Research Article

Isolation and Identification of Escherichia coli O157:H7 Lytic Bacteriophage from Environment Sewage

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Received 2 May 2021; Accepted 24 July 2021; Published 12 August 2021

Academic Editor: Zheng-Fei Yan

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Escherichia coli O157:H7 is one of the pathogenic bacteria causing foodborne disease. The use of lytic bacteriophages can be a good solution to overcome the disease. This study is aimed at isolating lytic bacteriophages from environmental sewage with E. coli O157:H7 bacterial cells. The sample used in this study was eight bacteriophages, and the technique used in identifying E. coli O157:H7 carriers of the stx1 and stx2 genes was PCR. The double layer plaque technique was used to classify bacteriophages. Plaque morphology, host specificity, and electron micrograph were used to identify the bacteriophages. The result obtained plaque morphology as a clear zone with the largest diameter size of 3.5 mm. Lytic bacteriophage could infect E. coli O157:H7 at the highest titer of 10 × 10^8 PFU/mL. Bacteriophages have been identified as Siphoviridae and Myoviridae. Phage 3, phage 4, and phage 8 could infect Atypical Diarrheagenic E. coli (aDEC) due to their host specificity. The Friedman statistical tests indicate that lytic bacteriophage can significantly lyse E. coli O157:H7 (p=0.012). The lysis of E. coli O157:H7 by phage 1, phage 2, phage 3, and phage 5 bacteriophages was statistically significant, according to Conover’s posthoc test (p<0.05). The conclusion obtained from this study is that lytic bacteriophages from environmental sewage could lyse E. coli O157:H7. Therefore, it could be an alternative biocontrol agent against E. coli O157:H7 that contaminates food causing foodborne disease.

1. Introduction

Foodborne diseases are diseases caused by consuming food and or drinks contaminated by various microorganisms or pathogenic microbes (foodborne pathogens) [1]. Foodborne diseases and cases of food poisoning are of wide concern in public health, especially in developing countries [2]. One of the key goals of national food safety programs is to reduce the number of cases of foodborne disease [3]. Escherichia coli O157:H7 is suspected of causing 63,000 instances of foodborne illness, 2,100 hospitalizations, 20 deaths, and a $271 million economic burden [4, 5]. E. coli O157:H7 was existed in the feces of asymptomatic children attending elementary school in Surabaya, East Java, Indonesia [6]. East Java is the largest contributor to the national cattle population, accounting for roughly 43% of the total, with beef production accounting for 20% of the total, or roughly 575,557 tonnes, and beef consumption accounting for 447,460 tonnes [7]. The Kalimas River, one of Surabaya’s main waterways, has been found to be contaminated by market sewage from a nearby site that lacks a wastewater treatment plant [8–10]. Therefore, interventions with a focus on food safety are needed to prevent the occurrence of foodborne pathogens in both children and people in general [11].

E. coli O157:H7 could cause disease by toxin releasing called shiga-like toxin (stx), which could result in food poisoning [12]. Shiga toxin producing E. coli causes more than 2.5 million diseases worldwide each year, resulting 269 deaths [11]. Lytic bacteriophages provide a natural and non-toxic method to reduce and control the growth of human pathogenic bacteria because bacteriophages are part of the environmental ecosystem [13] and as a component of human
Bacteriophages are mostly composed of nucleic acid, which is nontoxic. Therefore, it is beneficial as it has a low impact on the environment with relatively affordable costs. As a bacteriophage is used for a biocontrol agent, the interaction between bacteriophage proteins with biology or environmental components, animals, and the immune responses of a person which could be increased for potential negative effects needs to be considered (for example, when antibiotics make a deletion or elimination of the growth of bacteria, as a form of self defense, the bacteria will release a type of protein or toxin that can trigger a person’s immune response) [19].

2. Materials and Methods

2.1. Bacterial Stock Culture Preparation. Stock culture of E. coli O157:H7 isolate stored in Tryptic Soy Broth Media (Oxoid, United Kingdom) with 15% glycerol at -20°C was obtained from the Microbiology Laboratory, Soetomo General Academic Hospital, Surabaya, East Java, Indonesia. Stock culture of E. coli O157:H7 was subcultured on Mac Conkey Agar plate media (Oxoid, UK), incubated at 37°C for 24 hours and stored at low temperature.

2.1.1. Bacterial DNA Extraction. Bacterial DNA extraction referred to Geneaid (2017) procedural [20].

2.1.2. Amplification of Bacterial DNA Target. PCR was performed using 2 mL of template in a 20 mL volume of the PCR PreMix (Promega corporation). The PCR mixture consisted of 1 U thermostable DNA polymerase, 250 mM dNTP, 50 mM Tris-HCl (pH 8.3), 40 mM KCl, and 1.5 mM MgCl₂. PCR was carried out in a gene thermal cycler (Bio-Rad, Tokyo, Japan). The optimized cycle program of denaturation, annealing, and extension temperatures was as follows: 1 cycle of 2 min at 94°C; 35 cycles of 30 s at 94°C, 30 s at 55°C, and 1 min at 72°C; and 1 cycle of 5 min at 72°C.

Primer of shiga toxin genes of stx1 uses forward CAGT TAATGGTGTTGGCAAGG and reverse CACCA GACAAT GTAACCGCCTG of 1221 bp and stx2 forward ATCCCTA TTCCGGGGGATGCAG-3 and reverse GCCGTACCGTA TACACAGGAGC of 1247 bp [21]. The PCR products were analyzed using 2% agarose gel electrophoresis with ethidium bromide staining, with a 100 bp DNA Ladder Marker (Promega corporation). Electrophoresis was carried out at 100 volts for 35 minutes. Visualization of the band that appeared was done through a UV transilluminator and photographed [22] by Spectrolyne TC-312E/F, Japan.

2.2. Bacteriophage Preparation. The samples used for bacteriophage isolation were taken from traditional market sewage, abattoir, and Kalimas River of Surabaya, Indonesia (as seen on Table 1). Each 15 mL of sample was taken using a sterile tube.

2.2.1. Isolation of Bacteriophage. Sample filtration used the modified Thung et al. method, as much as 1 mL of liquid waste sample was diluted into 9 mL of Nutrient Broth (Oxoid, UK) media, centrifuged at 3000 rpm for 20 minutes, and then the supernatant was filtered using 0.45 μm millipore membrane (Minisart, Sartorius). The 4.5 mL filtrate was then mixed with 0.5 mL of E. coli O157:H7 at exponential phase culture (McFarland 0.5 ~ 1.5 × 10⁶ CFU/mL) and added 5 mL of Nutrient Broth. Next, the mixture was incubated for 24 hours in a waterbath at 37°C. It was then centrifuged at 3000 rpm at 4°C for 15 minutes. The supernatant was taken by a syringe and filtered using 0.22 μm millipore membrane (Minisart, Sartorius). The supernatant that has been filtered was inserted into a sterile tube and stored at low temperature [23].

2.2.2. Lytic Bacteriophage Morphology by the Double Layer Agar Method. Bacteriophage stocks were then inoculated using the modified method of Bonilla et al. by the double
layer plaque technique. A total of 100 μL of lytic bacteriophage stock were diluted into the Ringer buffer with serial 10-fold dilutions. Then, each serial dilution of lytic bacteriophages was taken as much as 100 μL and each was mixed with 100 μL of E. coli O157:H7 bacteria at exponential phase culture (McFarland 0.5) into a new sterile Eppendorf tube and incubated at 37°C for 30 minutes. Soft agar consisting 70% nutrient agar media that was previously made was then warmed at 56°C. After that, the previous mixture was added by pouring technique on nutrient agar media. Incubation was carried out at 37°C for 24 hours [24], and then the plaque formed was observed as lytic bacteriophage existence [23].

As lytic bacteriophages were observed, the purification of lytic bacteriophages was carried out by the plaque formed using Pasteur pipettes and enriched, so that more plaques would produce. Each plaque was transferred to 10 mL of E. coli O157:H7 bacterial culture at exponential phase (McFarland 0.5) and incubated for 24 hours, then centrifuged at 3000 rpm at 4°C for 20 minutes. The bacteriophage filtrate was then filtered using 0.22 μm millipore membrane (Minisart, Sartorius). The result was in the form of a bacteriophage filtrate, and then it was carried out in an NA plate media. The plaque formed was then removed and inserted into the Ringer buffer. The bacteriophage suspension was vortexed and left about 5 to 10 minutes at room temperature, and so then the bacteriophages could attach the E. coli O157:H7 bacteria. Then, centrifugation was done at 3000 rpm at 4°C for 20 minutes for two replication times. The supernatant was filtered using a 0.22 μm millipore filter membrane (Minisart, Sartorius) and then stored as bacteriophage stock at low temperature (4°C) [23].

2.2.3. Bacteriophage Purification. A modified method of Thung et al. of bacteriophage propagation was used. The plaque formed previously was then removed and inserted into the Ringer buffer (4 : 1). The bacteriophage suspension was vortexed and left about 5 to 10 minutes at room temperature, and so then the bacteriophages could attach the E. coli O157:H7 bacteria. Then, centrifugation was done at 3000 rpm at 4°C for 20 minutes for two replication times. The supernatant was filtered using a 0.22 μm millipore filter membrane (Minisart, Sartorius) and then stored as bacteriophage stock at low temperature (4°C) [23].

2.2.4. Bacteriophage Propagation. Bacteriophage propagation was using the modified method of Bonilla et al., and a total of 10 mL of E. coli O157:H7 bacteria cultured in Nutrient Broth medium (Oxoid, UK) at exponential phase (McFarland 0.5) were centrifuged at 3000 rpm at 4°C for 20 minutes. The pellets formed were each infected with 100 μL of lytic bacteriophages. Each mixture was incubated at 37°C for 30 minutes, and the mixture was then added to 10 mL of Nutrient Broth medium and incubated for 24 hours at 37°C. Then, each of them was centrifuged at 3000 rpm at 4°C for 20 minutes. The supernatant formed was taken with a syringe and filtered with a 0.22 μm filter membrane (Minisart, Sartorius). Each supernatant that had been filtered was inserted into a sterile tube and stored as a bacteriophage stock at low temperature (4°C) [24] at Department of Microbiology, Faculty of Medicine, Universitas Airlangga, Surabaya, East Java, Indonesia.

2.2.5. Bacteriophage Quantification. Bacteriophage quantification was measured by counting the amount of plaque formed in NA media plates as plaque forming units/mL (PFU/mL). Each lytic bacteriophage stock was diluted by 10-fold serial, and then 100 μL from each dilution of the bacteriophage isolate was taken and transferred to 100 μL of the E. coli O157:H7 bacterial culture, after incubated for 24 hours on Nutrient Broth medium (Oxoid, UK). The suspension was incubated for 30 minutes at 37°C. A total of 7 mL of soft agar that was previously made at 56°C were mixed. After that, each one of the suspensions was poured onto NA plate media (Oxoid, UK) and incubated at 37°C for 24 hours. After incubation, we then observed the plaque formed and expressed as PFU/mL [24].

2.3. Electron Micrograph of Bacteriophage. The Eijkman Institute in Jakarta, Indonesia, used transmission electron microscopy to identify the shape of the bacteriophage. A total of 10 μL of bacteriophage were dropped onto a 400 mesh grid and left for 30 seconds. On carbon-coated grids, bacteriophage samples were negatively stained with 5 μL of 2% (w/v) uranyl acetate. The grids were viewed using a JEM-1010 TEM (JEOL, Tokyo, Japan) [25].

2.4. Lysis of E. coli O157:H7 Bacteriophage. Lysis of E. coli O157:H7 by bacteriophages was using the modified method of Mirzaei and Nilsson and Aryal. One milliliter of E. coli O157:H7 bacteria that had been grown on MacConkey Agar plates was transferred onto Nutrient Broth media (Oxoid, UK) at exponential phase (McFarland 0.5), and each was infected with 1 mL of bacteriophage stock. Each mixture was then incubated for 2 hours, 4 hours, 6 hours, and 8 hours. The opacity assessment of McFarland densitometry was carried out to calculate the number of live bacteria [25, 26].
2.5. Bacterial Suspension Preparation for Host Specificity. The bacteria used for the host specificity test of lytic bacteriophages were Atypical Diarrheagenic E. coli isolates (aDEC1, aDEC2, aDEC3, aDEC4, aDEC5) (from hospital inpatient care of diarrhea) and keep storage clinical storage Salmonella paratyphi A, Salmonella paratyphi B, and Shigella flexneri isolates (from the Microbiology Laboratory of Medical Faculty of Universitas Airlangga Culture Collection, Surabaya, Indonesia). Atypical Diarrheagenic E. coli isolates (aDEC1, aDEC2, aDEC3, aDEC4, aDEC5), S. paratyphi A, S. paratyphi B, and S. flexneri isolates were stored in Tryptic Soy Broth Media with 15% glycerol at -20°C.

Stock culture of aDEC1, aDEC2, aDEC3, aDEC4, and aDEC5 was inoculated into Mac Conkey Agar plate media (Oxoid, UK) and incubated at 37°C for 24 hours. A positive result gives rise to a pink to rose red colonies, such as its ability to ferment lactose [27, 28] (Figure 1). Stock cultures of S. paratyphi A, S. paratyphi B, and S. flexneri isolates were inoculated into Salmonella Shigella Agar plate media (Oxoid, UK) and incubated at 37°C for 24 hours. A positive result gave rise to colorless for S. paratyphi A and S. flexneri, shown as colorless with black centers for S. paratyphi B as its ability to produce hydrogen sulfide (H₂S) [29] (Figure 2).

Atypical Diarrheagenic E. coli, S. paratyphi A, S. paratyphi B, and S. flexneri cultures were then identified by biochemical assay on Triple Sugar Iron Agar/TSIA (Oxoid, UK), Sulfate Indol Motility/SIM (Oxoid, UK), Simmons Citrate Agar/SCA (Oxoid, UK), and Urea broth (Oxoid, UK) [30] (see Table 2).

2.6. Host Specificity of Bacteriophages. In cultures of aDEC1, aDEC2, aDEC3, aDEC4, aDEC5, S. paratyphi A, S. paratyphi B, and S. flexneri, bacteriophage host specificity was observed using 100 μL of each bacterium previously grown in Tryptic Soy Broth Media (Oxoid, UK) by exponential phase (McFarland 0.5), mixed with 100 μL of lytic bacteriophages stock, and diluted into the Ringer buffer with serial 10-fold dilutions. Then, each serial dilution of lytic bacteriophage was taken as much as 100 μL into a new sterile Eppendorf tube and incubated at 37°C for 30 minutes for the lytic bacteriophages to attach. A total of 7 mL of soft agar that was previously made at 56°C was mixed by pouring technique on Nutrient Agar plate media, and incubation was carried out at 37°C for 24 hours. Then, the plaque formed was observed and counted [23].

2.7. Statistical Analysis. Statistical analysis used in this study was a nonparametric test in the form of the Friedman test. A significant result was continued with Conover’s posthoc.

3. Results and Discussion

3.1. Detection of Shiga Toxin Genes. The results of molecular identification of E. coli O157:H7 isolates carrying the stx1 and stx2 genes using the PCR technique show that only the stx1 gene was borne by E. coli O157:H7 isolates, as shown in Figure 3:

In contrast to the stx2 gene, this study discovered the stx1 gene formed by E. coli O157:H7 to the present in a 1220 bp
band approximately. This gene was also found in the feces of children in Southern Iran [31] and human feces with clinical manifestations in Japan [32]. As a result, *E. coli* O157:H7 must also be monitored as a foodborne pathogen.

### 3.2. Bacteriophage Sample

The samples were collected from the environmental sources as shown in the following table:

| Sample | Plaque diameter (mm) | Turbidity |
|--------|----------------------|-----------|
| Phage 1 | 2                    | Clear     |
| Phage 2 | 2                    | Clear     |
| Phage 3 | 3.5                  | Clear     |
| Phage 4 | 2                    | Clear     |
| Phage 5 | 1.5                  | Clear     |
| Phage 6 | 2                    | Clear     |
| Phage 7 | 0.7                  | Clear     |
| Phage 8 | 1                    | Clear     |

### 3.3. Isolation of Bacteriophage

Plaques in *E. coli* O157:H7 carrying shiga toxin gene cultures grown in the double layer plaque indicate the presence of lytic bacteriophages (see Figure 4).

Bacteriophages could affect bacterial lysis because they are able to recognize receptors on the surface of the host bacteria, so that bacteriophages are able to transfer their genetic material into the host cell and replicate in the host cell causing lysis [33]. From Table 3, it can be seen that the diameter size was around 0.7 to 3.5 mm. Topka et al. obtained bacteriophages with clear colors with diameters of around 2-3 mm [34]. Regarding this, Yazdi et al. conducted a study with diameters of around 1.5-2 mm [35].

There are several factors that could affect plaque formation. Rapidity to lyses bacterial cells was one factor that could determine plaque morphology and size. This factor could cause different appearances of plaques, either in morphology or in size [36]. Plaque diameter was closely related to propagation, and having the right size was desirable for an effective lytic bacteriophage [37].

The rate of plaque formation was influenced by environmental conditions (temperature, pH, and aeration) and the accessibility of the bacteriophage to the target bacteria [38, 39]. Previous studies also reported that cofactors such as Ca²⁺ ions can stabilize the fragile interface of the virion with its receptors [40, 41]. The size and amount of bacteriophage plaque resulting from host cell infection are related to the ability of the bacteriophage to replicate in the host cell. Plaque size is also influenced by several factors, such as agar.

**Table 3: Plaque diameter size of lytic bacteriophage.**

| Sample | Plaque diameter (mm) | Turbidity |
|--------|----------------------|-----------|
| Phage 1 | 2                    | Clear     |
| Phage 2 | 2                    | Clear     |
| Phage 3 | 3.5                  | Clear     |
| Phage 4 | 2                    | Clear     |
| Phage 5 | 1.5                  | Clear     |
| Phage 6 | 2                    | Clear     |
| Phage 7 | 0.7                  | Clear     |
| Phage 8 | 1                    | Clear     |

**Table 4: The concentration of lytic bacteriophage.**

| Sample | PFU/mL |
|--------|--------|
| Phage 1 | 9.2 × 10⁷ |
| Phage 2 | 3.4 × 10⁴ |
| Phage 3 | 3.0 × 10⁶ |
| Phage 4 | 10.0 × 10⁸ |
| Phage 5 | 3.9 × 10⁶ |
| Phage 6 | 4.3 × 10⁶ |
| Phage 7 | 5.4 × 10⁵ |
| Phage 8 | 3.0 × 10⁷ |

**Figure 3:** Detection of the shiga toxin gene of *E. coli* O157:H7 (M: marker; K: control; 1: stx1; 2: stx2).

**Figure 4:** Lytic bacteriophage morphology by the double layer plaque technique.
concentration, incubation conditions, and the log phase of host bacteria [42].

3.4. Concentration of Lytic Bacteriophage. The concentrations of the purified bacteriophages were then calculated based on the amount of plaque formed. The amount of plaque formed was then calculated in plaque forming units (PFU/mL), which is a measure of the amount of virus infective per volume of fluid. In addition, the lytic bacteriophage concentration was calculated as seen in Table 4:

Bacteriophage quantification was measured by counting the amount of plaque formed, and the concentration obtained was of $5 \times 10^{3}$ to $10 \times 10^{8}$ PFU/mL. The number of bacteriophages previously detected was $>10^8$ PFU/mL of Bacillus cereus bacteriophage [33], $10^{15}$ PFU/mL of Klebsiella pneumoniae bacteriophage [43], $5 \times 10^{12}$ PFU/mL of E. coli bacteriophage [44], $1.2 \times 10^{16}$ PFU/mL [45], and $5 \times 10^{6}$ PFU/mL for T4 bacteriophage [46], and $1.3 \times 10^{11}$ PFU/mL [23], $2.1 \times 10^{10}$ PFU/mL [34], and $2.62 \times 10^{10}$ PFU/mL of E. coli bacteriophage [33]. Marti et al. conducted a study with Salmonella spp. bacteriophages with titers of $5 \times 10^{8}$ PFU/mL [47], Bao et al. obtained titers of $5 \times 10^{7}$ PFU/mL [48].

Other studies have shown similar results with bacteriophage titres between $10^8$ and $10^{11}$ PFU/mL, this could be due to the optimum plating condition, and other parameters such as the buffer in which the bacteriophages were suspended, the incubation media [49], Ca$^{2+}$ ions which can stabilize the fragile interface of the virion with its receptors [40, 41], and the different bacteriophage environment surroundings could also be the influence [23, 50]. This study used Ringer buffer consisting of 1.55 g C$_3$H$_5$NaO$_3$, 3 g NaCl, 0.15 g KCl, and 0.1 g CaCl$_2$·2H$_2$O, while other studies used SM buffer consisting of 5.8 g NaCl, 2 g MgSO$_4$·7H$_2$O, 50 mM Tris-Cl (pH 7.5), and 5 mL gelatine in 1 L H$_2$O [23, 51, 52].

3.5. Electron Micrograph of Bacteriophage. The morphology of bacteriophages, such as the head and tail shapes, was discovered to be identical to the order of Caudovirales [53, 54], based on observations (see Figure 5).

Bacteriophages have a head and tail that vary in size, as seen in Table 5:

Other studies have shown similar results with bacteriophage titres between $10^8$ and $10^{11}$ PFU/mL, this could be due to the optimum plating condition, and other parameters such as the buffer in which the bacteriophages were suspended, the incubation media [49], Ca$^{2+}$ ions which can stabilize the fragile interface of the virion with its receptors [40, 41], and the different bacteriophage environment surroundings could also be the influence [23, 50]. This study used Ringer buffer consisting of 1.55 g C$_3$H$_5$NaO$_3$, 3 g NaCl, 0.15 g KCl, and 0.1 g CaCl$_2$·2H$_2$O, while other studies used SM buffer consisting of 5.8 g NaCl, 2 g MgSO$_4$·7H$_2$O, 50 mM Tris-Cl (pH 7.5), and 5 mL gelatine in 1 L H$_2$O [23, 51, 52].

**Table 5: Bacteriophage: morphology of electron micrographs.**

| Bacteriophage | Family          | Head diameter (nm) | Tail length (nm) | Tail diameter (nm) |
|---------------|-----------------|--------------------|------------------|-------------------|
| Phage 1       | *Siphoviridae*  | 113                | 200              | 17.4              |
| Phage 2       | *Siphoviridae*  | 133.3              | 288              | 10.3              |
| Phage 3       | *Siphoviridae*  | 50                 | 100              | 8.7               |
| Phage 4       | *Siphoviridae*  | 50                 | 100              | 6.3               |
| Phage 5       | *Myoviridae*    | 100                | 100              | 10                |
| Phage 6       | *Myoviridae*    | 100                | 71.4             | 14.3              |
| Phage 7       | *Myoviridae*    | 100                | 100              | 13.3              |
| Phage 8       | *Myoviridae*    | 83.3               | 83.3             | 16.7              |

**Figure 5: Electron micrograph of negatively stained bacteriophage.**
related to the multiplication rate of bacteriophages. The more titer [41]. The speed of plaque formation is considered due to an increased attachment rate at higher bacteriophage in a faster reduction in the bacterial count, which could be et al. stated that higher bacteriophage concentrations resulted was able to lysis E. coli O157:H7 caused by lytic bacteriophage: (phage 1). The following table shows the reduction in E. coli O157:H7 bacterium.

3.6. Lysis of E. coli O157:H7 by Bacteriophage. One mL of E. coli O157:H7 was injected with 1 mL of bacteriophage stock into Triptic Soy Broth media (Oxoid, UK) at 37°C at an incubation interval of 0 hour, 2 hours, 4 hours, 6 hours, and 8 hours. The lysis of E. coli O157:H7 is as seen on Table 6:

The ability of LBP to lyse E. coli O157:H7 can be seen. In comparison to the amount of E. coli O157:H7 in the control, which was 1.5 x 10⁸ CFU/mL without lytic bacteriophage injection, the lowest value of E. coli O157; H7 obtained was 3.7 x 10⁵ CFU/mL, with a maximum of 1.2 x 10⁸ CFU/mL (phage 1). The following table shows the reduction in E. coli O157:H7 caused by lytic bacteriophage:

After the 8-hour incubation period, lytic bacteriophage was able to lyse E. coli O157:H7 by 75% (see Table 7). Yazdi et al. stated that higher bacteriophage concentrations resulted in a faster reduction in the bacterial count, which could be due to an increased attachment rate at higher bacteriophage titers [41]. The speed of plaque formation is considered related to the multiplication rate of bacteriophages. The more bacteriophages are produced, and the more bacterial cells are lysed [38, 42]. In addition, the ability of lysis was also influenced by the multiplicity of infection (MOI), which is the ratio of bacteriophages to the number of target bacteria [53]. Bacterial growth will increase along with the decrease in MOI [33], but MOI cannot completely inhibit cell growth [55].

At first glance, the Myoviridae bacteriophage was faster than the Siphoviridae bacteriophage in lysing E. coli O157:H7 at the beginning of the infection time, but it was not seen at subsequent incubation times and obtained the same number of lysis as the Siphoviridae bacteriophage at the end of the incubation period (after 8 hours). Based on these characteristics, more research is needed on the lysis of bacteriophages with a higher number of Siphoviridae and Myoviridae bacteriophages, as well as a bigger number of E. coli O157:H7 bacterium.

3.7. Host Specificity of Bacteriophages. The host’s specificity is depicted in Figure 6 as plaque formation:

Phage 3, phase 4, and phase 8 were found to have plaque formation in aDEC1 (see Figure 6), with aDEC2, aDEC3, aDEC4, aDEC5, S. paratyphi A, S. paratyphi B, and S. flexneri, and lytic bacteriophage plaque development was not seen. In contrast to aDEC2, aDEC3, aDEC4, aDEC5, S. paratyphi A, S. paratyphi B, and S. flexneri, the presence of plaque in host specificity testing with aDEC1 indicates that phage 3, phase 4, and phase 8 infect other serotypes in one bacterial species, implying that the surface of aDEC1 bacterial cells has the same particular receptors against phase 3, phase 4, and phase 8. In this regard, Akhtar et al. discovered that Salmonella enterica lytic bacteriophages have a host specificity for S. typhimurium [56].
Jamal et al.'s study revealed that bacteriophages that lyse *K. pneumoniae* could not infect other bacteria, which suggests a narrow host range among other different bacterial strains [40]. It is similar to Abatangelo et al. who studied *Staphylococcus aureus* bacteriophages [57]. Bao et al. and Jurczak et al. found that *Salmonella enterica* bacteriophages could also lyse *E. coli* [48, 58]. Chen et al. found bacteriophages which lysed *Pasteurella multocida* A, and *P* capsular type was not able to lyse strains with D or F capsular type or other Gram-negative bacteria, including *E. coli*, *Salmonella* spp., and *Bordetella bronchiseptica* [59]. Lukman et al. also found a bacteriophage that can lyse EPEC and EHEC [33]. Further research on bacteriophage host specificity on another pathogenic *E. coli* is required based on these traits.

The narrow host specificity of bacteriophages may be viewed as a disadvantage. This characteristic restricts the number of bacteria where the selection for bacteriophage resistance mechanisms can occur in comparison to chemical antibiotics. This perhaps could be circumvented as cocktail bacteriophages with the aim of being able to lyse a wider range of bacterial species. Otherwise, it can be an advantage as it offers fewer side effects on natural flora. Therefore, the subject will not be susceptible to superinfection [19, 60].

**Figure 6**: Lytic bacteriophage 3, 4, and 8 plaque formation on *Atypical Diarrheagenic E. coli* 1.

**Table 8**: Friedman test of *E. coli* O157:H7 by bacteriophage.

| Factor |  |  |  |
|--------|---------------|---------------|---------------|
| Lysis of *E. coli* O157:H7 by bacteriophage | 0.012 | 0.993 |

**Table 9**: Conover’s posthoc comparisons.

|  |  |  |  |
|---|---------------|---------------|---------------|
| Phage 2 | 34.500 | 20.500 | 0.057 |
| Phage 3 | 34.500 | 25.500 | 0.214 |
| Phage 5 | 34.500 | 33.000 | 0.834 |
| Phage 6 | 34.500 | 17.500 | 0.022 |
| Phage 7 | 34.500 | 17.500 | 0.022 |
| Phage 8 | 34.500 | 31.500 | 0.676 |
| Phage 4 | 36.000 | 20.500 | 0.036 |
| Phage 5 | 36.000 | 33.000 | 0.676 |
| Phage 6 | 36.000 | 17.500 | 0.014 |
| Phage 7 | 36.000 | 17.500 | 0.014 |
| Phage 8 | 36.000 | 31.500 | 0.531 |
| Phage 3 | 34.500 | 25.500 | 0.214 |
| Phage 4 | 34.500 | 17.500 | 0.022 |
| Phage 5 | 34.500 | 33.000 | 0.834 |
| Phage 6 | 34.500 | 17.500 | 0.022 |
| Phage 7 | 34.500 | 31.500 | 0.676 |
| Phage 8 | 34.500 | 31.500 | 0.676 |
|  |  |  |  |
| Phage 1 | 0.012 | 0.993 |

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3.8. Statistical Analysis. Statistical analysis using JASP 0.14.1.0 programme described a nonparametric test. The Friedman test conducted a significant effect in the lysis of E. coli O157:H7 by lytic bacteriophages (p = 0.012), with estimating a large effect size of Kendall’s W by 0.993 [61] (see Table 8).

From Conover’s posthoc test, the p value was obtained for the effect of all groups as written in the following table:

Phage 1 had a statistically significant difference in E. coli O157:H7 quantity compared to phage 6 (p = 0.022), and LPB7 (p = 0.022), whereas phage 2 had a statistically significant difference in E. coli O157:H7 amount compared to phage 3 (p = 0.036), phage 6 (p = 0.014), and phage 7 (p = 0.014), according to Conover’s posthoc test. In addition, phage 5 had a significantly different impact in the lysis of E. coli O157:H7 relative to phage 6 (p = 0.036) and phage 7 (p = 0.036). Phage 6 had a significantly different effect in the lysis of E. coli O157:H7 compared to phage 8 (p = 0.057), and phage 7 had a significantly different effect in the lysis of E. coli O157:H7 compared to phage 8 (p = 0.057) (see Table 9).

On phage 6 and phage 7, the amount of E. coli O157:H7 was shown to be lower than on phage 1, the number of E. coli O157:H7 detected by phage 3, phage 6, and phage 7 was statistically lower than phage 2, and the number of E. coli O157:H7 detected by phage 6 and phage 7 produced a lower number of E. coli O157:H7 than phage 5.

4. Conclusions

Lytic bacteriophage identified from environmental sewage in Surabaya, Indonesia, shows plaque morphology as a clear zone with the largest diameter size of 3.5 mm, and lytic bacteriophage could infect E. coli O157:H7 carrying the stx1 gene at the highest titer of $10 \times 10^8$ PFU/mL. Bacteriophages have been identified as Siphoviridae and Myoviridae by electron micrograph.

It was studied that phage 3, phage 4, and phage 8 could infect Atypical Diarrheagenic E. coli 1 (aDEC1) due to their host specificity. The Friedman statistical tests indicate that lytic bacteriophage can significantly lyse E. coli O157:H7 (p = 0.012). The lysis of E. coli O157:H7 by phage 1, phage 2, phage 3, and phage 5 bacteriophages was statistically significant, according to Conover’s posthoc test (p < 0.05). The conclusion obtained from this study is that lytic bacteriophages from environmental sewage could lyse E. coli O157:H7. Therefore, it could be an alternative biocontrol agent against E. coli O157:H7 that contaminates food causing foodborne disease.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declared that there was no conflict of interest.

Acknowledgments

We would like to thank Mrs. Fariani Syahруl for providing the E. coli O157:H7 isolate, Mrs. Debi Arivo for contributing the enrichment of this manuscript, Mrs. Nur Ita Margayningsih for the assistance at the Transmission Electron Microscope Laboratory, Eijkman Institute for Molecular Biology, Jakarta, Indonesia, and Mr. Sugeng Harijono for microbiology laboratory assisted. This study was supported by the Education Fund Management Institute (LPDP), Ministry of National Finance, Indonesia.

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