



Isolation of Anti-*Escherichia coli* O157:H7 Bacteriophages and Determination of Their Host Ranges

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ABSTRACT

The aim of this study was to isolate, purify and determine host ranges of bacteriophages infecting *Escherichia coli* O157:H7 from different environment such as river/stream water, sewage, raw food, animal troughs, wastewaters of food processing plants, slaughterhouse and fish farms. For screening of *E. coli* O157:H7 bacteriophages, 92 samples were used. It was found that in respect to anti-*E. coli* O157:H7 bacteriophages, food processing wastewaters, sewage and slaughterhouse wastewaters are the richest sources, and streams, troughs and fisheries wastewater are rich in the middle, and raw foods were the poorest source. A total of 37 phages were isolated and purified. The phages counts of the purified samples were changed among 30×10^3 - 34×10^8 PFU/mL. The isolated phages were generally infective against *E. coli* O157:H7 and *E. coli* strains and 81.08% of the phages (30 out of 37) formed clear plaques and were capable to lyse at least 1 out of 5 *E. coli* O157:H7 strains. In addition to *E. coli*, some phages were capable to infect some *Salmonella enterica* serovars. This results show that inhibitor spectra of the phages were wide.

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Introduction

Escherichia coli belongs to the family Enterobacteriaceae, and is a Gram-negative, rod-shaped, motile, nonsporulating and facultative anaerobic bacterium. A majority of *E. coli* strains are nonpathogenic and exist harmlessly in the intestinal tract of humans and warm-blooded animals. They are routinely shed into the environment through feces and can contaminate water and soil, and, consequently fruits and vegetables (Bhunja, 2007; Ray, 2005).

Taxonomy of *E. coli* strains is based on specific virulence factors and phenotypic characters. Pathogenic *E. coli* strains are classified in six virotypes: enterohemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC) and diffusely adherent *E. coli* (DAEC). However, foodborne outbreaks have been particularly associated with EHEC and EAEC strains. EHEC or Shiga toxin-producing *E. coli* (STEC) strains that produce verocytotoxin or shiga-like toxin, the causative agent of diarrhea, hemorrhagic colitis, haemolytic-uremic syndrome and thrombotic thrombocytopenic purpura. Among the EHEC strains, *E. coli* O157:H7 has been widely recognized as the major cause of foodborne illness (Tarr et al., 2005; Wu et al.,

2011). The outbreaks of *E. coli* O157:H7 have been linked to contaminated meat, meat products, unpasteurized milk and leafy green vegetables, radish sprouts and fruits fertilized with contaminated animal manure was the source of contamination (Sartz et al., 2008; CDC, 2011).

Bacteriophages (phages) are viruses that only infect and lyse specific bacterial hosts and do not infect human, animal or plant cells. Also, they generally do not cross bacterial species and consequently do not affect desirable microflora present in food (Carlton et al., 2005). Furthermore, the use of phages to control pathogenic bacteria has attracted considerable interest in recent years due to the emergence of antibiotic resistant foodborne pathogenic bacteria. In fact, early in this century, phages were used to treat bacterial infections before the advent of antibiotics. Virulent phages may provide a natural, nontoxic, feasible approach for controlling human foodborne pathogens (FDA, 2006; Hagens and Loessner, 2014; Tolba et al., 2014).

The aim of this study was to isolate phages infecting *E. coli* O157:H7 strains from environment and determine their host range and to have a collection of *E. coli* O157:H7 specific phages.

Materials and methods

Bacterial Strains and Culture Conditions

E. coli O157:H7 strains used in phages screening were *E. coli* O157:H7 NCTC 12900, *E. coli* O157:H7 ATCC 43888, *E. coli* O157:H7 ATCC 35150, *E. coli* O157:H7 RSKK, *E. coli* O157 AİBÜ. The strains were kept at -80°C in brain heart infusion broth (BHI, Merck, Darmstadt, Germany) with 20% glycerol. Strains were obtained from our culture collection, culture collection of Biology Department of Ankara University and Veterinary Faculty of Kırıkkale University.

Collection and Preparation of Samples for Bacteriophage Screening

The samples used in bacteriophage screening were taken from river/stream water, sewage water, raw foods (milk, fruit, vegetable and meat), wastewater from food processing plants, slaughterhouse, and fish farms, and water from troughs found in Niğde, Aksaray, Ankara and Kayseri provinces. Water samples were centrifuged ($6.000 \times g$ for 15 min) and then the supernatants were passed through sterile cellulose nitrate membrane filter (0.45 and 0.22 μm) (Sartorius, Germany). Milk samples were centrifuged ($6.000 \times g$ for 15 min) after addition of lactic acid (10%) to precipitate casein and then the supernatants were filtered sterilized (0.45 μm pore size). Other semi hard and solid food samples (about 25 g) were put in sterile stomacher bags and at that time 100 mL of SM buffer (50 mM Tris-Cl, pH 7.5, 99 mM NaCl, 8 mM MgSO_4 , 0.01% gelatin) were added. After homogenization in a stomacher for 2 min, the samples were centrifuged and the filtrate was passed through a 0.45 μm sterile membrane filter. All filtrate samples gotten from water and food samples were used for bacteriophages isolation. A total of 92 samples were analyzed.

Bacteriophages Isolation

For isolation of bacteriophages, direct isolation and enrichment method were used. In the direct isolation method, filtered supernatants were directly used for bacteriophage screening against the test bacteria by the double agar layer plate method (Adams, 1959). In the enrichment protocol, 20 mL of the filtered samples were separately inoculated with 2 mL of actively grown culture of *E. coli* O157:H7 NCTC 12900, *E. coli* O157:H7 ATCC 43888, *E. coli* O157:H7 ATCC 35150, *E. coli* O157:H7 RSKK, *E. coli* O157:H7 AİBÜ in BHI broth and mixed with 3 mL of double strength BHI broth, and incubated at 35-37°C for 24 hours. Subsequently chloroform (50 $\mu\text{L}/\text{mL}$) was added into samples and vigorously mixed. After centrifugation ($5.000 \times g$ for 15 min), the supernatants were maintained at 5°C. The samples were tested by spot and double agar layer plaque assay against individual *E. coli* O157:H7 strains (McLaughlin et al., 2006).

Spot Testing and Double Layer Agar Plaque Assay

Spot testing was used to determine the presence of bacteriophage. The host bacterial lawn was made by using

soft BHI top agar (0.7 % agar) containing host bacterial suspensions and then overlaid on top of BHI agar (1.5 % agar) plates. When the agar overlays were solidified, the samples were spotted onto the lawns in plates. After incubation at 37°C for 24 h, all plates were observed for the clear zone formation.

The double-layer agar plaque method was used to determine the titer of phage as follow (Adams, 1959). One hundred micro liter of a dilution of the membrane filtered phage samples and 300 μL of the actively growing *E. coli* O157:H7 cultures (about 10^8 CFU/mL) were added into BHI soft agar (0.7% agar) at 45-50°C. After mixing, the contents of the tube were immediately poured onto BHI basal agar (1.5%) on Petri dishes and then the plates were incubated at 37°C for 24 h. At the end of incubation, phage numbers were counted (PFU/mL).

Purification of Bacteriophages

Bacteriophages are purified by removing a well isolated plaque using the wide end of a sterile Pasteur pipette and the phages were eluted with shaking for a minimum of 2 h in 100 μL SM buffer (50 mM Tris-Cl, pH 7.5, 99 mM NaCl, 8 mM MgSO_4 , 0.01% gelatin). After chloroform (50 $\mu\text{L}/\text{mL}$) extraction and centrifugation ($9.000 \times g$, 20 min, 4°C), the supernatant was transferred to a new sterile tube. The prepared serial dilutions of the phage samples were inoculated into an early-log phase host culture, and the lysate was replated as described above. Bacteriophage purification process was repeated at least three times through plaque assay, reinfection, centrifugation and resuspension to ensure purity of the phage. To determine the number of phage in the each sample, the double layer agar method were used (Stenholm vd., 2008).

Preparation of Phages Stocks

To prepare high titer phage stocks, 1 mL of overnight *E. coli* O157:H7 cultures in BHI broth at 37°C and 100 μL of the purified phage stock were put into 100 mL BHI broth and after mixing, they were incubated overnight at 37°C. After addition of chloroform (50 $\mu\text{L}/\text{mL}$) to lyse the bacterial cells, the amplified phages were centrifuged ($9.000 \times g$ for 13 min) and the supernatants were filtered with a disposable 0.45 or 0.22 μm pore size syringe membrane filter (Sartorius, Germany) (Kocharunchitt et al., 2009). The titre of the phage stock was determined by the double-layer plaque titration method (Adam, 1959).

Investigation of Host Range of Bacteriophages

Various species or strains or serovars of *E. coli*, *Salmonella*, *Shigella*, *Yersenia*, *Citrobacter*, *Enterobacter*, *Listeria*, *Staphylococcus*, *Bacillus* and *Enterococcus* was used to determine host ranges of anti-*E. coli* O157:H7 bacteriophages (Table 1). To determine host ranges of bacteriophage samples, 300 μL of logarithmic phase ($\text{OD}_{600}=0.3$) suspensions of host strains were put into BHI soft agar and then it was poured onto dried base plates. Subsequently, 10 μL of the 10^{-2} , 10^{-4} , 10^{-6} phage dilutions were spotted on the overlay. After incubation at 35-37°C for 24 h, the plates were examined for plaque formation.

Table 1 Bacteria used to isolate *E. coli* O157:H7 specific bacteriophages and to determine their host ranges

<i>Escherichia coli</i> O157:H7 NCTC 12900	<i>S. Typhimurium</i> MET-S1-625	<i>S. Anatum</i> DMC90
<i>E. coli</i> O157:H7 ATCC 43888	<i>S. Typhimurium</i> AIBU	<i>S. Telaviv</i> DMC62
<i>E. coli</i> O157:H7 ATCC 35150	<i>S. Typhimurium</i> ATCC 14028	<i>S. Montavide</i> DMC81
<i>E. coli</i> O157:H7 RSKK	<i>S. Enteritidis</i> DMC8	<i>S. Kentucky</i> DMC35
<i>E. coli</i> O157 AIBU	<i>S. Enteritidis</i> DMC22	<i>S. Carvalis</i> DMC86
<i>E. coli</i> DSa	<i>S. Enteritidis</i> ATCC 13075	<i>Listeria monocytogenes</i> ATCC19115
<i>E. coli</i> BL21	<i>S. Enteritidis</i> MET-S1-411	<i>Staphylococcus aureus</i> ATCC 25923
<i>E. coli</i> CFAI	<i>S. Enteritidis</i> MET-S1-512	<i>Bacillus cereus</i> ATCC 10875
<i>E. coli</i> ATCC 25922	<i>S. Enteritidis</i> MET-S1-742	<i>Yersinia enterocolitica</i> O:9 AU
<i>Salmonella</i> Typhimurium LT2 SR II	<i>Salmonella</i> Virchow DMC8	<i>Citrobacter freundii</i> AU
<i>S. Typhimurium</i> Tr90	<i>S. Infantis</i> DMC7	<i>Enterobacter aerogenes</i> AU
<i>S. Typhimurium</i> Wild type 14028	<i>S. Thompson</i> DMC47	<i>Enterococcus faecalis</i> ATCC 29212

MET, Middle East Technical University; AIBU, Abant İzzet Baysal University; AU, Ankara University

Results and Discussion

Isolation of *E. coli* O157:H7 Specific Bacteriophages

In the present study, *E. coli* O157:H7 specific bacteriophages which were lytic and broad spectrum were isolated from natural sources and purified and developed a collection of phages to biocontrol foodborne pathogenic *E. coli* O157:H7. A total of 92 samples were used for the screening of bacteriophages. Of the 92 samples, 12 were

taken from river/stream water, 13 from sewage, 10 from waste water of fish farms, 18 from raw foods (milk, fruit, vegetable and meat), 11 from wastewater of food processing plants, 13 from wastewater of slaughterhouse, and 8 from water of troughs. The results of phage screening were given in Table 2.

Table 2 Isolated bacteriophages infecting *E. coli* O157:H7 serovars

Sample	Name of phage	Host Serovar	Number of phage (PFU/mL)
Rivers/streams	Eco-Phage-1	<i>E. coli</i> O157:H7 NCTC 12900	22.1×10 ⁷
	Eco-Phage-2	<i>E. coli</i> O157:H7 RSKK	34.0×10 ⁸
	Eco-Phage-3	<i>E. coli</i> O157:H7 NCTC 12900	36.0×10 ⁴
Sewage	Eco-Phage-4	<i>E. coli</i> O157:H7 RSKK	44.0×10 ⁸
	Eco-Phage-5	<i>E. coli</i> O157:H7 NCTC 12900	31.5×10 ⁶
	Eco-Phage-6	<i>E. coli</i> O157:H7 ATCC 43888	47.0×10 ⁷
	Eco-Phage-7	<i>E. coli</i> O157:H7 RSKK	52.5×10 ⁵
	Eco-Phage-8	<i>E. coli</i> O157:H7 ATCC 35150	80.0×10 ⁶
	Eco-Phage-9	<i>E. coli</i> O157:H7 RSKK	80.0×10 ⁶
	Eco-Phage-10	<i>E. coli</i> O157:H7 AİBÜ	12.2×10 ⁶
	Eco-Phage-11	<i>E. coli</i> O157:H7 ATCC 35150	15.0×10 ⁷
	Eco-Phage-12	<i>E. coli</i> O157:H7 NCTC 12900	21.0×10 ⁴
	Eco-Phage-13	<i>E. coli</i> O157:H7 ATCC 43888	34.5×10 ⁴
Fisheries wastewater	Eco-Phage-14	<i>E. coli</i> O157:H7 RSKK	16.0×10 ⁷
	Eco-Phage-15	<i>E. coli</i> O157:H7 RSKK	70.0×10 ⁵
Food processing wastewater	Eco-Phage-16	<i>E. coli</i> O157:H7 ATCC 35150	38.5×10 ⁴
	Eco-Phage-17	<i>E. coli</i> O157:H7 RSKK	24.0×10 ⁷
	Eco-Phage-18	<i>E. coli</i> O157:H7 ATCC 35150	12.1×10 ⁷
	Eco-Phage-19	<i>E. coli</i> O157:H7 NCTC 12900	28.5×10 ⁷
	Eco-Phage-20	<i>E. coli</i> O157:H7 RSKK	72.0×10 ⁶
	Eco-Phage-21	<i>E. coli</i> O157:H7 ATCC 35150	22.0×10 ⁵
	Eco-Phage-22	<i>E. coli</i> O157:H7 NCTC 12900	61.0×10 ⁶
	Eco-Phage-23	<i>E. coli</i> O157:H7 ATCC 43888	23.2×10 ⁷
	Eco-Phage-24	<i>E. coli</i> O157:H7 NCTC 12900	89.0×10 ⁶
	Eco-Phage-25	<i>E. coli</i> O157:H7 RSKK	30.0×10 ⁸
	Eco-Phage-26	<i>E. coli</i> O157:H7 ATCC 43888	82.0×10 ⁵
	Eco-Phage-27	<i>E. coli</i> O157:H7 AİBÜ	49.0×10 ⁵
Slaughterhouse wastewater	Eco-Phage-28	<i>E. coli</i> O157:H7 RSKK	37.5×10 ⁵
	Eco-Phage-29	<i>E. coli</i> O157:H7 AİBÜ	30.0×10 ³
	Eco-Phage-30	<i>E. coli</i> O157:H7 RSKK	54.0×10 ⁵
	Eco-Phage-31	<i>E. coli</i> O157:H7 AİBÜ	12.4×10 ⁶
	Eco-Phage-32	<i>E. coli</i> O157:H7 ATCC 35150	28.0×10 ⁵
	Eco-Phage-33	<i>E. coli</i> O157:H7 AİBÜ	37.0×10 ⁵
	Eco-Phage-34	<i>E. coli</i> O157:H7 RSKK	66.0×10 ⁵
Trough	Eco-Phage-35	<i>E. coli</i> O157:H7 RSKK	24.0×10 ⁵
	Eco-Phage-36	<i>E. coli</i> O157:H7 AİBÜ	13.0×10 ⁴
	Eco-Phage-37	<i>E. coli</i> O157:H7 ATCC 43888	37.5×10 ⁷

As seen Table 2, food processing waste waters, sewage and slaughterhouse wastewaters are the richest sources in respect to anti-*E. coli* O157:H7 bacteriophages. Conversely, streams, troughs and fisheries waste water were found to be quite poor. In addition, *E. coli* O157:H7 bacteriophages were not detected in milk, meat, fruits and vegetables. Thirty seven *E. coli* O157:H7 specific bacteriophages were isolated. Some isolated phages were given in Fig. 1.

Many researchers reported that the presence of bacteriophages is strictly associated with their natural host bacterial cells. *E. coli* O157:H7 is a natural inhabitant of the gastrointestinal tract of animals and human, and abundant in feces. As a result, they are natural members of the microbial ecosystem and therefore they are easily transmitted to sewage, waste water, soil and food. Consequently, various waste effluents such as sewage, naturally provide the best source for isolation of phage infecting *E. coli* O157:H7 (Oot et al., 2007; Synnott et al.,

2009; Viazis et al., 2011). Similar to our results a number of researchers isolated phages specific for *E. coli* O157:H7 have previously been isolated from natural and their host environments (Kudva et al., 1999; Morita et al., 2002; Oot et al., 2007; Synnott et al., 2009; Niu et al. 2009b; Viazis et al., 2011; Raya et al., 2011; Litt and Jaroni, 2017).

It was found that for isolation of bacteriophages, the enrichment method was better than direct method since the number of phages in the samples examined by the enrichment process increased considerably compared to the direct method. This increase was especially pronounced in case where the count of phages was low (e.g. river/stream waters) (Fig. 2). Some researchers also reported that the phage titer of the sample isolated were increased by using enrichment method (McLaughlin et al., 2006; Oot et al. 2007; Viazis et al. 2011; Akhtar et al., 2014).

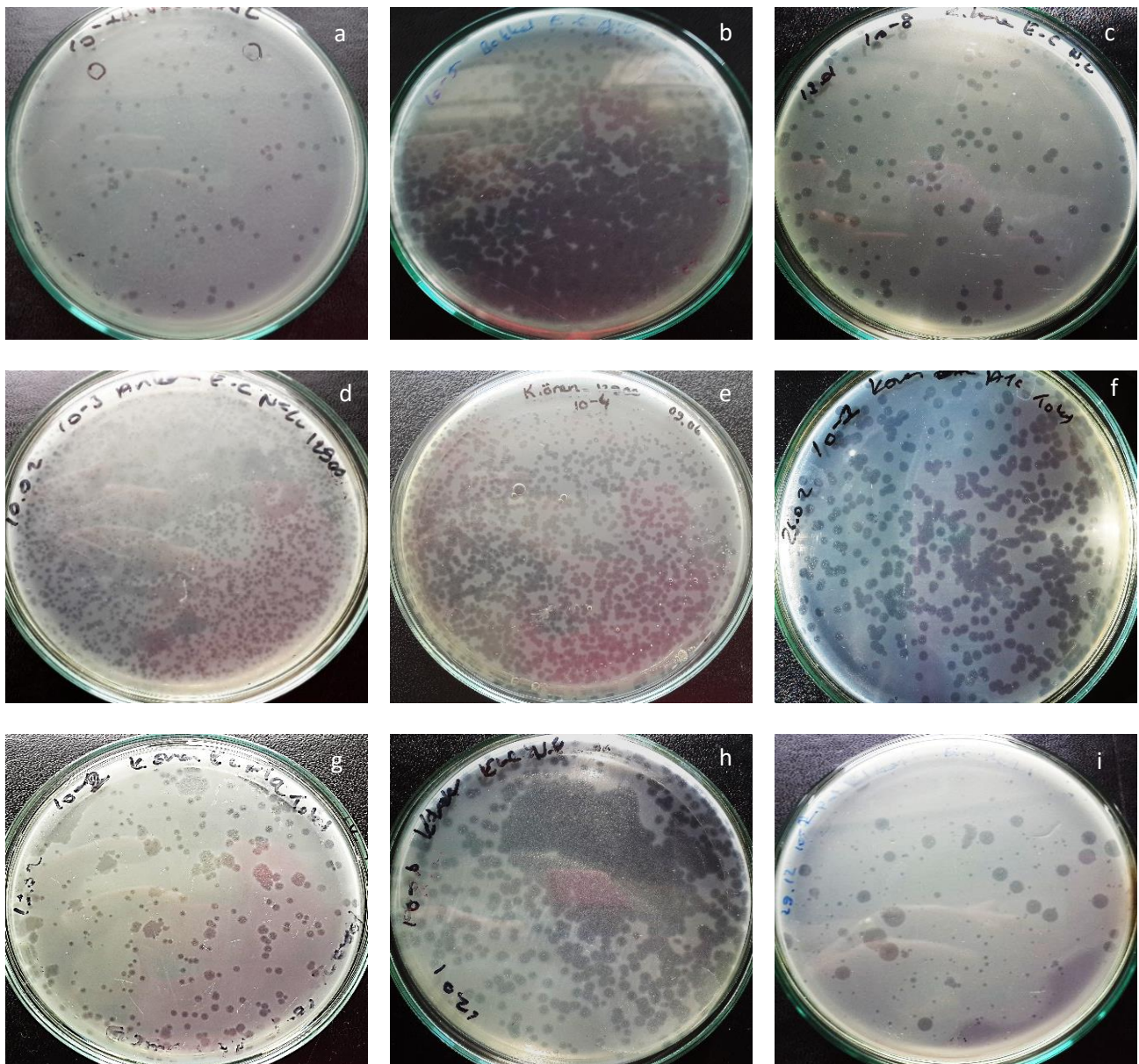


Fig 1 Some of isolated *E. coli* O157:H7 specific bacteriophages. Bacteriophage from food processing wastewaters (a), (b) and (c); bacteriophages from sewages (d), (e) and (f); bacteriophages from slaughterhouse wastewaters (g), (h) and (i).

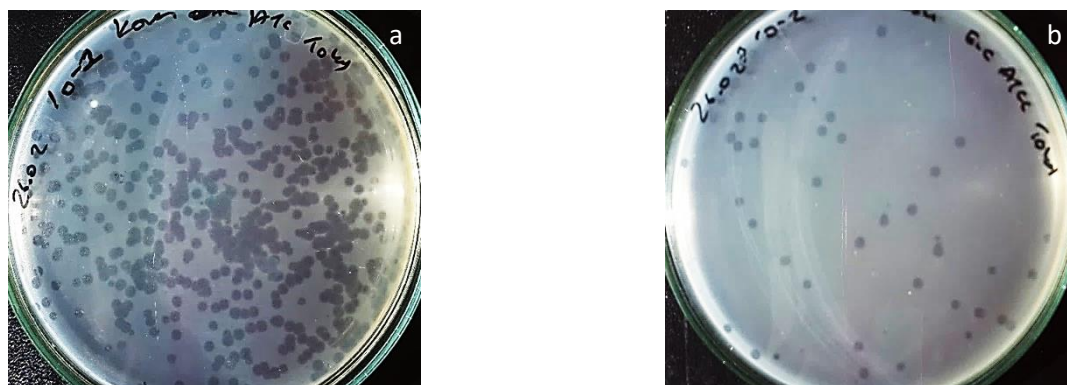


Fig. 2 Comparison of enrichment method (a) versus direct (b)



Fig. 3 Purification of *E. coli* O157:H7 bacteriophages

Purification of Phage and Preparation of Phage Stocks

A single plaque method was used to purify isolated phages (Adam, 1958). In purification procedure, a single plaque was taken from Petri plate containing maximum of 4-5 phage plaques and this process was repeated at least 3 times (Fig. 3). The purified bacteriophages were stored at -80°C in SM buffer with 20% glycerol. It was determined that the bacteriophage titer of the sample stocks were between 30×10^3 - 44×10^8 PFU/mL. The isolation environment of 37 purified phages and their naming was summarized in Table 2.

Host Ranges of Bacteriophages

To examine their host ranges, the isolated *E. coli* O157:H7 phages were tested against 5 different *E. coli* O157:H7 strains, 4 other *E. coli* strains, 6 *S. Typhimurium*, 6 *S. Enteritidis* serovars, 8 other *Salmonella enterica* serovars, and 7 different Gram-negative and Gram-positive species (Table 3 and 4). All 37 isolated anti-*E. coli* O157:H7 phages were capable of lysing their host strains throughout the purification procedure and they were generally infective against *E. coli* O157:H7 and *E. coli* strains. 81.08% of isolated phages (30 out of 37) formed clear plaques and were capable to lyse at least 1 out of 5 *E. coli* O157:H7 strains. It was observed that 18.92% of the phages (Eco-phage 2, 10, 14, 20, 28, 35 and 36) were lysogenic and did not form clear zone against their host cells. The isolated phages were able to lyse minimum 1 to maximum 8 of 36 strains tested with highly variable host ranges. Host

ranges of some phages, Eco-Phage-7, 11, 13, 17, 21, 23, 24 30, 34 and 35 were wide and effective against 4 to 8 strains tested, but the remaining phages have narrow inhibitory spectrum, being just able to 1 to 3 strains tested (Table 3). Eco-phage-13, 17, 24 and 34 lysed 80 % of the *E. coli* O157:H7 (4 out of 5 different strains) and 12 Eco-phage were able to lyse 60% of the *E. coli* O157:H7 strains tested (3 out of 5 different strains) (Table 3).

It was observed that *E. coli* O157:H7 specific phages do not have strain specificity. The isolated total 37 phages were infective against at least one of the *E. coli* O157:H7 strains tested and 14 of 37 Eco-phages were highly infective against strains of *E. coli* O157:H7 NTCT 12900, ATCC 4388 and ATCC 35150 strains. Furthermore, 15 out of 37 Eco-phages (40.54%) were infective against other *E. coli* strains tested besides *E. coli* O157:H7 strains (Table 3).

Double layer agar testing against 20 strains of different serovars of *Salmonella* was used to investigate the cross-species infectivity of each phage. Phage lysis of *Salmonella* ranged from 0 to 50% of the 20 strains tested (Table 3 and 4). Eco-phage-12 and 23 were effective against two different *Salmonella* Enteritidis strains (Table 3), Eco-phage-13 was effective against one *Salmonella* Typhimurium strain (Table 4), and Eco-phage-11, 13, 17, 21, 23, 30 and 34 effective against different other *Salmonella enterica* serovars (Table 4). None of the isolated Eco-phages were found to be infective against *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus cereus*, *Yersinia enterocolitica*, *Citrobacter freundii*, *Enterobacter aerogenes* and *Enterococcus faecalis*.

Table 3 Host ranges of *E. coli* O157:H7 specific phages on *E. coli* strains and *Salmonella* Enteritidis

EP	<i>E. coli</i> O157:H7					<i>E. coli</i>					<i>Salmonella</i> Enteritidis				
	12900	43888	35150	AİBÜ	RSKK	DSα	BL21	CFAI	25922	DMC8	DMC22	13075	411	512	742
1	+++	+++	++-	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	+++	-	-	-	-	-	-	-	-	-	-
3	+++	+++	+++	-	-	-	-	-	-	-	-	-	-	-	-
4	-	-	+++	-	+++	-	-	-	-	-	-	-	-	-	-
5	+++	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	+++	+++	-	-	-	-	-	-	+++	-	-	-	-	-	-
7	+++	-	-	-	+++	+++	++-	++-	-	-	-	-	-	-	-
8	-	-	+++	-	-	-	-	-	-	-	-	-	-	-	-
9	-	-	-	-	+++	-	-	-	-	-	-	-	-	-	-
10	-	-	-	+++	-	-	-	-	-	-	-	-	-	-	-
11	+++	++-	+++	-	-	-	-	-	-	-	-	-	-	-	-
12	+++	+++	+++	-	-	-	-	-	-	-	-	-	-	-	-
13	+++	+++	+++	+++	-	-	-	-	-	-	-	-	++-	-	-
14	-	-	-	-	+++	-	-	+--	-	-	-	-	-	-	-
15	-	-	-	-	+++	-	-	-	-	-	-	-	-	-	-
16	+++	+++	+++	-	-	-	-	-	-	-	-	-	-	-	-
17	++-	+--	++-	-	+++	-	-	-	+++	-	-	-	-	-	-
18	-	-	+++	-	-	-	-	-	-	-	-	-	-	-	-
19	+++	+++	+++	-	-	-	-	-	-	-	-	-	-	-	-
20	-	-	-	-	+++	-	-	-	-	-	-	-	-	-	-
21	++-	+++	+++	-	-	-	-	-	-	-	-	-	-	-	-
22	+++	+++	+++	-	-	-	-	-	-	-	-	-	-	-	-
23	+++	+++	+--	-	-	-	-	-	+++	+++	-	-	-	-	-
24	+++	+++	+++	-	+--	-	-	-	-	-	-	-	-	-	-
25	-	-	-	-	+++	-	+--	-	-	-	-	-	-	-	-
26	+++	+++	+++	-	-	-	-	-	-	-	-	-	-	-	-
27	-	-	-	+++	-	-	-	+++	-	-	-	-	-	-	-
28	-	-	-	-	+++	-	-	-	+++	-	-	-	-	-	-
29	-	-	-	+++	-	-	-	+++	-	-	-	-	-	-	-
30	-	-	-	-	+++	+++	-	-	++-	-	-	-	-	-	-
31	-	-	-	+++	-	-	-	-	+++	-	-	-	-	-	-
32	-	-	+++	-	-	-	-	+--	+++	-	-	-	-	-	-
33	-	-	-	+++	-	-	-	-	-	-	-	-	-	-	-
34	+++	+++	+++	-	+++	-	-	-	-	-	-	-	-	-	-
35	-	-	-	-	+++	+++	-	+++	+++	-	-	-	-	-	-
36	-	-	-	+++	-	-	-	-	+++	-	-	-	-	-	-
37	+++	+++	-	-	-	-	-	-	++-	-	-	-	-	-	-

EP: Eco-Phage, +++, 10², 10⁴ and 10⁶ diluted phage samples were inhibitor positive; ++-, 10², 10⁴ diluted phage samples were inhibitor positive but 10⁶ dilution sample was inhibitor negative; +--, 10² dilution sample was inhibitor positive, 10⁴ and 10⁶ dilution sample was inhibitor negative; -, 10², 10⁴ and 10⁶ diluted phage samples were inhibitor negative

Wide host range phages specific to *E. coli* O157:H7 strains with clear plaques were predominantly isolated from wastewater from food processing and slaughterhouse, sewage and troughs. Eco-phage-13 had the broadest host range, with lytic ability against 8 different bacterial strains (lysed 22.22% of the strains tested), 4 of them from *E. coli* O157:H7, 1 from *Salmonella* Enteritidis (Table 3), 1 from *Salmonella* Typhimurium, and 2 from other *Salmonella* serovars (Table 4). This was followed by Eco-phage-23, 7, 34, 11, and 21, being infective against 4 to 6 different bacterial strains (Table 3 and 4).

Host specificity is a common property of bacteriophages, but, it is a restrictive factor in their usage in food industry to prevent and/or inhibit the growth of foodborne pathogenic bacteria (Bielke et al., 2007). In our study, many isolated Eco-phages showed wide host ranges, being infective against at least 3 different *E. coli* O157:H7 strains tested.

Viazis et al. (2011) informed that lytic ability of 70 EHEC bacteriophages ranged from 5.3 to 31.6% and 8 out of 70 phages (38, 39, 41, AR1, 42, CEV2, ECB7 and ECA1) lysed from 94 to 98% of the *E. coli* O157:H7 strains screened using spot testing. The same researchers also determined the cross-genus activity of the EHEC phages against the 27 strains of different serovars of *Salmonella* and they found that phage ECB7 lysed 48.2% of the *Salmonella* strains, while the rest of the phage lysed from none to 22.2% of the strains of this pathogen. Villegas et al. (2009) reported that although the EHEC O157:H7-specific bacteriophage wV8 had genome characteristics very similar to the *Salmonella* specific phage Felix O1, it could not lyse any strains from 12 *Salmonella* serovars through spot testing.

Raya et al. (2006) reported that CEV1 phage was active against 17/19 *E. coli* O157:H7 strains tested, but not active 9 strains from 4/5 phylogenetic groups of the *E. coli* reference (ECOR) collection, lab strains B and K-12,

and strains from serogroups O43, O126, O153, O158, and *Salmonella enterica* serovars tested. Conversely, Ibrahim (1969) found that *E. coli* phages were also effective against some *Salmonella* serovars besides their host cells.

Some researchers reported that phages specific to one *E. coli* O157:H7 strain can also infect other O157:H7 strains (Bach et al., 2003; Litt and Jaroni, 2017; Raya et al., 2006; Sheng et al., 2006; Viazis et al. 2011). ϕ 241

phage, specific for *E. coli* O157:H7, is O157 antigen-specific and therefore just only infect strains that have O157 antigen, and *E. coli* strains lacking O157 antigen were resistant to the phage infection, regardless of the presence or absence of H7 antigen (Lui and Breidth, 2015). Results from the present study are similar to these studies, revealing that isolated phages are virulent against other *E. coli* O157:H7 strains with high target specificity.

Table 4 Host ranges of *E. coli* O157:H7 specific phages on *Salmonella* Typhimurium and other *Salmonella enterica* serovars

EP	<i>Salmonella</i> Typhimurium						Other <i>Salmonella enterica</i> serovars							
	LT-SRII	Tr90	AİBÜ	Wild type	625	14028	S. Virchow DMC8	S. Infantis DMC7	S. Thompson DMC47	S. Anatum DMC90	S. Telaviv DMC62	S. Montavide DMC81	S. Kentucky DMC35	S. Carvalis DMC86
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	-	-	-	-	-	-	-	-	-	-	-	-	+++	-
12	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	-	-	-	-	-	+++	-	-	+++	-	-	-	+++	-
14	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	-	-	-	-	-	-	-	-	-	-	-	-	+++	-
18	-	-	-	-	-	-	-	-	-	-	-	-	-	-
19	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	-	-	-	-	-	-	-	-	-	-	-	-	+++	-
22	-	-	-	-	-	-	-	-	-	-	-	-	-	-
23	-	-	-	-	-	-	-	-	-	-	-	-	+++	-
24	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25	-	-	-	-	-	-	-	-	-	-	-	-	-	-
26	-	-	-	-	-	-	-	-	-	-	-	-	-	-
27	-	-	-	-	-	-	-	-	-	-	-	-	-	-
28	-	-	-	-	-	-	-	-	-	-	-	-	-	-
29	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30	-	-	-	-	-	-	-	-	-	-	-	-	+++	-
31	-	-	-	-	-	-	-	-	-	-	-	-	-	-
32	-	-	-	-	-	-	-	-	-	-	-	-	-	-
33	-	-	-	-	-	-	-	-	-	-	-	-	-	-
34	-	-	-	-	-	-	-	-	-	-	-	-	+++	-
35	-	-	-	-	-	-	-	-	-	-	-	-	-	-
36	-	-	-	-	-	-	-	-	-	-	-	-	-	-
37	-	-	-	-	-	-	-	-	-	-	-	-	-	-

EP: Eco-Phage, +++, 10⁻², 10⁻⁴ and 10⁻⁶ diluted phage samples were inhibitor positive; ++, 10⁻², 10⁻⁴ diluted phage samples were inhibitor positive but 10⁻⁶ dilution sample was inhibitor negative; +-, 10⁻² dilution sample was inhibitor positive, 10⁻⁴ and 10⁻⁶ dilution sample was inhibitor negative; -, 10⁻², 10⁻⁴ and 10⁻⁶ diluted phage samples were inhibitor negative

Conclusion

Thirty seven bacteriophages specific to *E. coli* O157:H7 were isolated from sewage and wastewaters of food processing, slaughterhouse and fisheries, stream and trough using spot agar test against 5 different *E. coli* O157:H7 strains. Phages specific to *E. coli* O157:H7

strains with wide host range and lytic activity were mainly isolated from wastewater from food processing and slaughterhouse, sewage and troughs. These *E. coli* O157:H7 specific phages have broad host ranges and were able to lyse 1 to 8 of 36 strains tested and 15 out of

37 Eco-phages (40.54%) were infective against other *E. coli* strains tested. Additionally, 8 out of 37 isolated *E. coli* O157:H7 specific phages were capable to lyse some *Salmonella enterica* serovars.

These *E. coli* O157:H7 specific phages can be used as a possible alternative to chemical antimicrobials against foodborne pathogenic bacterium *E. coli* O157:H7 and can be used as therapeutic agents against animal infections caused by *E. coli* O157:H7.

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