JMID/
Journal of Microbiology and Infectious Diseases

2023; 13 (1):38-46
doi: 10.5799/jmid.1265400

RESEARCH ARTICLE

Characterization of SP-T1 Phage for Potential Biological Control of *Salmonella enteritidis*

Melih Enes Altinok[1], Mustafa Ture[2], Ayse Cebeci[2], Ilhan Altinok[3]

[1]*Trabzon Science High School, 61040 Ortahisar, Trabzon, Turkey*
[2]*Fish Health Department, Central Fisheries Research Institute, Yomra, Trabzon, Turkey*
[3]*Department of Fisheries Technology Engineering, Faculty of Marine Science, Karadeniz Technical University, Surmene, Trabzon, Turkey*

ABSTRACT

Objectives: *Salmonella enteritidis* is a pathogen that causes gastroenteritis and septicemia in humans and animals, causing worldwide health implications. This pathogen often causes fatal foodborne diseases in humans and animals. In recent years, new treatment strategies for bacterial infections have been studied. Bacteriophages might be used as an alternative strategy for battling against bacterial diseases.

Methods: A novel SP-T1 phage was isolated, and its lytic activity against *Salmonella enteritidis* strains and other enteric bacterial species was evaluated. In addition to its kinetic and genetic characteristics, it is in vivo cytotoxicity and ability to inhibit the development of biofilms were investigated.

Results: The gene sequence of the major capsid protein confirmed that the SP-T1 phage belonged to the family *Siphoviridae*. There was no evidence that phage had a lytic effect on bacteria other than the type of *Salmonella* used in this investigation. The phage exhibited a short latent period of 15 to 20 min and a burst size of 10 plaque forming unit per infected cell. The phage was resistant to temperatures between 15 and 37 °C and pH levels between 5 and 11. The SP-T1 phage exhibited no adverse effects on EPC cell culture and reduced biofilm formation.

Conclusions: Because of its short latent period, appropriate burst size, ability to inhibit biofilm formation, and sensitivity to various temperatures and pH levels, the phage found in this study would be an excellent candidate for phage therapy. These findings are beneficial for improving the microbiological safety and quality of food and creating efficient bacteriophage-based regulation in the food chain. *J Microbiol Infect Dis 2023; 12(4):38-46.*

Keywords: *Biofilm formation, Lytic bacteriophages, Salmonella enterica serovar Enteritidis*

INTRODUCTION

Etiological agents of *Salmonella* are serotypes of *Salmonella* species that are members of the *Enterobacteriaceae* family. Salmonellosis, after campylobacteriosis, is the second most prevalent zoonotic disease in the EU, and *Salmonella* is a significant cause of foodborne disease outbreaks [1]. *Salmonella enterica serovar Typhimurium* and *S. enterica serovar Enteritidis* are the most common *Salmonella* serovars. These bacteria are one of the primary causes of foodborne illness, and they may survive for years in conditions such as dry surfaces, plastics, and dry foods [2]. People become infected by ingesting contaminated drinks and food items and coming into contact with an infected or carrier person. In addition, the expense of treating *Salmonella*-related infections causes financial concerns for governments [3]. Every year, roughly 93.8 million cases of foodborne *Salmonella*-related disease and 155,000 deaths are documented worldwide [4].

Correspondence: Ilhan Altinok, Department of Fisheries Technology Engineering, Faculty of Marine Sciences, Karadeniz Technical University, Trabzon, Turkey
E-mail : Email: ialtinok@gmail.com
Received: 11 December 2022 Accepted: 18 February 2023

Phages are considered an alternative treatment to commercial antibiotics as they exhibit bactericidal characteristics, are simple to manufacture, and are relatively inexpensive [8]. The United States Department of Agriculture (USDA) and the Food and Drug Administration (FDA) have authorized the use of bacteriophages on ready-to-eat beef and poultry products and confirmed that they are generally regarded as safe [5]. The use of bacteriophage-based control to improve the microbiological safety of food is a promising method [6]. Foodborne diseases pose a significant threat to global public health. In the food sector, pathogenic bacteria may exist as biofilms, becoming a constant source of cross-contamination. Most pathogenic bacteria associated with the foodborne disease can adhere to and form biofilms on a wide range of surfaces, including wood, metal, and plastic. These surfaces can be cleaned with appropriate disinfectant, but some bacterial biofilms are resistant to disinfectant [7].

On the other hand, meat, poultry products, fruits, and vegetables such as lettuce and parsley can be contaminated with foodborne bacteria and cannot be cleaned with disinfectant. In this context, there has been a rise in interest in utilizing bacteriophages as possible biofilm-attacking agents. Therefore, the objectives of this study were to isolate a novel *Salmonella enteritidis*-specific lytic bacteriophage and to determine its phenotypic, kinetic, genetic, and biofilm-inhibiting properties.

METHODS

Isolation and Enrichment of Bacteriophage

The bacteriophage isolation, detection, and enrichment method was modified from that published by [8]. *Salmonella* enterica subsp. *enterica serovar Enteritidis* (*Salmonella enterica*, ATCC 13076) was used as the host strain for bacteriophage isolation. First, 50 mL of water from the municipal sewage system of Trabzon, Turkey, was centrifuged at 4000 x g for 20 minutes before being filtered through a 0.22 µm membrane filter (Isolab, Germany). Then filtered water was mixed with 50 mL of tryptic soy broth (TSB, double concentration), and 1 mL of an overnight culture of *S. enteritidis* was added to the combination and incubated in a shaking incubator for 24 hours at 30°C. After incubation, the sample was centrifuged for 20 minutes at 4000 x g before filtered through a 0.22 µm filter to remove any residual bacterial cells. This process was repeated three times to boost the bacteriophage concentration. Spot testing confirmed the presence of bacteriophages infecting *S. enteritidis*. In this case, 0.1 mL of *S. enteritidis* culture (10^8 CFU/mL) was mixed with 5 mL of soft tryptic soy agar (TSA) held at 40°C and spread throughout the surface of solidified hard TSA. The plate was dried at room temperature for about 30 minutes. When the top agar solidified, 15 µl of the filtrate was put over the surface of the *S. enteritidis* culture. After 24 hours of incubation at 30 ^{0}C, the plate was assessed for the formation of plaques. According to the International Committee on the Taxonomy of Viruses (ICTV), the isolated novel phage was named *Salmonella* phage (SP-T1), providing the host genus and strain designation [9].

Titer Determination

The SP-T1 phages were diluted with distilled water between 101 and 106 times using the dilution procedure. The diluted phage was mixed with *S. enteritidis*, and the phage titer was calculated as a plaque forming unit (PFU)/mL after overnight incubation at 30°C using the double-layer agar (DLA) method.

Phage Sensitivity at Different Temperatures And pHs

Phage stability at different temperatures (15, 25, and 37 °C) and pH (3, 5, 7, 9, and 11) was tested using the method reported by Ture et al. [15]. The pH of the TSB medium was adjusted using 1M HCL or 1M NaCl to reach the proper value. Pre-incubating phage suspensions tested phage sensitivity to pH and temperature at the appropriate pH and temperature for one hour, and surviving phages were determined using the DLA procedure.

Host Range

The lytic activity of the SP-T1 bacteriophage against five different *S. enteritidis* strains, *Escherichia coli, Aeromonas hydrophila, Citrobacter gillieni, Lactococcus garvieae*, and *Enterococcus faecalis* was studied. After spreading 50 µl (about 10^8 CFU/mL) of each overnight culture of bacteria grown in TSB onto TSA, 15 µl of phage (10^8 PFU/mL) was added to the solid agar and incubated overnight at 30°C. This experiment was done twice for each type of bacteria [10]

Phage Adsorption Rate

Overnight culture of *Salmonella enteritidis* was infected with SP-T1 bacteriophage at a multiplicity of infection (MOI) of 0.1 and then incubated at 30 °C. After 0, 5, 10, 15, 20, 25, and 30 min post-incubation, samples from the host–phage mixture were collected and centrifuged at 10,000 xg for 5 min. The supernatant was diluted with 1% chloroform containing distilled water. A spot assay was used to quantify the phage concentration in the supernatant [10].

The One-step growth Curve

Salmonella enteritidis overnight culture was centrifuged at 4,000 xg for 5 minutes, and the pellet was suspended in 1 mL of TSB medium. The suspension was then mixed with SP-T1 phage at a MOI of 0.1 before being incubated for 15 minutes at 30°C. Next, the mixture was centrifuged at 10,000 xg for 5 min, and the pellet was resuspended in 1 ml TSB and kept at 30°C to remove unattached phage particles. The phage was determined after collecting samples every 5 minutes for 1 hour using a spot assay. A one-step curve analysis was used to determine the burst size and latent period [8].

Toxic Effect of The Phage on The Cell

The Epithelioma Papulosum Cyprini (EPC) cell line was used as a model to investigate the cytotoxic effect of SP-T1 phage. In a 24-well plate, EPC isolates were seeded with minimum essential medium (MEM, Sigma) supplemented with 1% penicillin-streptomycin and 10% fetal calf serum and incubated at 22°C for 24 hours. The cells were subsequently infected with 100 µl of SP-T1 phage diluted to 1x104-107 PFU/ml and incubated at 22°C for 24 hours. The presence of CPE was studied in both the control and phage-containing groups. Following washing, 500 µl of 0.25% trypsin-EDTA solution (0.5 mM EDTA-tetra sodium) was added to each well and incubated at 22°C for 5 min to separate the cells. Next, ten microliters of cell suspension were incubated with ten liters of trypan blue solution for two minutes. An inverted light microscope was used to count the total number of cells on a Thoma slide at 100x magnification (Olympus, CK30) (Olympus, CK30). Then cell concentration and vitality were determined [8].

Effect of Phage on Biofilm Formation

The effect of the SP-T1 phage on biofilm formation by *S. enteritidis* was evaluated using the colorimetric method reported by Islam et al. [2] with slight modifications. In brief, 200 µl of *S. enteritidis* in TSB was inoculated onto a 96-well plate at a final concentration of 10^8 CFU/mL and incubated for 72 hours at 30 °C. Following incubation, phage was introduced to the bacterial solution at final titers of 10^8 and 10^9 PFU/mL, resulting in biofilms treated with phage SP-T1 at two different titers. In the control group, distilled water (DW) was used instead of phage lysate. The samples were incubated at 30 °C for another 15 hours. Following phage treatment, each well was gently washed four times with DW and allowed to dry in the laminar air-flow cabinet. Following the wash, pure methanol was added and kept for 10 min. Then, the methanol was pipetted away, and the plates were allowed to dry similarly. Afterward, 250 µL of 1% crystal violet solution was added to each well and left for 30 minutes. The crystal violet was removed after incubation, and 250 µL of 33% acetic acid was added. A spectrometer (ELISA Reader, Biotek) was used to measure the OD values of phage-treated and control samples at a wavelength of 600 nm.

Phage Genome Isolation And Restriction Endonuclease Analysis

SP-T1 phage was treated with DNase I and RNAse A at 37°C for 30 min. The phage's genomic DNA was extracted and purified reported by Ture et al. [10]. Electrophoresis and spectrophotometry were used to assess the purity and concentration of the extracted DNA, respectively. The restriction enzymes EcoRI, HindIII, and BamHI followed the manufacturer's instructions to perform a restriction fragment length polymorphism (RFLP) (Promega, Thermofisher). The phage genome was treated with RNAse A, DNAse I, and S1 endonuclease for one hour at 37°C (Thermofisher) to illustrate the structure of the phage genome. The DNA fragments were separated on an agarose gel stained with SYBR Green dye in Tris–Acetate–EDTA solution. The phage genome size was estimated by comparing the combined fragment sizes to the 1 Kb Plus DNA Ladder (Thermofisher). The DNA fragments were separated on a 1% agarose gel stained with SYBR Green dye in Tris–Acetate–EDTA solution. The phage genome size was calculated by comparing the combined

fragment sizes to the 1 Kb Plus DNA Ladder (Thermofisher).

Genetic and Phylogenetic Characterization

SP-T1 phage DNA polymerase gene was amplified with JerDP1 primers [16] (F-5' ACATCAAAACGGCGGTGTGC 3', R-5' AGCATGGCTAAGGATAAACG). AmpliTag Gold 360 Master Mix (Thermo Fisher Scientific) was used under the following PCR conditions: initial denaturation at 95 °C for 10 minutes, denaturation at 95 °C for 30 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 1 minute in 35 cycles, and final extension at 72°C for 10 minutes. A 1.5 percent (w/v) agarose gel stained with SYBR Green dye in 1TBE buffer was used to evaluate the PCR result. The purified PCR product was directly sequenced on an ABI PRISM 3500 Genetic Analyzer with the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) per the manufacturer's instructions. A partial DNA polymerase gene sequence was blasted for similarity (https://blast.ncbi.nlm.nih.gov/Blast.cgi). A phylogenetic tree was created based on the DNA polymerase gene using a sequencing of the phage and several phages from three families obtained from GenBank. CLUSTAL W was used for multiple sequence alignment, and the BIOEDIT program (version 7.1.11) was used to refine the alignment [17,18]. A bootstrap analysis with 1000 repeats was conducted to assess the dependability of the tree topology [19].

Statistical analysis

The paired t-test and one-way ANOVA were used for statistical analysis. When significant differences existed between samples, the Tukey test was used to identify them. All statistical analyses were conducted using the SPSS (SPSS Inc., Chicago, IL, USA) software with a significance threshold of $P \leq 0.05$.

RESULTS

A novel SP-T1 phage infecting *S. enteritidis* was isolated in a wastewater treatment plant. The SP-T1 phage was confirmed to be a lytic phage since it formed distinct plaques with a diameter of 0.3 to 0.5 mm on *S. enteritidis* lawn on TSA. Approximately 10^9 PFU/mL of phage were obtained as a consequence of the enrichment procedure (Figure 1).

SP-T1 phage demonstrated lytic activity exclusively against all *S. enteritidis* strains but not against *Escherichia coli, Aeromonas hydrophila, Citrobacter gillenii, Lactococcus garvieae*, or *Enterococcus faecalis*.

The thermal stability of the SP-T1 phage was evaluated between 15 and 37℃. The titer of the SP-T1 phage incubated for 1 hour at various temperatures did not vary significantly (Figure 2). The ideal pH for the SP-T1 phage was identified by examining its stability from pH 3 to pH 11 in TSB. The viability of phages did not vary between pH 5 and pH 11 (Figure 3). Only at pH 3 the activity drops by 25% relative to other pH levels. The result revealed that the SP-T1 phage had a high rate of adsorption. About 70% of the phage particles were adsorbed after 10 minutes, and over 90% were absorbed within 30 min (Figure 4). The infection dynamics of SP-T1 phage were analyzed using a single-step growth curve. Based on the curves, the phage's latent duration and burst size were estimated to be between 15 and 20 minutes and 10 infected cells (PFU per infected cell), respectively (Figure 5).

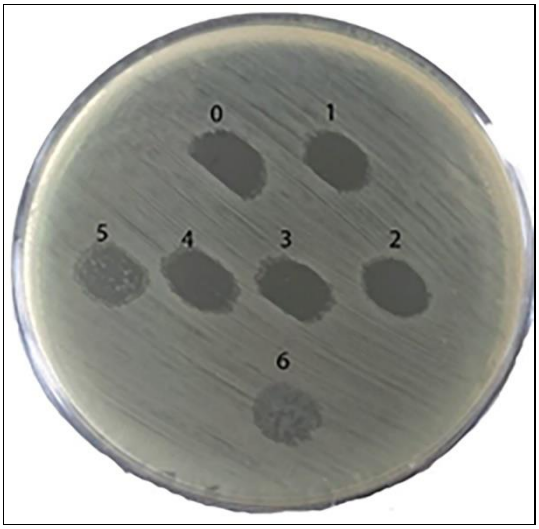

Figure 1. Lytic effect of SP-T1 on *Salmonella enteritidis*. The phage concentration ranges from 10^0 to 10^6. While a value of 0 indicates the greatest concentration that has not been diluted, a value of 6 indicates the most diluted concentration.

SP-T1 phage introduced to EPC cell culture at various dilutions had no adverse effects on EPC cell culture. It did not result in cell death (Figure 6). The SP-T1 phage significantly reduced the existing *Salmonella* biofilm. When 10^8 and 10^9 PFU/mL of phage were added to *Salmonella* biofilms, those biofilms shrunk by 59% and 78% in 12 h, respectively (Figure 7).

SP-T1 phage genome was resistant to the BamHI enzyme but susceptible to the EcoRI and HindIII enzymes. The double-stranded DNA genome of the phage was 40 kbp in size. Amplification of a 700-bp fragment revealed that the SP-T1 phage was a member of the *Siphoviridae* family and belonged to the *Jerseyvirus* genus. A blast search revealed that the SP-T1 phage was 95% identical to other *Salmonella* phages belonging to the *Jerseyvirus* genus. The phylogenetic tree also showed that the phage was more closely related to the *Siphoviridae* family than to the *Podoviridae* or *Myoviridae* families.

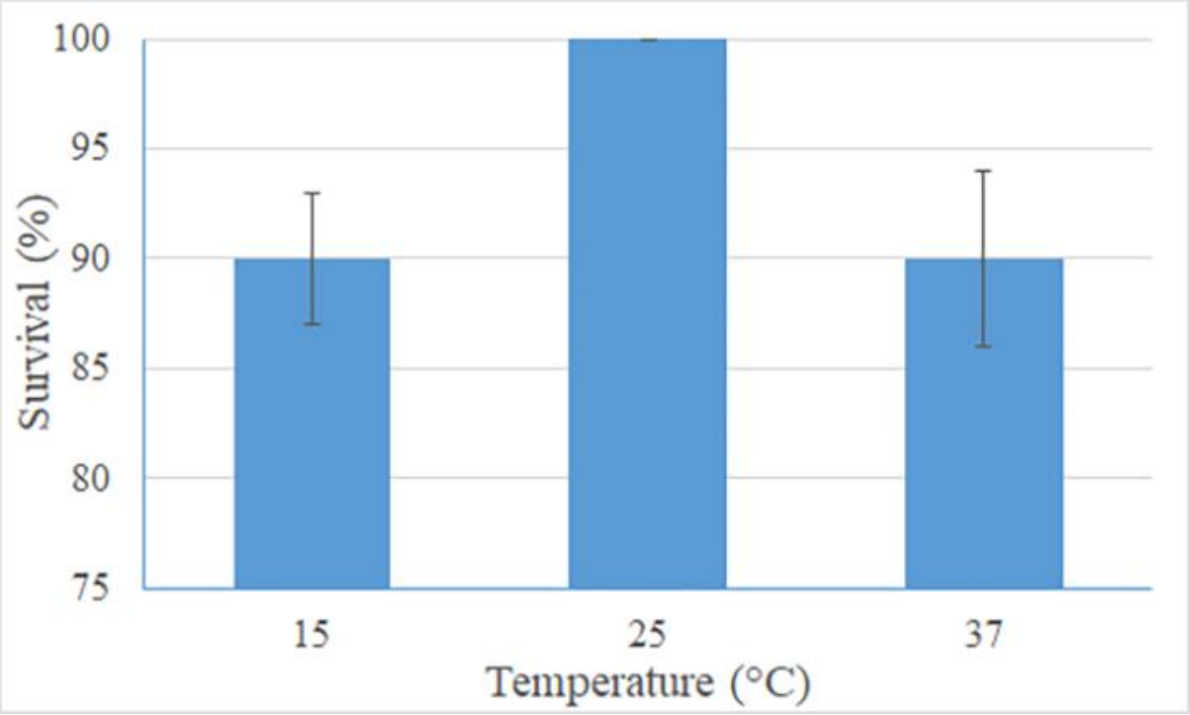

Figure 2. Impact of temperature on the survival of SP-T1 phage.

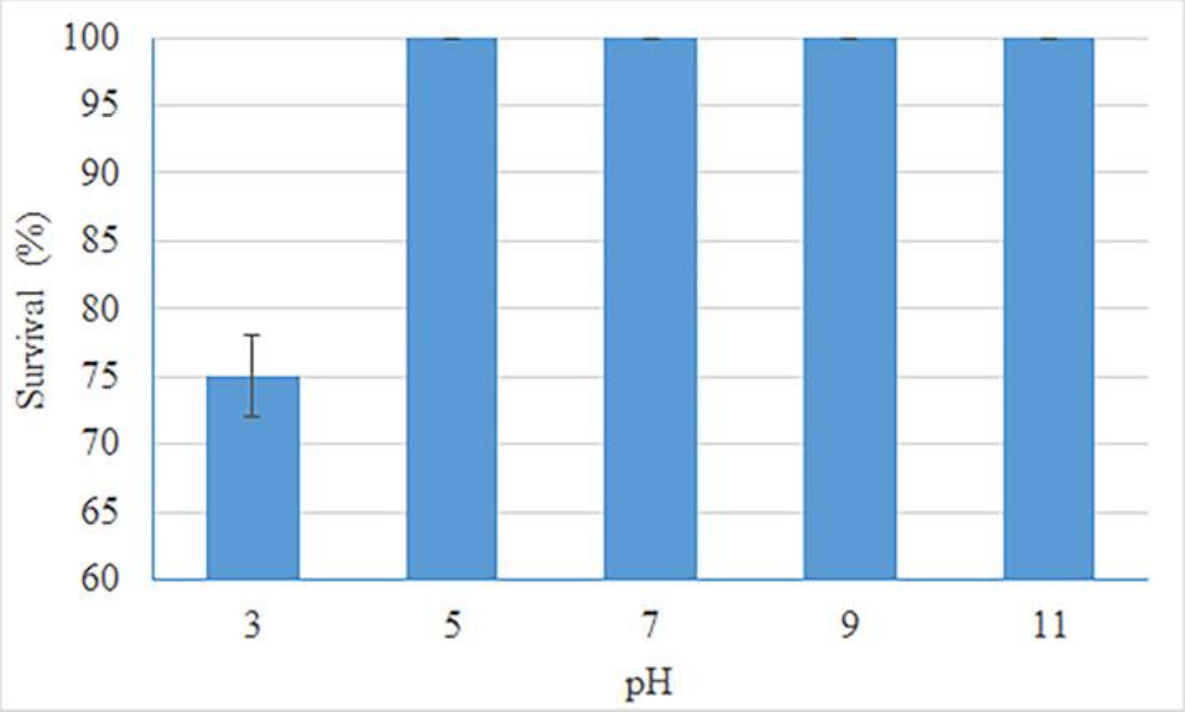

Figure 3. Impact of pH on the survival of SP-T1 phage.

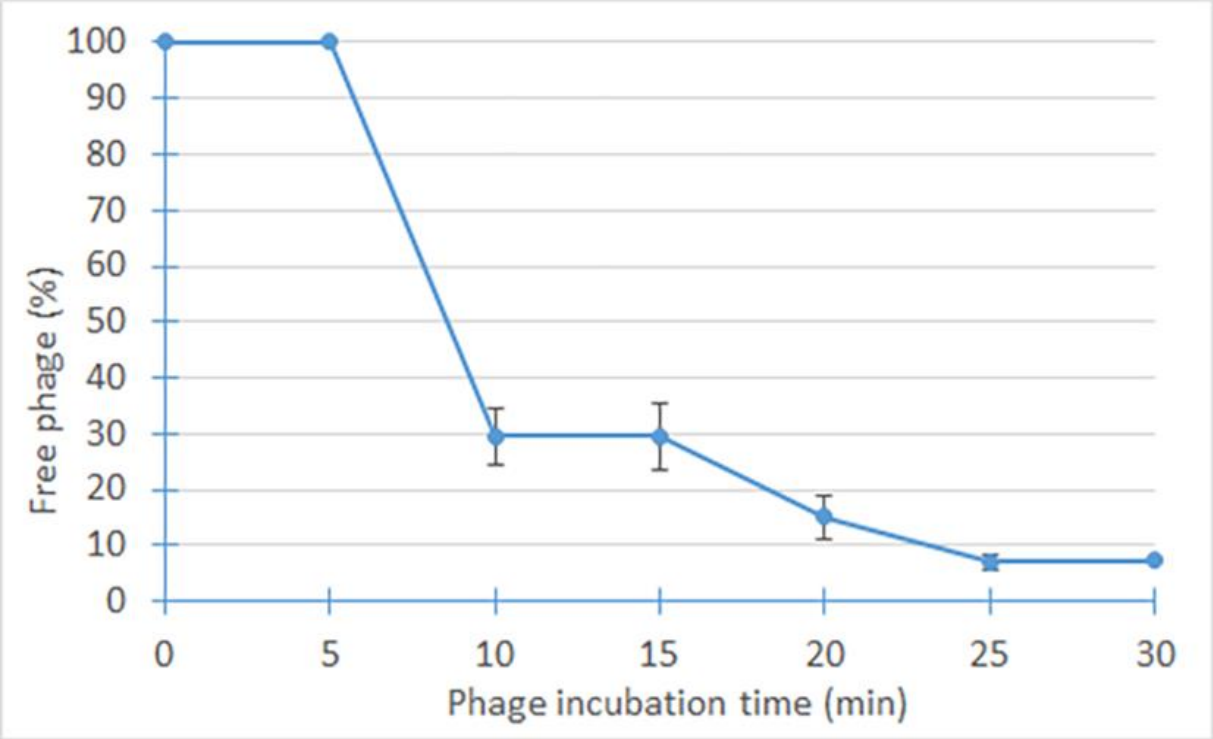

Figure 4. Adsorption rate of phage

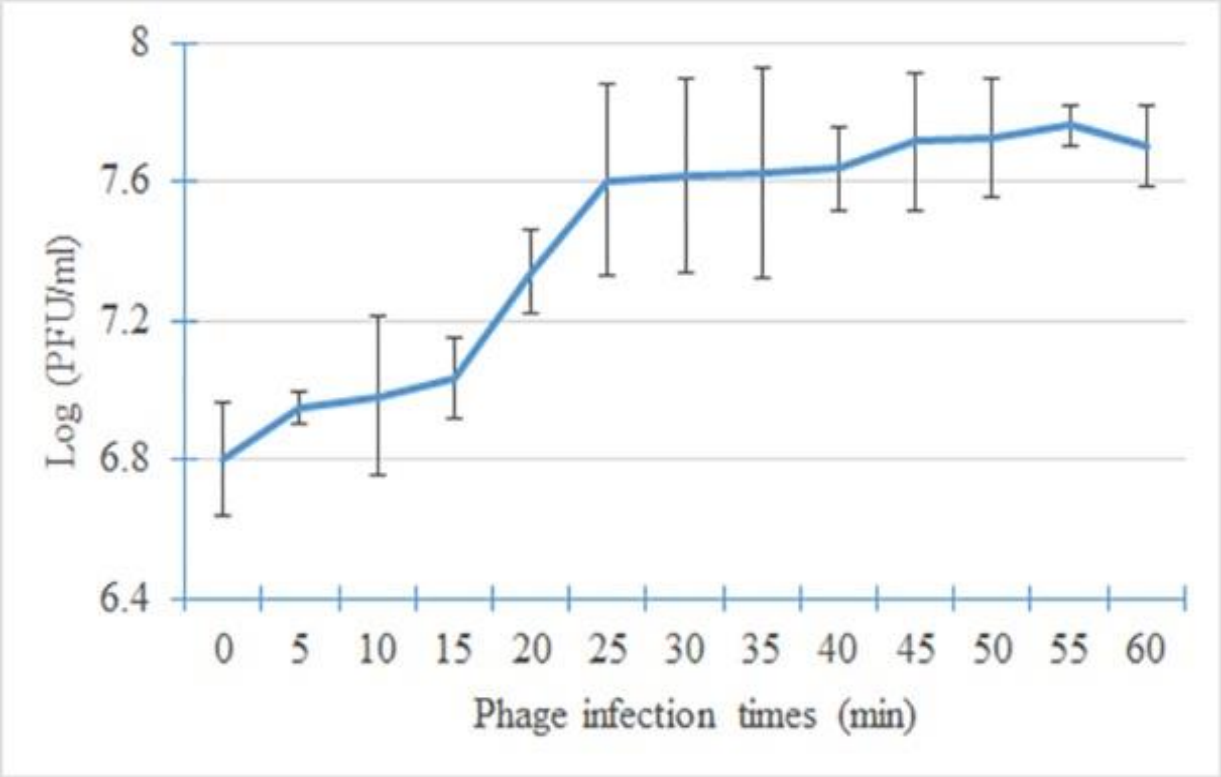

Figure 5. One-step growth curve of phage

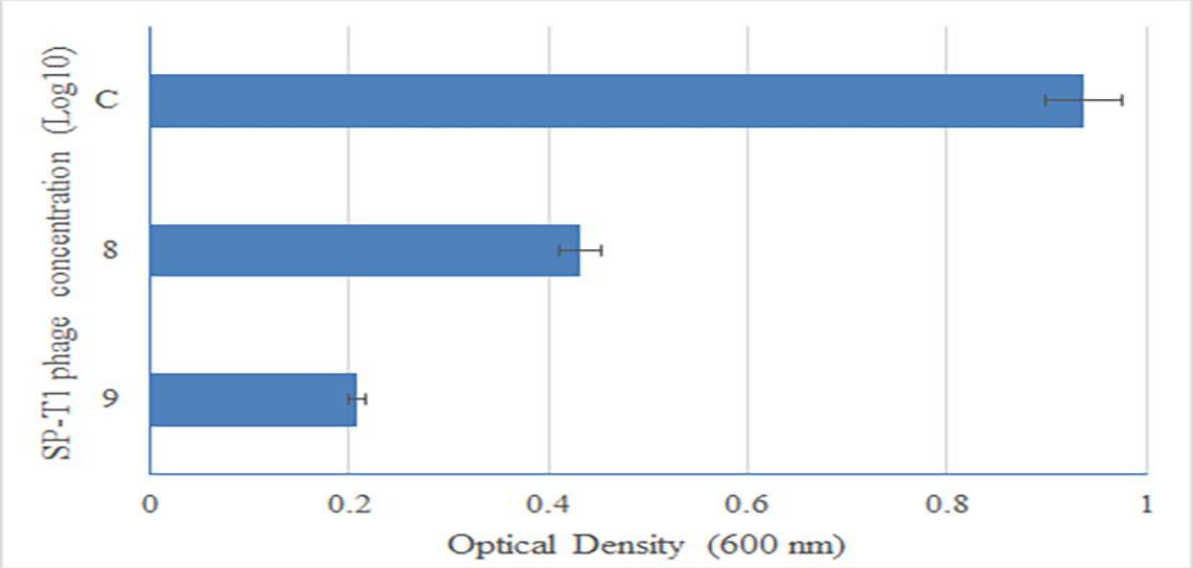

Figure 6. *Salmonella enteritidis* biofilm inhibition 12 hours after $9log_{10}$ and $8log_{10}$ SP-T1 phage infection. C, represents a control in which bacteria were not exposed to phage.

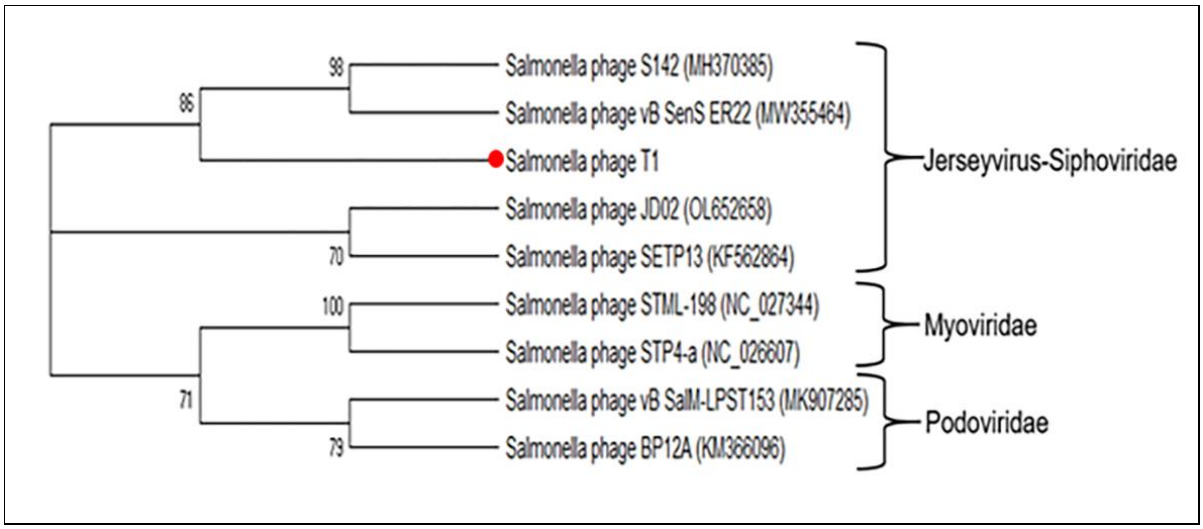

Figure 7. A phylogenetic tree based on the DNA polymerase gene sequences. The Bootstrap values for branches were given as percentages. The phage sequences' GeneBank accession numbers are in parentheses.

DISCUSSION

Infections caused by pathogenic microorganisms are often treated with antimicrobial agents. However, antibiotic overuse in treating a disease may develop antimicrobial resistance in pathogenic and nonpathogenic microorganisms [20,21]. Therefore, SP-T1 phage, a novel, ecologically friendly bacteriophage, was identified within the scope of this study to combat *S. enteritidis*, the causal agent of foodborne *Salmonella* disease in humans.

Phage treatment for biological control and the prevention of bacterial infections has gained favor in recent years [22]. The SP-T1 phage isolated in this study showed potent antibacterial activity against *S. enteritidis*. In addition, the SP-T1 phage-displayed exceptional pH tolerance and heat stability. Recent research indicates that *Salmonella* species might live and procreate in the pH range of 4 to 9 [23]. Therefore, pH does not restrict SP-T1 phage activity. Due to the SP-T1 phage's pH tolerance and non-cytotoxic action on cultured cells, it might be administered orally to treat *Salmonella*-related disorders and survive in the gastrointestinal tracts of humans and animals.

Salmonella enteritidis can grow at temperatures ranging from 15 to 37°C. Variations in relevant

temperatures did not affect SP-T1-phage growth and antibacterial activity. The optimal temperature at which the phage-displayed antibacterial activity was found to be 25°C, while at 15°C and 37°C, the activity of the SP-T1 phage was only lowered by 10%. This circumstance facilitates the use of SP-T1 phage in a variety of conditions.

The most significant characteristic of phages is that they are often species- or even strain-specific [8]. In a prior investigation, the *Salmonella* lytic phage, SS3e, infected a variety of *Salmonella enterica* serovars and other enteric bacteria, including *E. coli, Serratia marcescens*, *Enterobacter cloacae*, and *Shigella sonnei* [24]. In contrast, our study revealed no lytic action against other enteric bacteria, including *A. hydrophila, L. garvieae, C. gillenii,* and *Enterococcus faecalis*. When bacteriophages infect non-target bacteria, they may have a detrimental impact on phage treatment. When administering phage treatment to people, the phage should not infect beneficial bacteria in the digestive system. SP-T1 phage was shown to be efficient exclusively against *Salmonella enteritidis*.

The infection kinetics of the SP-T1 phage was determined by analyzing a single-step growth curve. The latent period and burst size are the time for the first release of phage particles and the ratio of phage titers in the plateau phase to initial phage titers. Based on the curves, it was determined that the latent period of the phages was around 15 minutes. This is a hallmark of the majority of lytic phages identified against *Salmonella enteritidis*.: a time of dormancy [2,24]. In addition, our study determined the burst size of the SP-T1 phage to be 10 PFU per infected cell. There are substantial variances in the lytic phages' burst size values. The burst size of *Salmonella* phage SS3e was reported to be 98 virions per cell in a recent investigation [24]. In our study, phages displayed fast adsorption, with over 90 percent of phage particles being adsorbed within 30 minutes. In general, lytic phages have a quick adsorption rate, which our findings verify.

RFLP analysis indicates that the genomic size of the phage is 40 kb, which is consistent with earlier research on *Salmonella* phages in the *Siphoviridae* family [25].

In this study, a novel phage SP-T1 was obtained with good potential to prevent and control *Salmonella enteritidis*. It has also been shown that this phage can be used as an eco-friendly alternative to antibiotics to prevent the bacterial infection caused by Salmonellosis. In further studies, the SP-T1 phage can be tested in-vivo, and the genome characteristics of the phage can be studied. Introducing SP-T1 phage decreased *Salmonella* biofilm formation. In addition, SP-T1 phage dramatically decreased biofilms adhering to the microplate after 12 hours of activity.

On the other hand, Andreatti Filho et al. [26] observed a decrease in the bacteriophage activity against *Salmonella* samples. The contaminated food contact surface and the microorganism can interfere with bacteria's persistence in manufacturing environments through biofilm formation [23]. Thus, bacteriophage therapy provides an extra option for a more accurate and dependable method. However, its implementation requires more study to understand the link between microorganisms and their environments to make the technology commercially viable.

Conclusion

SP-T1 phage provides potential characteristics for treating foodborne disease-related bacteria and is regarded as efficient in reducing biofilm development and dispersal of mature biofilms. The SP-T1 phage may be used as a spray to prevent or eliminate the existence of *S. enteritidis* biofilms on the surface of equipment or food, such as fruits and vegetables. Because the SP-T1 phage is resistant to low pH, it may also be used orally to treat *S. enteritidis* infection. Thus SP-T1 phage provides a more natural approach than conventional safety and food preservation techniques.

ACKNOWLEDGMENTS

We want to thank the Republic of Turkey Ministry of Agriculture and Forestry Central Fisheries Research Institute (SUMAE), Trabzon, for providing the essential equipment and materials. We would also like to thank Elif Aygur and Nihal Caliskan for their assistance.

Conflict of Interest: The authors declare that there are no conflicts of interest.

Author Disclosure Statement: No competing financial interests exist.

Ethical Approval: Ethical approval was not required.

REFERENCES

1. EFSA. *Salmonella* [Internet]. European Food Science Authority 2022 [cited 2022 May 14]. Available: https://www.efsa.europa.eu/en/topics/topic/*Salmonella*

2. Islam MS, Hu Y, Mizan MFR, Yan T, Nime I, Zhou Y, et al. Characterization of *Salmonella* phage LPST153 that effectively targets most prevalent *Salmonella* serovars. Microorganisms 2020; 8(7): 1089.

3. Coburn B, Grassl GA, Finlay BB. *Salmonella*, the host and disease : a brief review. Immunol Cell Biol 2007; 85: 112-118.

4. Eng SK, Pusparajah P, Ab Mutalib NS, Ser HL, Chan KG, Lee LH. *Salmonella*: A review on pathogenesis, epidemiology and antibiotic resistance. Front Life Sci 2015; 8(3): 284-293.

5. Sulakvelidze A, Alavidze Z, Morris J. Bacteriophage therapy. Vol. 45, Antimicrob Agents Chemother 2001; 45: 649-659.

6. Lee S, Kim MG, Lee HS, Heo S, Kwon M, Kim GB. Isolation and characterization of listeria phages for control of growth of Listeria monocytogenes in milk. Korean J Food Sci Anim Resour 2017; 37(2): 320-328.

7. Thung T, Jayarukshi K, Premarathne K, et al. use of a lytic bacteriophage to control *Salmonella enteritidis* in retail food. LWT- Food Sci Technol 2017; 78: 222-225.

8. Scher K, Romling U, Yaron S. Effect of heat, acidification, and chlorination on *Salmonella* enterica serovar typhimurium cells in a biofilm formed at the air-liquid interface. Appl Environ Microbiol 2005; 71(3): 1163-1168.

9. Ture M, Cebeci A, Altinok I, Aygur E, Caliskan N. Isolation and characterization of Aeromonas hydrophila-specific lytic bacteriophages. Aquaculture 2022; 558: 738371.

10. Adriaenssens EM, Rodney Brister J. How to name and classify your phage: An informal guide. Viruses 2017; 9(4): 70.

11. Ture M, Altinok I, Cebeci A, Caliskan N. Characterization of novel bacteriophage HP-T19 that targets Hafnia alvei. Aquac Res 2022; 53(2): 694-699.

12. Newase SK, Gupta A, Dastager SG, Kapadnis BP, Shashidhar R. Development and evaluation of taxon-specific primers for the selected Caudovirales taxa. Virus Res 2019; 263: 184–188.

13. Thompson JD, Higgins DG, Gibson TJ. Clustal W. Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 1994; 22(22): 4673–4680.

14. Hall TA. BIOEDIT: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/ NT. Nucleic Acids Symp Ser 1999; 41: 95-98.

15. Felsenstein J. Confidence Limits on Phylogenies: An Approach Using the Bootstrap. Evolution (N York) 1985; 39(4): 783-791.

16. Capkin E, Terzi E, Altinok I. Occurrence of antibiotic resistance genes in culturable bacteria isolated from Turkish trout farms and their local aquatic environment. Dis Aquat Organ 2015; 114(2): 127-137.

17. Ture M, Altinok I, Alp H. Effects of cage farming on antimicrobial and heavy metal resistance of Escherichia coli, Enterococcus faecium, and Lactococcus garvieae. Microb Drug Resist 2018; 24(9): 1422-1430.

18. Doss J, Culbertson K, Hahn D, Camacho J, Barekzi N. A review of phage therapy against bacterial pathogens of aquatic and terrestrial organisms. Viruses 2017: 9; 50.

19. Acevedo-Villanueva KY, Akerele GO, Hakeem WG Al, Renu S, Shanmugasundaram R, Selvaraj RK. A novel approach against *Salmonella*: A review of polymeric nanoparticle vaccines for broilers and layers. Vaccines 2021; 9: 1041.

20. Kim S, Kim SH, Rahman M, Kim J. Characterization of a *Salmonella enteritidis* bacteriophage showing broad lytic activity against Gram-negative enteric bacteria. J Microbiol 2018; 56(12): 917-925.

21. Kang HW, Kim JW, Jung TS, Woo GJ. wksl3, a new biocontrol agent for Salmonella enterica serovars Enteritidis and *typhimurium* in foods: Characterization, application, sequence analysis, and oral acute toxicity study. Appl Environ Microbiol 2013; 79(6): 1956-1968.

22. Andreatti Filho RL, Higgins JP, Higgins SE, et al. ability of bacteriophages isolated from different sources to reduce *Salmonella enterica serovar Enteritidis* in vitro and in vivo. Poult Sci 2007; 86(9): 1904-1909.