Original Article

Iranian Journal of Medical Microbiology

Iran J Med Microbiol: Volume 13, Number 3 (July - August)

Journal homepage: www.ijmm.ir

Isolation of *Escherichia coli* Specific Lytic Phages from Wastewater and Evaluation of Its Antimicrobial Effect in Chicken Meat

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Article Information

Article Subject:
Food Microbiology

10.30699/ijmm.13.3.180

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Abstract

Background and Aims: Nowadays due to the harmfulness of chemical preservatives, the use of natural preservatives has increased. Bacteriophages are bacterial mandatory parasites that are harmless for human and animals and can, therefore, be used as appropriate antimicrobial agents in food. The aim of this study was to isolate the *Escherichia coli* lytic phage from wastewater, identify and evaluate its efficiency in controlling *E. coli* infection in chicken meat.

Materials and Methods: *Escherichia coli* (PTCC: 1330) was obtained from Iranian Science and Technology Research organization. Phage isolated from the wastewater of Malayer dairy factory and its antimicrobial effect was investigated through plaque formation. TEM microscopy was used to observe the phage morphology and to determine its possible family. Host spectrum against 6 *E. coli* strains and its effect against *Salmonella enterica*, *Staphylococcus aureus* and *Yersinia enterocolitica* were also evaluated .The effect of *E. coli* lytic phage on the amount of inoculated *E. coli* to contaminate the chicken meat was examined.

Results: The isolated phage was tailless and had round capsid, possibly belonging to the *Tectiviridae* family. The target phage had antimicrobial activity against 6 selected *E. coli* strains, unlike the other genera tested. The effect of the phage on the *E. coli* contamination in chicken meat showed that the bacterial count was reduced from 3 $\log_{10}$ to 1.8 $\log_{10}$ after 24 h and reached less than 1 log cycle after 4 days.

Conclusion: The isolated phage had strong antimicrobial effect against *E. coli*. Therefore, it can be a good preservative candidate for use in foods.

Keywords: Bacteriophage, *Escherichia coli*, Tectiviridae, Chicken

Received: 2019/05/14 Accepted: 2019/08/24 Available online: 2019/08/24

How to cite this article:

Faraji Kafshgari S, Maghsoodlou Y, Khomeiri M, kashiry M, Babaei A. Isolation of Escherichia coli specific Lytic Phages from Waste Water and evaluation of its antimicrobial effect In Vitro and Chicken meat . Iran J Med Microbiol. 2019; 13 (3) :180-193

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Introduction

Escherichia coli is one of the most important contaminants in chicken meat causing urinary tract infection, septicemia, neonatal meningitis and traveler's diarrhea as its most common complications (3,4). Therefore, it is necessary to develop new methods for the detection and control of this bacterium (5). Bacteriophages are bacterial specific viruses that have direct effect on target cells and specific function and do not infect eukaryotic cells as their advantages (10). On the other hand, their disadvantages are phage resistance, the possibility of virulent phage mutation and transformation to lysogenic phage (11). The aim of this study was to isolate the *E. coli* specific lytic phage and identify its possible family as well as to evaluate its efficacy to reduce *E. coli* in chicken meat.

Materials and Methods

E. coli bacteria PTCC (1330) and ATCC (33876, 35218, 25922), DHα5 (SCC: 1785) and BL21 (PTA: 5976); Staphylococcus aurous (ATCC: 2392), Yersinia enterocolitica (PTCC: 1785) and Salmonella enterica (ATCC: 14028) were used in this study.

Isolation, Purification and Storage of Phage

A total of 100 mL of wastewater was mixed with 50 mL of LB liquid medium, into which the host bacterium was inoculated. After incubation at 37°C and shaking in 100 rpm for 24 h, 50 mL of this solution was centrifuged for 15 min and the supernatant was then filtered with a 0.45 nm filter and then serial dilution was prepared. Then 100 µL of dilutions 6, 7 and 8 were mixed with 2.5 mL of melted LB agar medium and 200 µL primary bacteria and incubated in LB agar plate for 24 hours at 37°C. The lytic plaque with the same shape and size were selected and mixed with 500 µL LB medium and incubated for 15 minutes after which the serial dilution was prepared. 100 µL of each microtube was removed and mixed with 200 µL of the primary bacteria and 2.5 mL of LB agar medium and poured into LB agar plate and incubated at 37°C for 24 hours. From the stock phage in the broth, 500 µL was removed and mixed with 500 µL of sterile glycerol in the microtube. Phage storage was performed at -70°C (15).

Morphology and Detection of Phage Family by Transmission Electron Microscopy (TEM)

TEM imaging was performed to investigate of morphology of the isolated phage. The TEM images were matched with previously identified standard phages to identify their families (16).

Evaluation of Antimicrobial Efficacy of the Isolated Phage *in vitro*

After activation of *Escherichia coli* in LB medium, 100 µL of this suspension was added to 5 mL of semi-solid LB medium and then transferred to a plate and gave 15 minutes time to the medium to harden. The serial dilution was then prepared from phage and 10 µL of the phage solution was added to the surface of activated bacteria and spread on the plate surface and incubated for 24 hours at 37°C. The number of phage particles in the suspension was determined using the following equation (17).

The number of phage particles=

$$\frac{the\ number\ of\ pluqe}{dilition \times volume\ of\ phage\ suspension}\ (\frac{PFU}{ml})$$

Host Spectrum and Phage Specificity

Host spectrum and specificity of phage against 5 different strains of *E. coli* as well as *S. enterica*, *Yersinia enterocolitica* and *Staphylococcus aureus* species were evaluated by plaque assay (18).

Evaluation of the Isolated Phage Efficiency in Reducing *Escherichia coli* in Chicken Meat

A total of 25 g of chicken fillet was sterilized by gamma irradiation (KG 10) (19) and immersed into 100 mL of bacterial suspension (10^3 CFU/g). Phage solution (10^8 PFU/mL) was also added. Counting of living bacteria was immediately done in all samples after the addition of bacteria and phage (20).

Results

Isolation and Determination of Phage Particles

Between 10^8 and 10^{10} PFU/mL of *E. coli* specific phage was isolated from the wastewater which appeared as clear zones (lysed areas) in the culture medium.

Phage Morphology and Identification of Its Possible Family

The isolated phage was tailless and had a spherical capsid or head about 70-60 nm, and was probably a member of the *Tectiviridae* family (Fig 2).

Phage Specificity and Host Spectrum

Among of the 5 selected strains, the isolated phage had antimicrobial effect on the 4 out of the 5 tested strains of *E. coli* whilst had no antimicrobial effect on the other species tested in this article (Table 1).

Fig 1. The effect of isolated lytic phage on *Escherichia coli* and plaque formation

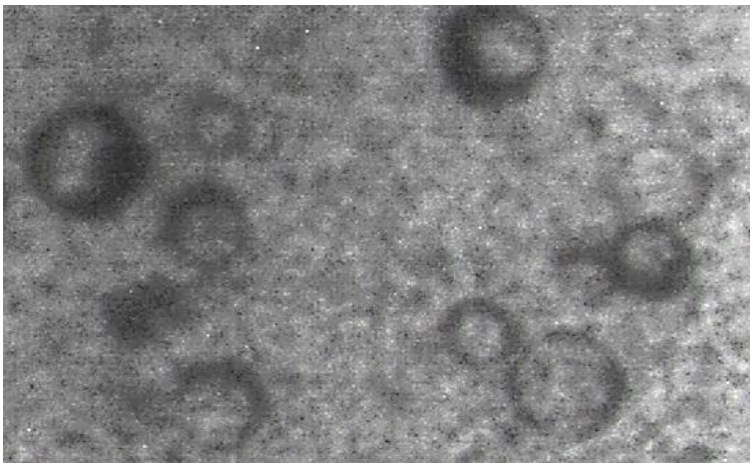

Fig 2. TEM of *Escherichia coli* lytic phage structure

Table 1. The activity of the isolated phage against different bacteria

Bacteria	Plaque
E. coli PTCC 1330	+
Salmonella enterica subsp. Enterica serovar typhimurium ATCC 14028	-
Staphylococcus aureus ATCC 2392	-
Yersinia enterocolitica subsp. enterocolitica PTCC 1785	-

Table 2. The activity of isolated phage against different strains of Escherichia coli

Strains of *Escherichia coli*		Plaque
E. coli PTCC	1330	+
E. coli ATCC	25922	+
E. coli ATCC	35218	-
E. coli ATCC	33876	+
E.coli DHα5 SCC	2197	+
E. coli BL21 PTA	5976	+

No antimicrobial effect – , Antimicrobial effect +

Evaluation of the Isolated Phage Antimicrobial Efficacy in Chicken Meat

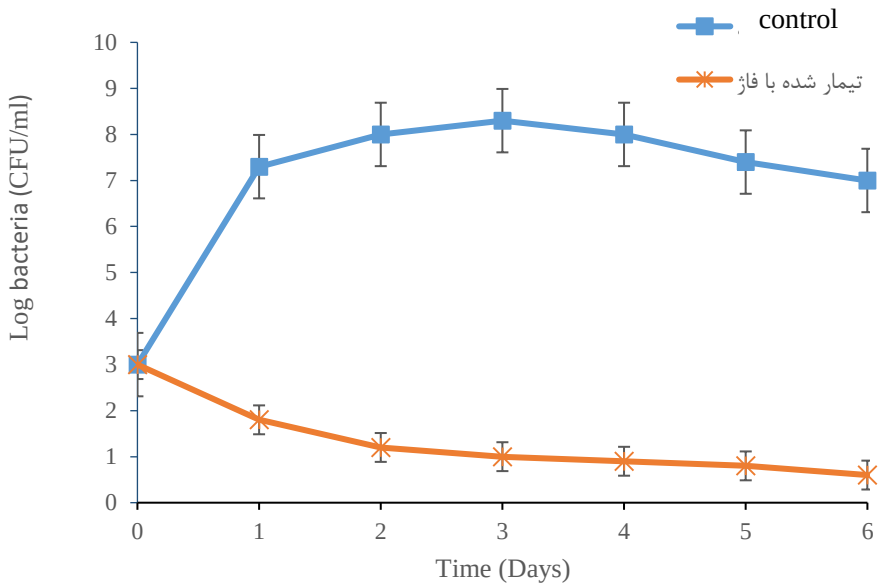

Fig 3. Effect of *Escherichia coli* specific lytic phage on *Escherichia coli* contamination in chicken

Evaluation of the Isolated Phage Antimicrobial Efficacy in Chicken Meat

The isolated phage reduced the bacterial population in chicken meat by 1.2 log in the first 24 h. The reduction of host bacterial continued with lower speed in other days. So that on the fourth day after inoculation, the *E. coli* levels reached approximately less than 1 log cycle.

Discussion

The aim of this study was to isolate, identify and evaluate the antimicrobial effect of *E. coli* specific lytic phage in chicken meat. The number of isolated phage particles was estimated to be. 10^8 to 10^{10} PFU/mL. The isolated phage had antimicrobial effect against 5 out of the 6 *E. coli* strains tested and had relatively broad host range and was probably a member of the *Tectiviridae*

family. In this study, the use of *E. coli* specific lytic phage (10^8 PFU/mL) in chicken meat decreased 1.2 log in the first 24 h after inoculation, and continued with lower speed to 48 h and then became almost constant, so that on the fourth day after inoculation, *E. coli* population reached approximately less than 1 log cycle. Generally, most of the discovered phages in the world have long or short tail and belong to the *podoviridae*, *myoviridae* and *syphoviridae* families and little information is available on the isolation and identification of tailless phages. Chai *et al.* (2016) isolated phage φHN161 from the wastewater that was tailless and had a spherical capsid which belonged to the *Tectiviridae* family. It had the potential to destroy *E. coli* O161. Moreover, the isolated phage in the present study was morphologically similar to the phage discovered by these researchers and therefore it is likely that it also belongs to the *Tectiviridae* family **(30)**. Fiorentin *et al.* (2005) used lytic phages of *S. enteritidis* (10^9 PFU/mL) to reduce the count number of *S. enteritidis* (10^6 CFU/mL) inoculated into chicken skin. According to their results, a significant decrease in the number of Salmonella bacteria was observed in the samples treated with phage after 3, 6 and 9 days **(32)**. The results of this study also showed a significant decrease in the population of *E. coli* inoculated into chicken meat after the phage treatment.

Conclusion

In this study, the isolated *E. coli* specific lytic phage from wastewater was tailless and had spherical capsid possibly belonging to the *Tectiviridae* family. This phage had antimicrobial effect on 4 out of the 5 *Escherichia coli* strains tested in this study but had no antimicrobial effect on *S. enterica*, *Yersinia enterocolitica* and *S. aureus* species. It also reduced the amount of chicken meat inoculated *E. coli* from 3 log to 1.8 log after 24 h and to less than 1 log cycle after 4 days. Therefore, it can be used as an *E. coli* biocontrol agent in foods.

Acknowledgments

The authors of this article thank of the department of Food Science and Engineering, Faculty of Food Industries, University of Agricultural Sciences and Natural Resources, and the department of Biology, University of Malayer.

Conflict of Interest

The authors reported no conflict of interest.