JOURNAL OF WOMEN MEDICAL & DENTAL COLLEGE

# Discovery and Analysis of Novel Phage DSI29 with Potent Activity Against *Escherichia coli*

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**Received date:** 16-07-2025 **Publication date:** 28-07-2025

## Abstract

*Escherichia coli* is a common foodborne pathogen that can result in life-threatening diseases and represents a serious burden to food safety management. With the advent of the post-antibiotic era, bacteriophages have generated great attention as promising biocontrol agents for their selective action against bacteria and lysis them. Here, we describe the isolation and charcaterization of a new *E. coli*-infecting phage DSI29, isolated from a freshwater sample in Joypurhat, Bangladesh. DSI29 phage has a wide host range and a lytic performance to *E. coli*, implying that the phage is applicable for food safety. Subsequent work revealed its stability to different pH and temperatures, rendering it appropriate for a broad range of environments. Because of its pure lytic lifestyle and powerful bacteriolytic activity, the DSI29 has promising potential as a biocontrol agent in food manufacturing systems. These results reveal the potential of phage therapies in controlling *E. coli* contamination, a sustainable alternative to conventional antibiotics.

**Keywords** Phage; DSI29, Escherichia, Characterization, Biological control, Foods

## 1. Introduction

*E. coli* O157:H7 is one of the most infamous serotypes worldwide and linked to foodborne disease outbreaks. It is a member of the gram-negative, heterotrophic bacteria and is well adapted for life in calorie-dense environments, such as the mammalian intestine and feces [1]. Outbreaks have been linked to the consumption of raw or undercooked animal products, such as raw milk and beef [2,3]. Water and food being contaminated and animal contact etc is reasons for how *E. coli* O157:H7 can be passed on. Outbreaks with these symptoms are known to have secondary transmission between the cases [4,5]. *E. coli* O157:H7 has been linked to outbreaks in America, Canada, Japan, the United Kingdom, China, and Germany and is known to have started initially in the US [6,7].

Pathogens that can be acquired from food are a major concern within the entirety of the food production process. Of particular importance, is the development of drug resistance to these pathogens which is often caused by the misuse or overuse of antibacterial agents [8]. After such pathogens have developed resistance, they are capable of spreading along the entire food supply chain obstructing efforts directed towards food safety and exacerbating risks for consumers. It is crucial to remember that antibacterial drugs comprise both naturally produced antibiotics from microorganisms and synthesized antimicrobial chemical compounds. Increasingly, research has shown the continuum of food production to have a greater wound presence of antibiotic and disinfectant resistant foodborne pathogens such as *E. coli* [9-11]. Based on such issues, there is growing interest in biological controls as alternative methods for

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https://doi.org/10.56600/jwmdc.v3i1.111

controlling *E.ou soon coil*. The aim is to adopt control strategies that effectively minimize pathogen loads without adding to the burden of antimicrobial resistance.

Phages are natural antibacterial agents that can be utilized to control food deterioration and harmful bacteria. From farm to table, they work well at every point of the food production chain [12]. Currently, the FDA has designated many commercially available phage products for food applications as generally recognized as safe (GRAS), including SalmoFreshTM, ListShieldTM, and PhageGuard STM [13]. To address more phage-host-food product combinations and offer more defined phages exhibiting biocontrol potential for food application, more study is necessary. The purpose of this study is to isolate, identify, and assess the effectiveness of phage DSI29 as a zoonotic E. coli control method in a variety of foods.

## 2. Materials and Method

### 2.1. Bacteria strains and growth conditions

The host used to isolate the phages was *E. coli* ATCC 25922. In order to evaluate the phages' host and lytic range, 14 different strains of E. coli (listed in Table 1) were used. These strains were kept in a 20% (v/v) glycerol solution at -80 °C. All bacterial strains were first cultivated using the streak plate method on Luria-Bertani (LB) agar plates at 37 °C before investigations began. To verify the purity of the bacterial stocks, isolated colonies were then cultured for an entire night at 37 °C in LB broth. The double-layer agar plate method, which included a 0.7% agar overlay on a 1.5% agar base, was then used to determine the phage titer.

### 2.2. Phage isolation and purification

Using well-established techniques, Phage DSI29 was isolated from freshwater samples taken from a river in Joypurhat, Bangladesh [14]. The phage isolation enrichment were done as described by [15], with modification. This was achieved through the inoculation of 2.5 mL of the host bacterial strain, 10 mL of LB broth, and 5 mL of a 0.22 µm filtered sample. This mixture was incubated at 37 °C overnight after that. Following incubation, the bacteria were pelleted by centrifugation (10,000 rpm, 10 min, 4 °C) and the resulting supernatants were filtered through a 0.22 µm filter. The existence of phages was subsequently verified by spot tests using this collected supernatant, also known as phage lysate. To guarantee the purity of the phage isolate, any phage plaques that emerged were carefully selected, and each plaque was propagated at least three times. The phage propagation process was carried out using previously known techniques [16]. 100 µL of the host bacterium (*E. coli* ATCC 25922) culture and 100 µL of the phage stock (about 9 log PFU/mL) were combined in 50 mL of LB broth to create high-titer phage stocks. At 37 °C, this mixture was incubated for 12 hours. After centrifuging the culture for 10 minutes at 10,000× g, the supernatant was filtered using a disposable, sterile 0.22 µm syringe filter. Until they were required, the purified phages were kept at 4 °C [17].

### 2.3. Lytic Spectrum Determination by Spot Test

A spot test was used to assess the phages' lytic capacity against different bacteria. In short, bacterial lawns were spotted with 5 µL aliquots of phage lysates. The 14 *E. coli* strains indicated above were cultured overnight in LB medium containing 0.7% agar to create these lawns, which were then placed on top of LA plates. After spotting of the phage lysates, the plates were incubated at 37 °C over night. Bacteriolytic activity was exhibited as a clear lysis zone in a bacterial lawn.

### 2.4. Determination of Host Range by Efficiency of Plating (EOP)

Efficiency of plating (EOP) of phage DSI29 after we identified phage DSI29 for its broad lytic spectrum in spot tests and high ability to lyse in killing experiments, performed further study to determine its EOP. The EOP, which is also referred to as the phage's host range, was analyzed using modified methods from previous reports [18]. The tested bacterial strains were grown at 37 °C until they reached their exponential phase. In double-layer plate experiments, 100 µL of the diluted phage lysate was mixed with 100 µL of the bacterial culture after incubation. In this investigation, dilution factors between $10^6$ and $10^9$ were used. The number of plaques was counted after the plates were incubated for eight hours at 37 °C. The relative EOP was subsequently calculated using the formula: (average PFU on test bacteria / average PFU on host bacteria). The average EOP value was classified as EOP 0.5 to 1.0, high efficiency; EOP 0.1 to <0.5, moderate efficiency; 0.001 to <0.1, low efficiency; and <0.001, inefficient.

**Table 1. Host range of phage DSI29**

| Bacteria | Strains | DSI29/Spot Test | DSI29/EOP | Sources |
|---|---|---|---|---|
| *E. coli* | ATCC 25922 | + | 1 | ATCC |
| *E. coli* | ATCC 43890 | + | 1 | ATCC |
| *E. coli* | BL21 | + | 1 | Lab stock |
| *E. coli* | ATCC 43890 | + | 0.9 | Lab stock |
| *E. coli* | CB1 | + | 1 | Lab stock |
| *E. coli* | CB2 | + | 1 | Lab stock |
| *E. coli* | CB3 | + | 1 | Lab stock |
| *E. coli* | CB4 | + | 1 | Lab stock |
| *E. coli* | CB5 | + | 1 | Lab stock |
| *E. coli* | CB6 | + | 1 | Lab stock |
| *E. coli* | CB7 | + | 0.9 | Lab stock |
| *E. coli* | CB8 | + | 1 | Lab stock |
| *E. coli* | CB9 | + | 1 | Lab stock |
| *E. coli* | CB10 | + | 1 | Lab stock |

EOP 0.5 to 1.0, high efficiency; EOP 0.2 to <0.5, moderate efficiency; 0.001 to <0.2, low efficiency; and <0.001, inefficient. ATCC, American Type Culture Collection.

### 2.5. pH Tolerance of phage DSI29

100 µL of phage suspension (7 log PFU/mL) was introduced separately to 900 µL aliquots of LB medium in order to evaluate the pH stability of the phage. NaOH or HCl were used to pre-adjust these LB aliquots to pH values between 2 and 13. The phage titer was measured after the resultant mixes were incubated for 60 minutes at 37 °C.

### 2.6. Temperature Tolerance of phage DSI29

By warming 500 µL of phage suspension (7 log PFU/mL) to temperatures between 30 and 80 °C, the thermal stability of the phage was evaluated. After 30 and 60 minutes of incubation, aliquots of the samples were obtained for counting. The samples were incubated for 60 minutes. The double-layer agar method was used to determine the phage stock titer.

### 2.7. Statistical analysis

Three separate biological studies, each with three technical replicates, provided data for the final study. Prism 6.01 for Windows (GraphPad software, San Diego, CA, USA) was used to perform statistical analysis. A nonparametric one-way analysis of variance (ANOVA) and Bonferroni's multiple-comparison posttest were used for multivariate comparisons. Statistical significance was defined as a significance level of $p < 0.05$.

## 3. Results

### 3.1. Phage isolation and characterization

Using *E. coli* ATCC 25922 as the host strain, phages were effectively isolated from freshwater samples obtained in Joypurhat, Bangladesh. The isolated phage, known as DSI29, showed the capacity to stop *E. coli* ATCC 25922 from growing on double-layered agar plates. Visual examination additionally verified that phage DSI29 generated transparent plaques on an *E. coli* ATCC 25922 strain lawn (Figure 1).

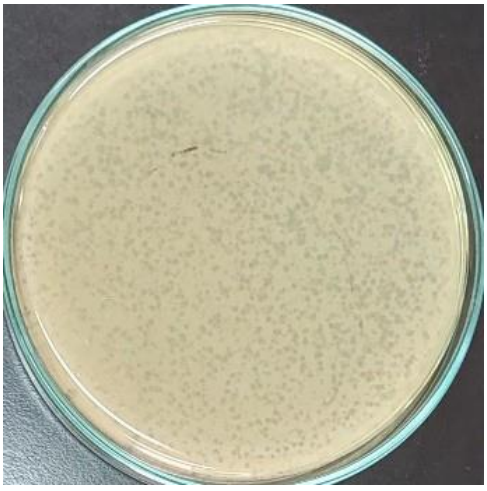

**Figure 1.** Phage plaques for morphology

### 3.2. Lytic ranges of candidate phages DSI29

Freshwater samples taken from a river in Joypurhat, Bangladesh, yielded phage DSI29. This isolated phage showed that it could efficiently lyse its host throughout the purification procedure. Phage DSI29 was evaluated against 14 typical strains of *E. coli* during spot testing (Table 1). Interestingly, all 14 of these *E. coli* strains were lysed by DSI29. Phage DSI29 demonstrated notable lytic activity against a range of *E. coli* strains, according to further Efficiency of Plating (EOP) tests (Table 1). The high plating efficiency was indicated by the EOP values, which ranged from 0.9 to 1.0 (Table 1). These thorough host range investigations show that phage DSI29 has a wide host range and can effectively lyse every strain of *E. coli* that was investigated in this work, including those that are frequently linked to food contamination. These encouraging findings led to the selection of phage DSI29 for additional characterization.

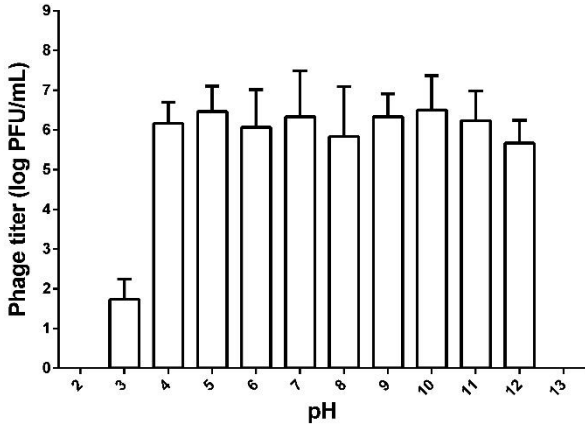


**Figure 2.** pH stability of Phage DSI29.

### 3.3. Temperature and pH stability test DSI29

Phages are interesting biocontrol agents for food production since they are typically resistant to environmental stressors like pH, temperature, salt, and conventional disinfectants. Therefore, we looked at whether phage DSI29 might remain infectious at different pH and temperature levels. Phage DSI29 remained comparatively stable across a wide pH range of 4 to 12, according to our pH stability tests (Fig. 2). But when incubated at pH 3.0 or lower and at pH 13 or higher, DSI29 was totally inactivated (Figure 2). Phage DSI29 demonstrated thermal stability by withstanding temperatures of up to 60 °C without seeing any titer loss. Titers started to decrease above 50 °C, and after incubation at 60 °C, about 50% of phages lost their ability to infect. As expected, exposure to extremely high temperatures, such as 80 °C, caused all viable phages to be lost (Figure 3). These results imply that DSI29's strong resistance to a broad range of pH and temperature conditions supports its potential for a variety of uses as a biocontrol agent in settings related to food processing and storage.

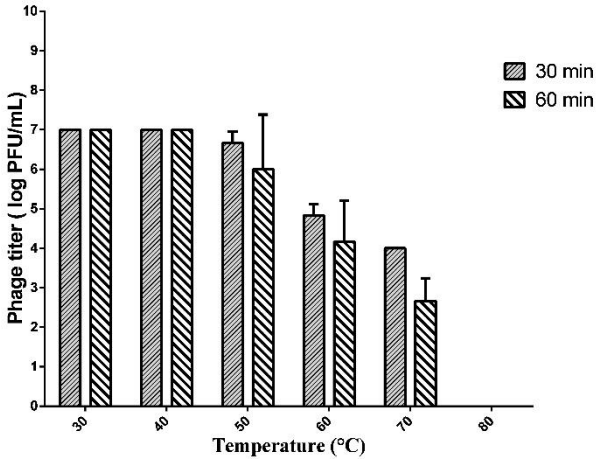


**Figure 3.** Thermal stability of Phage DSI29.

## 4. Discussion

Foodborne illness outbreaks caused by *E. coli* O157:H7 continue to be a major problem for the food business. Certain phages may efficiently lyse a broad variety of strains within a particular bacterial species, but their specificity which permits non-target bacterial populations to remain undisturbed is a major benefit of utilizing phages [14]. A promising "green" method for the biocontrol of several foodborne pathogenic bacteria is provided by phages. When they can lyse a wider variety of harmful bacteria, spanning several species or even genera, their usefulness is greatly increased. These broad-host-range phages are especially useful in complicated and varied food and food processing settings where there may be several bacterial pathogens. However, today there is currently not a coherent, consistent methodology of classifying the varying levels of a, so called, wide host range when it comes to phage research. Without this feature, comparison and classification of recently discovered phages based upon the type of lytic spectrum may prove challenging. Recent discoveries emphasize the need in more unified classification method such as the phase lineage of biota

with broad host ranges, which demonstrate that across the community, phages with different host specificities are continuously being evolved [19,20] community-wide single-cell metagenomics of extreme environments [21] indicate that broad host range phages are common in natural environments [22,23]. Host range study is an inevitable way to select lytic phages to apply on foods as biocontrol agents. It is this characteristic that defines what strains of bacteria a phage may infect and subsequently lysis. As monovalent phages possess by definition a very limited host range, it is again likely that they can only infect a few types of bacteria. Polyvalent phages, however, are more broad in activity, and often are able to infect and lyse a large proportion of strains in a given bacterial species. Consuming polyvalent phages can be especially helpful in regulating the types of bacteria present in the complex food environment due to their expansive lytic properties [24]. In the isolated phage DSI29 present in freshwater sample used in this study, it was evident that it infected various strains of *E. coli* with variable plaque-forming efficiencies (EOP). It means that the wide infection spectrum of phage DSI29 is likely to be associated with the binding of a rich set of receptors on the surface of *E. coli* phage DSI29 as we found, is good bacteria control against various strains of the bacteria *E. coli*. Spot testing depicted that on all the 14 strains of the *E. coli* tested, DSI29 produced clear lysis zones. And also, phage DSI29 efficiently infects these 14 strains of *E. coli* as well as its major host, based on Efficiency of Plating (EOP) results. This extensive activity may be due to high density or quality of *E. coli* observed in the places where phage DSI29 was found.

The virulent phage DSI29 has been selected as an option of biocontrol use in our food systems in the future due to its outstanding capacity to have a broad host range, making it able to lyse all *E. coli* strains within our collection. A phage can be extremely useful in the long food development process chain because of its ability to remain stable under numerous environmental conditions. This study has determined the capacity of the isolated E. coli phage DSI29 to maintain infectivity in the different conditions of pH or temperature. Phage DSI29 was exceptionally tolerant as it maintained a stable titer over 1 hour at pH as low as 4 or as high as 12. Although the ideal pH range for many phages, including the T2 phage, is normally between 6 and 8 (T2 loses 50% of its infectivity between pH 5 and 9), DSI29 is especially resilient due to its wider pH stability [25]. Phage DSI29 demonstrated extraordinary thermal stability in addition to its remarkable pH tolerance. Interestingly, the isolated phage DSI29's titer did not significantly decrease after being held for 60 minutes at temperatures between 30 and 60 degrees Celsius. One essential quality for its possible application in a variety of food processing and storage settings is its resistance to heat.

## 5. Conclusion

*E. coli* O157:H7 is still considered one of the most challenging food safeties bio-contaminants. In this study, the isolated phage DSI29 was shown to have broad host range activity against multiple strains of E. coli, as evidenced by clear lytic zones and high EOP. The ability to bind to a wide range of bacterial receptors makes DSI29 useful in biocontrol measures within food processing environments. Its stability over a broad pH range of 4-12 and temperatures of 30-60°C makes DSI29 useful in many industrial situations. Rather than being narrow-host-range like most phages, DSI29's broad nature makes it useful in decreasing E. coli contamination in food systems. This study emphasizes the need for phage-based solutions in Bangladesh, where such solutions are limited. Follow-up studies should improve phage formulation design for real-world food situations, including system integration and large-scale efficacy trials. As a whole, phage DSI29 illustrates the growing need for sustainable methods for food safety issues, in this case, highlighting the absence of antibiotics, which are increasingly not preferred due to their detrimental effect on technologies used for pathogen control.

**Conflict Of Interest**

The authors declare no conflict of interest

**Acknowledgement**

None

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