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LYTIC BACTERIOPHAGES EFFECTIVE AGAINST *ESCHERICHIA COLI* O157:H7, A FOODBORNE PATHOGEN

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ABSTRACT
Bacteriophages are considered as alternative antibacterial agents in the food industry and phage therapy due to the widespread of multidrug-resistant pathogen bacteria. The objective of this study was to isolate lytic bacteriophages target to foodborne pathogen *Escherichia coli* O157:H7. For screening of bacteriophages, 51 samples were collected from sewage, wastewaters of fish farms, slaughterhouses and food factories, and 18 bacteriophages were isolated. The titer of the purified phages samples were changed among 1.7×10^4-1.02×10^8 PFU/ml. All of the isolated phages had lytic activity against *E. coli* O157:H7 strains and thus formed clear plaques. Four of 18 phages were found to have inhibitory effects against other *E. coli* strains including CFAI, ATCC 25922 and DSα in addition to *E. coli* O157:H7. It was observed that only two phages were infective against *Salmonella* Kentucky DMC35. The Eco-OH-phages were highly infection ability with EOP values from 0.5 to 0.1 against *E. coli* O157:H7 strains.
Keywords: Bacteriophage, *Escherichia coli* O157:H7, isolation, host range, efficiency of plating

GIDA KAYNAKLI PATOJEN *ESCHERICHIA COLI* O157:H7'YE KARŞI ETKİLİ BAKTERİYOFAJLAR

ÖZ
Bakteriyofajlar, çoklu ilaç dirençli patojen bakterilerin yaygın olması nedeniyle gıda endüstrisinde ve faj terapisinde alternatif antibakteriyel ajanlar olarak kabul edilmektedirler. Bu çalışmanın amacı, gıda kaynaklı patojen *Escherichia coli* O157:H7'ye etkili litik bakteriyofajları izole etmektir. Bakteriyofajların taranması için kanalizasyon, balık çiftliklerinin, kesimhanelerin ve gıda fabrikalarının atık sularından toplam 51 örnek toplanmış ve 18 bakteriyofaj izole edilmiştir. Saflaştırılmış faj örneklerin titreleri 1.7×10^4-1.02×10^8 PFU/ml arasında değişmiştir. İzole edilen fajların tümü, *E. coli* O157: H7 suşlarına karşı litik aktiviteye sahip olduğu ve bu nedenle berrak plaklar oluşturduğu gözlenmiştir. On sekiz fajdan dördünün, *E. coli* O157:H7'ye ek olarak CFAI, ATCC 25922 ve DSa dâhil olmak üzere diğer *E. coli* suşlarına karşı enfektif oldukları bulunmuştur. Sadece iki fajın *Salmonella* Kentucky DMC35'e karşı enfektif olduğu gözlenmiştir. Eco-OH-fajları, *E. coli* O157:H7 suşlarına karşı 0.5 ila 0.1 arasında EOP değerleri ile yüksek enfeksiyon kabiliyetine sahip olduğu bulunmuştur.
Anahtar kelimeler: Bakteriyofaj, *Escherichia coli* O157:H7, izolasyon, konak hücre aralığı, plak etkinliği

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INTRODUCTION

E. coli, a member of Enterobacteriaceae, is a Gram-negative, oxidase-negative, catalase-positive, generally motile, facultative anaerobic and rod-shaped bacterium. Most *E. coli* strains are nonpathogenic and colonize harmlessly in the intestinal tract of warm-blooded animals and humans as a normal flora. However, some strains of *E. coli* are pathogenic to humans and cause foodborne illness. Enterohemorrhagic *E. coli* (EHEC), shiga toxin (Stxs) producer, is defined as the pathogenic *E. coli* causing hemorrhagic colitis and hemolytic uremic syndrome. The serotype in the EHEC group that often cause diseases in humans are O157:H7. *E. coli* O157:H7 is an important foodborne pathogen that lives commensally in the rumen of food animals. It spreads to the soil and water through feces, and from these, it passes to foods such as fruit, vegetables and meat and contaminates them. *E. coli* O157:H7 outbreaks have been associated with beef, uncooked sausages, raw milk and dairy products, cider, sprouts, lettuce and spinach. *E. coli* O157:H7 infections often result in enterohemorrhagic enteritis, bloody diarrhea and hemolytic uremic syndrome (Callaway et al. 2006; Lim et al., 2010; Estrada-Garcia et al., 2013; Jang et al., 2017; CDC, 2019). *E. coli* O157:H7 infection is a major public health concern in the world since it can survive and persist in numerous environments such as soil, water, and food as well as in animal reservoirs. Also, *E. coli* O157:H7 resists acidic conditions using different acidic resistance systems. Briefly, this pathogen survives well in diverse environments, from its natural reservoir in healthy cattle to the farm environment and food (Lim et al., 2010).

Nowadays, the food industry and the pharmaceutical industry are in search of new products or methods, as consumers prefer foods that do not contain chemical preservatives and the number and proportion of antibiotic resistant pathogenic bacteria increase. Bacteriophages are one of the most important agents that can be alternatives to chemical preservatives and antibiotics. Bacteriophages or phages are one of the member of viruses, and they only infect bacterial cells without any negative effect on human or animal cells. They are extremely abundant in the ecosystem and found everywhere where the bacteria are located. Their proliferation depends on infecting the host bacterial cell and using the bacterial intracellular synthetic machinery of their host cell. In other word, they are mandatory parasites that need the host cellular machinery to proliferate. Virulent or lytic bacteriophages can cause the lysis of host bacteria; therefore, they are natural biopreservative agents for the control of bacterial pathogens in food and therapy agents to kill pathogenic bacteria that cause illness in animals. Phage therapy is widely being reconsidered as an alternative to antibiotics. The phage preparations that are currently approved by the FDA and tracked for use in the food industry and in the prevention of bacterial infections in animals are Listex™ P100 and Listshield™ (*Listeria monocytogenes*), EcoShield™ (*E. coli*), SalmoFresh (*Salmonella*) and SalmoPro® (*Salmonella*). These approvals by the FDA have accelerated research on the isolation and use of phages against foodborne pathogens (Mahony et al., 2011; Sarhan and Azzazy, 2015).

The range of bacteria that phages can infect is different. Some phages can infect only one or a few strains, while others can infect bacteria of different genus (Ross et al., 2016). Differences in the host cell range of phages are determined by specific phage receptors located on the surface of the host cell (OmpC, lipopolysaccharide O-antigen, lipoteichoic acid etc.) (Letarov and Kulikov, 2017).

Nearly a century ago, d'Herelle (1919) showed the effectiveness of bacteriophages against pathogen bacteria. Due to the emergence of antibiotic resistant bacteria today, phage biocontrol and phage therapy have become popular again. Multidrug-resistant *E. coli* O157:H7 strains were isolated from human clinical samples and foods and become life threatens (Meng et al., 1998; Schmidt et al., 1998; Zhao et al., 2001). Studies conducted under in vitro conditions have demonstrated that lytic bacteriophages very effectively inhibit the growth of *E. coli* O157:H7 in the different foods including meat (Tomat et

al., 2013), vegetables (O'Flynn et al., 2004; Tanji et al., 2005; Raya et al. 2006; Abuladze et al., 2008; Sharma et al., 2009; Viasis et al., 2011a; Hudson et al., 2013).

In the food industry and phage therapy, it is important to isolate new phages with high lytic activity and a wide range of target cells and to create adequate phage stocks against pathogenic bacteria. This study was conducted to isolate new lytic bacteriophages with wide host range using *E. coli* O157:H7 strains as host system from sewage and wastewaters from different sources.

MATERIALS AND METHOD

Bacterial strains and culture conditions

E. coli O157:H7 NCTC 12900, *E. coli* O157:H7 ATCC 43888, *E. coli* O157:H7 ATCC 35150 *E. coli* O157:H7 RSKK and *E. coli* O157 AİBÜ were used as host bacterial cells to isolate phages. *E. coli* O157:H7 strains were cultivated in brain heart infusion broth (BHI) (Lab M, Lancashire, UK) and stored at -80°C in BHI broth with 20% glycerol.

Collection of wastewater

Samples used for bacteriophage screening were collected from sewage, ponds, stream, sea and wastewaters of fish farms, slaughterhouses and food factories in Niğde, Ordu, İzmir, Bursa, Adana, and Kayseri provinces and kept at 4°C before processing. The water samples were processed using the method given by Yıldırım et al. (2018). Briefly, after water samples were centrifuged at 8000 × g for 15 min, the supernatants were collected and re-centrifuged at 8000 × g for 20 min. After the supernatants are passed through 4 layers of coarse filter paper, they were passed through 0.45 µm millipore syringe membrane filters (Sartorius, Germany). In the present study, 50 different wastewater samples and one raw milk sample were used to screen the lytic bacteriophages against five different *E. coli* O157:H7 strains.

Isolation of Bacteriophages

For isolation of bacteriophages, two different methods were used: direct isolation and enrichment method. In the direct isolation method, filtrate samples were directly used for the presence of lytic activity against *E. coli* O157:H7 strains by using spot agar test and the double agar layer plate method. In the enrichment method, 10 ml of the filtrate samples were inoculated with 2 ml of actively grown culture of five different *E. coli* O157:H7 strains in Luria–Bertani (LB) broth and mixed with 3 ml of double strength LB broth. After incubation at 35°C overnight, chloroform (50 µL/mL) was added into the samples and vigorously mixed. The samples were centrifuged at 8000 × *g* for 20 min and the supernatants were collected, filtered through 0.45 µm millipore syringe membrane filters and maintained at 5°C (Mclaughlin et al., 2006; Yildirim et al., 2018).

Bacteriophage Activity Test

The filtrate samples were tested for the presence of phages infecting *E. coli* O157:H7 NCTC 12900, *E. coli* O157:H7 ATCC 43888, *E. coli* O157:H7 ATCC 35150, *E. coli* O157:H7 RSKK and *E. coli* O157 AİBÜ by using spot agar overlay and double layer agar plaque assay (Adams, 1959).

For spot agar overlay, 15 µl of individual phage lysates was spotted onto a lawn of actively growing target bacterium cell. The plates were left to dry at room temperature for 30 min and then incubated at 35°C overnight. In samples showing the inhibition region in the spot test, a double layer agar plate assay was used to both confirm the presence of infective phage and to determine the titer of phage.

In the double layer agar plaque assay, 100 µl of filtrate was mixed with 300 µl of actively growing target bacterial culture in a sterile vial and added to test tube containing 4 ml of molten soft LB agar held at 45-47°C, and then poured onto LB agar basal plates. After incubation overnight at 35°C, the plates were examined for the presence of plaques or any lytic activity on bacterial lawn.

Purification of bacteriophages

Phage plagues were purified using serial dilution purification method. Briefly, an isolated phage plaque was taken using a sterile Pasteur pipette and placed in 100 µL SM (50 mM Tris-Cl, pH 7.5, 99 mM NaCl, 8 mM $MgSO_4$, 0.01% gelatin)

buffer and vortexed very well. After chloroform (50 µl/ml) extraction and centrifugation (9,000 × g, 20 min, 4°C), the supernatant was transferred to a new sterile tube. Actively grown host cells (OD_{600}=0.5) were infected with the serially diluted phage samples and incubated at room temperature for 15 min in order to allow adsorption of phages to host cell. Phage-host cell mixture was added to 4 ml of 47-50°C LB soft agar (0.6% agar) and overlaid onto LB agar plates. The plates were incubated overnight at 35°C. To ensure that each plaque originated from a single bacteriophage type, the plaques were purified by at least four rounds of dilution and re-isolation.

Phage stocks preparation

For the assay, 1 ml of *E. coli* O157:H7 culture and 100 µL of the purified phage sample were added into 100 mL of LB broth and incubated overnight at 35°C. At the end of incubation, chloroform (50 µL/mL) was added and then they were centrifuged at 9,000 × g for 15 min. The supernatants were collected and filtered with a disposable 0.45 µm pore size syringe membrane filter (Sartorius, Germany). The double-layer plaque titration method was used to determine the number of phage in each sample. Bacteriophage lysates were stored at 4°C over chloroform.

Host range of bacteriophages

To determine the host range, the isolated bacteriophages were tested 37 strains/serovars of pathogenic bacteria including, *E. coli* O157:H7 (n=5), other *E. coli* (n=4), *Salmonella* Entereditis (n=6), *S.* Typhimurium (n=5), other *Salmonella enterica* serovars (n=8), *Listeria monocytogenes* ATCC 19115, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 10875, *Yersinia enterocolitica* O:9 AÜ, *Citrobacter freundii* AÜ, *Enterobacter aerogenes* AÜ, *Enterococcus faecalis* ATCC 29212, *Bacillus cereus* ATCC 10875 and *Enterococcus faecalis* ATCC 29212 (Table 1). LB soft agar containing 300 µl of actively grown (OD_{600}=0.3) test bacterium was poured onto dried base plates. Subsequently, 10 µL of the 10^{-2}, 10^{-4}, 10^{-6} phage dilutions were spotted on the overlay and incubated at 35°C for 18 h. At the end of the incubation, the plates were examined in terms of whether plaques were formed. In other word, the interaction of bacteriophage with target bacteria has been revealed as the presence of plaque formation. Phage plaques were evaluated for lysis profile and clarity.

Table 1. Titer of the isolated phage samples

Phage positive samples	Host cell *E. coli* O157:H7 strain	Phage titers of the samples (PFU/mL)	Phage code	Phage titer of the purified samples (PFU/mL)
Wastewater of milk plant-1	ATCC 35150	20.8×10^{6}	Eco-OH1	6.80×10^{9}
Wastewater of slaughterhouse-1	NCTC 12900	1.70×10^{4}	Eco-OH2	2.60×10^{8}
Wastewater of fish farm-1	NCTC 12900	3.50×10^{5}	Eco-OH3	4.90×10^{8}
Wastewater of slaughterhouse-2	NCTC 12900	5.60×10^{4}	Eco-OH4	2.60×10^{8}
Sea-1	ATCC 35150	3.35×10^{6}	Eco-OH7	3.70×10^{9}
Sea-2	ATCC 43888	5.75×10^{5}	Eco-OH9	9.20×10^{10}
Sewage-1	NCTC 12900	3.55×10^{7}	Eco-OH10	2.00×10^{10}
Sewage-1	ATCC 43888	3.95×10^{7}	Eco-OH11	2.60×10^{9}
Sewage-2	ATCC 35150	1.02×10^{8}	Eco-OH13	2.10×10^{9}
Pond-1	ATCC 43888	2.65×10^{6}	Eco-OH14	6.50×10^{9}
Wastewater of fish farm-2	ATCC 35150	5.85×10^{6}	Eco-OH15	1.28×10^{10}
Sewage-3	NCTC 12900	4.40×10^{7}	Eco-OH16	1.80×10^{10}
Sewage-3	ATCC 43888	2.00×10^{7}	Eco-OH17	9.10×10^{10}
Sewage-4	ATCC 35150	2.80×10^{4}	Eco-OH18	3.00×10^{8}
Sewage-5	ATCC 35150	6.55×10^{6}	Eco-OH19	2.50×10^{9}
Wastewater of slaughterhouse-3	NCTC 12900	2.80×10^{6}	Eco-OH20	4.40×10^{9}
Sewage-6	NCTC 12900	3.60×10^{7}	Eco-OH21	5.90×10^{8}
Stream-1	ATCC 43888	7.85×10^{5}	Eco-OH22	1.13×10^{9}

Efficiency of plating (EOP)

In this analysis, only phages that were effective against other bacteria in addition to their host cell were used. All bacterial strains to be tested were cultivated overnight (18 hours) at 35°C and 200 µl of each of those cultures (OD_{600}=0.4) was used in double layer plaque assays together with 100 µl of diluted phage lysate. After incubation overnight at 35°C, the number of phage plaque was counted. The EOP test for a particular phage was performed in parallel to all the bacterial strains tested. EOP was calculated as follows average the number of phage plaque on the test bacterium/average the number of phage plaque on the host bacterium. EOP was classified as "high", "moderate", and "low" efficiency based on the productive infection on the target bacterium. EOP was considered as "high" efficiency only if the phage-bacterium combination against the test bacterium had a productive infection of at least 50% (EOP ≥ 0.5) compared to the host bacterium; 0.1>EOP< 0.5 was recorded "moderate" efficiency; 0.01<EOP≤ 0.1 was recorded as "low" efficiency; and EOP< 0.01 was considered as inefficient (Mirzaei and Nilsson, 2015).

Statistical analysis

Statistical analysis of the data gotten from three replications was accomplished using SPSS (version 11.0; SPSS, Chicago, IL, USA) package program and variance analysis (ANOVA).

RESULTS AND DISCUSSION

The basic approach in bacteriophage isolation is to obtain an environmental sample that is likely to contain a high percentage of the target bacteria. Phages are reported to be the most abundant organism on earth, and their total number on the planet is estimated to be more than 10^{31}. Since phages are a mandatory requirement for a host, their abundance and distribution are likely to rely on host organisms. Therefore, in order to understand the viral abundance, it is necessary to determine where most of their hosts are located. The most common locations of bacteria are human and animal intestines, sewage, soil, wastewater and marine environments (Abedon, 2008; Khan and Nilsson, 2015; Xu et al., 2016; Yu et al., 2016; Clokie et al., 2019).

In this study, total 50 wastewater samples and 1 raw milk sample were used for screening lytic phages infective against foodborne pathogen *E. coli* O157:H7. 10 of 50 the samples were obtained from river/stream water, 6 from sewage water, 7 from wastewater of fish farms, 3 from wastewater of food processing plants, 13 from wastewater of slaughterhouse, 3 from pond water, 2 from sea, 1 from hospital wastewater and 1 from swamp. Of the 50 samples examined, only 17 samples produced lytic plagues against *E. coli* O157:H7 strains tested (Table 1), and 7 samples yielded lysogenic (turbid) plaques. In the remaining 28 samples, turbid or lytic plagues were not detected.

As a result of phage screening, samples containing bacteriophage specific to *E. coli* O157: H7 are presented in Table 1. The isolated phages were designated as Eco-OH1-22. It was observed that sewage samples were good sources of lytic bacteriophages (Table 1). Many researchers have reported that various wastewaters, especially sewage, are the best source of bacteriophages specific for foodborne pathogen bacteria such as *E. coli, Salmonella* (Oot et al., 2007; Niu et al. 2009; Synnott et al., 2009; Viazis et al., 2011; Raya et al., 2011; Hudson et al., 2013; Litt and Jaroni, 2017; Yıldırım et al., 2018; Xu et al., 2019). *E. coli* O157:H7-specific phage positive samples were given Fig. 1. It was determined that the phage titers of the screened samples varied between 1.70×10^{4}-1.02×10^{8} PFU/mL (Table 1).

The raw milk sample tested was found to have an infective effect against four different *E. coli* O157:H7 strains (Fig. 1b). When the serial dilutions prepared from the raw milk sample were tested against *E. coli* strains, the infective effect disappeared. In other word, the serial dilutions of the raw milk sample did not show inhibitory activity against any *E. coli* O157:H7 strains (Fig. 1c). Therefore, it was assumed that the infective effect of raw milk was not due to phages, but to the preservative added to raw milk.

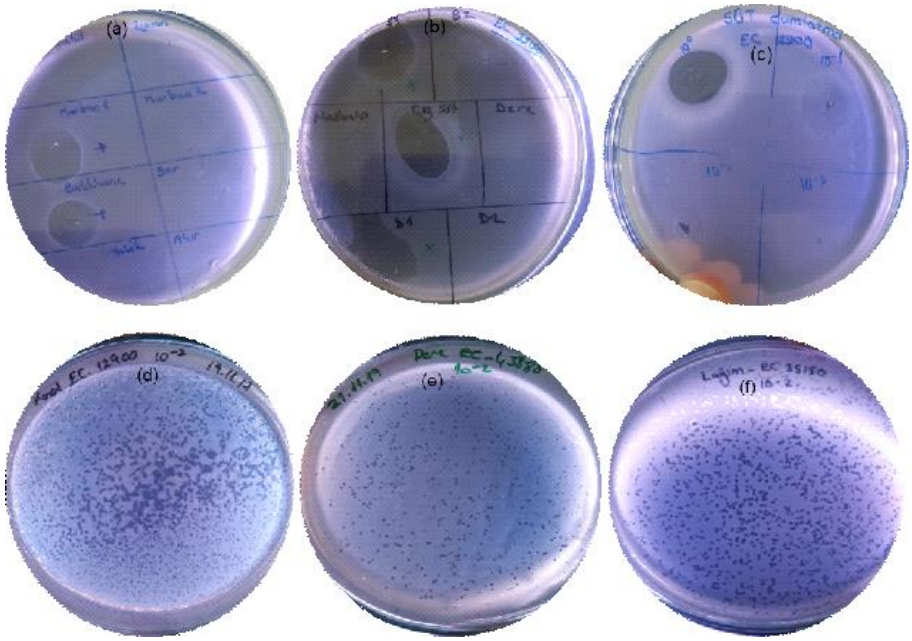

Fig. 1. *E. coli* O157:H7-specific phage positive samples. Samples tested against *E. coli* O157:H7 NCTC 12900, *E. coli* O157:H7 ATCC 43888 and *E. coli* O157:H7 ATCC 35150 strains. (a) samples from sea, wastewaters of fish farms and slaughterhouse; (b) samples from sewage, pond, slaughterhouse wastewater, milk, stream and wastewater of food factories; (c) dilutions of milk sample; (d) sewage sample; (e) stream sample; (f) sewage sample.

Isolated lytic bacteriophages infective against *E. coli* O157:H7 were purified by using single plaque method and then stock samples were prepared (Adam, 1958). The titer of the purified crude phage samples were among 2.6×10^{8}-9.2×10^{10} PFU/mL (Table 1).

It is usually believed that many phages can only infect a closely related spectrum of bacteria (Weinbauer, 2004). This is due to many factors including the specificity of host-binding proteins of phages, biochemical interactions during infection, and bacterial phage-resistance mechanisms (Hyman and Abedon, 2010). The host specificity of bacteriophages is a limiting factor in their use in the food industry and therapy to prevent the growth of pathogenic bacteria. Therefore, for food biopreservation and phage therapy, host cell killing tends to be the key determination. Different methods are used to measure the host cell range. Hyman and Abedon (2010) defined seven different types of host range including adsorptive, penetrative, bactericidal, productive, plaguing, spotting and lysogenic. There are two common ways of determining whether the phage is able to infect: plaguing and spotting (Hyman and Abedon, 2010; Abedon, 2011). In the plaguing technique, serial dilutions of phage lysate are mixed with target bacteria and placed on plate surface by soft agar overlay while in spot testing, a small volume of phage lysate is placed on a growing lawn of bacteria. Both method depends on whether a phage is able to form plaques on a particular species or strain of host bacteria. Both methods were used in order to accurately determine the host cell range of the phages. 10^{0}, 10^{-2}, 10^{-4} and 10^{-6} dilutions of phage samples were used to determine the host cell range by spot assay and the results of phage host range were shown in Table 2. As seen from the table, the *E. coli* O157:H7 specific phages were mostly effective on *E. coli* O157:H7 NCTC 12900, ATCC43888 and ATCC 35150 strains and none of the phage samples had an inhibitory effect on *E. coli* O157:H7 RSKK. Only two phages showed inhibitory effect against *E. coli* O157:H7 AİBÜ.

The phages encoded Eco-OH7, Eco-OH9, Eco-OH14, Eco-OH15 and Eco-OH22 were effective against other *E. coli* strains (Table 2). Furthermore, it has been observed that Eco-OH19 and OH20 phages were effective on some *Salmonella* serovars in addition to *E. coli* strains (Table 3). The remaining phages were infective only on *E. coli* O157:H7 strains. It was determined that the Eco-OH9 and Eco-OH14 phages had the largest host cell range. Eco-OHP9 and Eco-OH14 phage was infective against 5 different bacteria, including 3 *E. coli* O157:H7 strains and 2 other *E. coli* strains. Many phages such as Eco-OH1, Eco-OH7, Eco-OH9, Eco-OH10, Eco-OH11, Eco-OH13, Eco-OH14, Eco-OH16, Eco-OH19-OH22 have been found to have a strong infective effect against *E. coli* strains even at low concentrations (10^{-4} and 10^{-6} dilution) of the phage samples. These results show that Eco-OH phages have strong lytic activity against their host cells. Examples of inhibitory effects of some phages against host cells were given in Fig. 2. None of the phages isolated in the present study were capable of lysing *Listeria monocytogenes* ATCC 19115, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 10875, *Yersinia enterocolitica* O:9 AÜ, *Citrobacter freundii* AÜ, *Enterobacter aerogenes* AÜ and *Enterococcus faecalis* ATCC 29212.

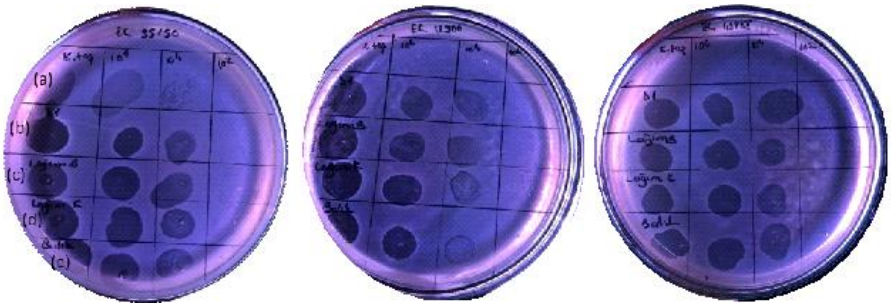

Fig. 2. Infective effect of Eco-OH18 (a), Eco-OH7 (b), Eco-OH12 (c), Eco-OH13 (d) ve Eco-OH15 (e) against *E. coli* O157:H7 ATCC 35150, NCCT 12900 and ATCC 43888, respectively.

Topka et al. (2019) reported that vB-EcoS-95 phage was able to infect different *E. coli* strains, but, it was not able to infect *E. coli* O157:H7 strains and other bacterial species, including *Shigella flexneri* or *Salmonella enterica, Pseudomonas aeruginosa, Enterococcus faecalis, Staphylococcus sciuri*. ϕ241 phage (Lu and Breidth, 2015) and FAHEc1 phage (Hudson et al., 2013) isolated from sewage specific to *E. coli* O157:H7 only infect *E. coli* strains that have O157 antigen.

Some researchers reported that bacteriophages target to one *E. coli* O157:H7 strain can also infect other O157:H7 strains (Bach et al., 2003; Raya et al., 2006; Sheng et al., 2006; Viazis et al. 2011; Litt and Jaroni, 2017; Yıldırım et al., 2018).

Viazis et al. (2011b) informed that 38, 39, 41, AR1, 42, CEV2, ECB7 and ECA1 bacteriophages specific to *E. coli* O157:H7 lysed different strains of *E. coli* O157:H7 and also the phage ECB7 lysed 48.2% of the *Salmonella* strains. CEV1 phage target to *E. coli* O157:H7 was also active against *Salmonella enterica* serovars tested in addition to *E. coli* strains (Raya et al., 2006). Ibrahim (1969) reported that *E. coli* phages were also effective against some *Salmonella* serovars.

Liao et al. (2019) stated that vB_EcoM-Ro111lw and vB_EcoM-Ro121lw phages were infective against multiple serogroups of STEC strains in addition to generic *E. coli* ATCC 13706, ATCC 43888 and DH5a strains, whereas vB_EcoS-Ro145lw and vB_EcoM-Ro157lw had narrow host ranges. They were only infective against Shiga toxin producing *E. coli* O145 strains, but not effective against *E. coli* O157:H7 strains and *Salmonella* serovars tested. In addition, EOP results revealed that vB_EcoM-Ro111lw was the only phage showing medium or high infection efficiency against different serogroups (O26 and O111) of STEC strains.

vB_Eco4M-7 and ECML-117 phages were effective in infecting most *E. coli* O157 strains, including various *E. coli* O157:H7 strains, but not infective against the tested *Shigella flexneri, Salmonella enterica, Bacillus* sp., *Pseudomonas aeruginosa, Enterococcus faecium*, *Staphylococcus aureus, Klebsiella* sp., and *Acinetobacter* sp. strains (Necel et al., 2020). Chang and Kim (2011) reported that EP23 phage infected the three *E. coli* strains and two *Shigella sonnei* strains.

Swap et al. (2018) informed that bacteriophage chee130_1 lysed enteropathogenic *E. coli*, enteroinvasive *E. coli,* enterotoxigenic *E. coli* and *Shigella sonnei* strains whereas bacteriophage 24 lysed *Salmonella* serovars, enterotoxigenic *E. coli,* enterohemorrhagic *E. coli* O103:H2, and *Shigella dysenteriae* strains. Both bacteriophages showed lysis on enterotoxigenic *E. coli* (ETEC) type strain H10407.

Results from the present study are similar to these studies, revealing that some isolated phages are infective against *E. coli* O157:H7 strains with high target specificity and some isolated phages are infective against other *E. coli* strains and some *Salmonella enterica* serovars in addition to *E. coli* O157:H7 strains.

EOP analysis was conducted only for the plaguing assay-positive strains. The twenty phages displaying a wide host range in the spot assays were subjected to a more thorough assessment of productive infection as defined by EOP. EOP analysis was performed to determine the effectiveness of each phage against a variety of target bacteria. EOP values of the phages were given in Table 4. It was determined that the Eco-OH-phages were highly infection ability with EOP values from 0.5 to 0.1 against *E. coli* O157:H7 strains. Nine of 20 phages were "highly" effective (EOP≥ 0.5) against 3 of the 5 *E. coli* O157:H7 strains tested. Eco-OH9, Eco-OH16 and Eco-OH17 phages were found to had medium infection efficiency to lyse ATTC 35150 strain, and Eco-OH7 and Eco-OH9 phages had medium infection efficiency to NCTC 12900 and ATTC 35150 strains, respectively. Eco-OH15 was the only phage that had medium EOP values against *E. coli* O157:H7 İABU strain. It was found that Eco-OH9 and Eco-OH14 had the widest spectrum of lytic ability. Eco-OH14 had the most efficient with a wide range of lysis ability among all the isolated phages since it showed high infection efficiency against *E. coli* O157:H7 NCTC 12900, *E. coli* O157:H7 ATTC 43888, *E. coli* O157:H7 ATTC 35150, and *E. coli* DSα, and medium infection efficiency against *E. coli* CFAI strain. Eco-OH9, Eco-OH14 and Eco-OH15 phages had the capacity to infect *E. coli* DSα strain with very high efficiency (EOP≥ 0.5).

CONCLUSION

The current study was conducted to isolate lytic bacteriophages infective against *E. coli* O157:H7 strains. Among the studied samples, it was determined that the richest in terms of bacteriophage specific to *E. coli* O157:H7 were sewage samples, followed by wastewaters from slaughterhouses and fish farms, respectively. Isolated some phages were found to be highly specific, thus infecting only *E. coli* O157:H7 strains, but some phages infect other bacteria in addition to *E. coli* O157:H7 strains. The isolated Eco-OH-phages were highly infection ability with EOP values from 0.5 to 0.1 against *E. coli* O157:H7 strains. As a conclusion, phages with good host cell ranges and good infection efficacy such as Eco-OH9 and Eco-OH14 can be good candidates in the control of *E. coli* O157:H7 in food industry and phage therapy.

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STATEMENT OF CONFLICT OF INTEREST

We declare that there are no conflicts of interest among the authors, among other authors related to this manuscript and/or other institutions/ councils etc.

AUTHORS' CONTRIBUTIONS

ZY and TSŞ designed the research. TSŞ and NNU collected wastewater samples. TSŞ screened

wastewater samples to isolate *E. coli* O157:H7 specific bacteriophages, purified the isolated bacteriophages and determined their titers and host ranges. NNU carried out efficiency of plating analysis. TSŞ and ZY made statistical analyzes. ZY wrote the paper. All authors have read and approved the final article.

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