect lasting at least 12 hours ($p < 0.05$), results comparable to those obtained by Yan *et al*. (2020) and Islam *et al*. (2020b). However, phage cocktails have demonstrated greater efficacy (>2 log reduction in 4 h) and reduced emergence of resistant mutants (Pereira *et al.* 2016).

The main contribution of this work lies in the isolation and detailed characterization of FS01, highlighting its biological properties, stability profile, and infection dynamics. Its specificity, rapid replication kinetics, and controllable sensitivity offer advantages for targeted applications against *Salmonella typhimurium*. Nonetheless, its susceptibility to certain environmental factors limits its use under extreme conditions, warranting the evaluation of protective formulations or combinations with other phages to enhance its versatility.

Conclusions

Bacteriophage FS01, isolated from wastewater, exhibits promising biological properties for biocontrol applications against *S. typhimurium*, particularly in food safety contexts. Its lytic capability, stability under moderate temperature (≤60 ºC) and pH (6-9) conditions, along with sustained bacterial growth inhibition for 12 hours, support its potential use in phage-based formulations for decontaminating surfaces, ingredients, or ready-to-eat products. To facilitate practical implementation, future studies should evaluate: (1) efficacy in real food matrices, (2) performance in multivalent phage cocktails, (3) formulation development to enhance stability against adverse conditions (e.g., encapsulation or lyophilization), and (4) impacts on beneficial microbiota and resistance emergence. Such investigations will establish its technological viability, sustained efficacy, and regulatory compliance for food industry applications.

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