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Isolation of a bacteriophage with lytic activity on enteropathogenic *Escherichia coli* from a waste water source in Tehran

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ABSTRACT

Introduction: Enteropathogenic *Escherichia coli* (EPEC) strains cause a gastrointestinal disease in the developing countries. Over the years, antimicrobial resistance of EPEC have been a major issue. Bacteriophages have been a therapeutic option for bacterial infection. Our aim was to assay lytic activity of a siphophage on an EPEC strain**. Materials and Method:** EPEC strain ATCC 43887 was used for phage isolation using by double layer agar method. Bacteriophage morphology was visualized by transmission electron microscope. **Result:** a siphophage was detected by transmission electron microscope images. The phage formed small clear circular plaques. The results of lytic activity of siphophage on reference EPEC strain showed the phage could lyse the strain. **Conclusion:** the phage had lytic activity on reference EPEC strain. The phage belonged to siphoviridae family. It look the phage can lyse clinical EPEC strains.

Keywords: Antimicrobial resistance, Enteropathogenic *Escherichia coli*, Bacteriophage, Siphoviridae

1.Introduction

Enteropathogenic *Escherichia coli* (EPEC), a gram-negative bacterium and opportunistic pathogen, is one of the most common Enterobacteriacea family members, which are widely caused childhood gastrointestinal infection in developing countries(1). Often, EPEC-caused diarrhea has watery form with abdomen cramp in children. Apart from the frequency of EPEC strains, these strains have

infected about 79000 children in world every year. Antimicrobial resistance of EPEC strains have been increasing and become a main concern in hospital settings. Studies on multidrug resistance EPEC-caused infections therapy were to render alternative therapies such as bacteriophages therapy.

Bacteriophage, virus killing bacterial host, has slightly harm on human cells and even human's microbiota(2). The advantage of phage therapy compared to antibiotics attributes properties including lower bacterial resistance, lack side effects on human cells and stability at different temperatures and pH values. However, cases of bacterial resistance to phages has been reported (3, 4). The specificity of those on their host and lack of adverse effects on human cells have been become as an alternative therapy in some countries such as Eastern Europe, Soviet Union and Georgia(5). Recently, phage therapy on laboratory animals with burns and pulmonary infections has successfully been carried out(6).

Most of *Escherichia coli* phages are isolated from different sewage sources such as municipal sewage and hospital sewages. *Escherichia coli* phages have different morphology, some of which have long tail, while others had short tail. Almost all of *Escherichia coli* phages set into *Myoviridae*, *Podoviridae* or *Siphoviridae* family. We decided to evaluate lytic effect a phage of siphoviridae family on an EPEC strain.

2. Material and Method

2.1 Bacterial strain and isolation of bacteriophage

We used from an EPEC ATCC 43887 (*eae*+/*stx*$^-$) as phage host. We isolated a bacteriophage from waste water of Tehran as described by Bhetwal et al with a few modification (7). Briefly, wastewater samples were centrifuged at 6000 rpm for 5 minute and then supernatant was passed through 0.45µm syringe filter. The bacterial strain cultured (10^8 cfu/ml) was added to filtered sample and then suspension was incubated at 37°C for 18h. Suspension was centrifuged at 6000 rpm at 10 min. phage isolation was performed using by Double layer agar method and plaques was purified until five times.

2.2 Phage morphology

A purified plaque coated on carbon grid and was negatively stained with 1% uranyl acetate. Sample was dried and fixed, then was viewed using Zeiss Transmission Electron Microscope (TEM) (Zeiss, Germany) at 80 kV(8).

2.3 Phage titration

Phage titration was performed for further study using double-layer agar method described by Mattila *et al* (9). Briefly, 6 tubes with 0.9 ml of phosphate buffered saline (PBS) (1.6 g NaCl, 0.04 g KCl, 0.22 g Na_2HPO_4 and 0.04 g KH_2PO_4 in 100 mL of distilled water) were provided. One hundred microliters of purified phage was added into the first tube and well mixed, and then the suspension was serially diluted until the sixth tube.

2.4 Lytic effect Bacteriophage on the strain

To assay, double layer agar method was used. Briefly, EPEC ATCC 43887 was incubated at 37 °C for 16–18 h in 3 ml of tryptone soy broth (TSB). Bacteriophage (1×10^{10} pfu/ml) was added to 0.1 ml of the strain and incubated at 25 °C for 15 min. Then that was added into molten nutrient agar and agitated gently. Suspension was spread on agar plate and incubated at 37 °C for 24 h(9).

2.5 One-step growth

To assay one-step growth curve of phage, the method of Lu et al. was performed with a few modification (10). Briefly, siphophage at multiplicity of infection (M.O.I) 0.1 was effected on the EPEC for 60 min at 37°C and then centrifuged at 10,000 rpm for 50 sec. The pelleted cells were re-suspended in 5 ml of preheated nutrient broth and incubated at 37 °C. Samples were taken every 10 min and phage titer (pfu/ml) were immediately obtained. Experiments were repeated at least three times with duplicate samples.

3. Result

3.1 Characterization of phage and plaque morphology:

After being purified plaques until five times, clear circular plaques with <1mm diameter were observed (Figure 1). Purified plaques measuring

was performed by a ruler. Transmission electron microscope visualized phage has an icosahedral head (67 nm) with a long non-contractile tail (140 nm) (Figure 2) and belongs to siphoviridae family.

3.2 Lytic potential assay of siphophage

Plaques formed on the agar plate showed phage was able to lyse the reference EPEC strain at 37°C in 18h. Also the results of phage titration showed optimum phage concentration was 10^{10} pfu/ml that could lyse the EPEC strain.

3.3 One-step growth

As shown in Figure 3 latent phase of the phage was 10 min. Phage titer started to be decreased after 30min and finally reached to 10^7 pfu/ml at 35 min. Adsorption ratio was >80 % at 8 min.

4. Discussion

Among *E. coli* pathotypes, EPEC is the second most prevalence pathotype that cause traveler's diarrhea, like enteroaggregative *E.coli* (EAEC), in most developing countries. Since years ago, the strain has been considered as one of major bacterial strains producing gastrointestinal infections(11, 12). Schaumburg et al. reported EAEC and EPEC are the most prevalent agents causing diarrhea(13).The use of broad-spectrum antibiotics as a treatment solution for fight against EPEC is not beneficial enough anymore. High antimicrobial resistance of EPEC especially has seriously resulted challenges such as increasing mortality of hospitalized patients(14, 15). Therefore, interest in phage therapy has been renewed in hospital and community settings as an alternative treatment. Haines *et al.* assayed lytic activity a 6-phage cocktail on antimicrobial resistant *Escherichia coli* and *Klebsiella pneumoniae* strains. Their study showed phage cocktail was able to significantly lyse these strains(16).

Although many studies have been performed on the effect of phages on *E.coli*, our sight about the mechanisms of phage infection on antimicrobial resistance bacteria particularly EPEC, is limited. The present study showed the siphophage was able to easily lyse host. Similarity, in Vahedi *et al.* study bacteriophages isolated from sewage could lyse EPEC(17). Yazdi et al. study showed latent period of phage is 40 min, while our study showed siphophage had 20 min latent period (18).Conversely, The results of Huang *et al.* in China showed Abp1 phage had no effect on any of *E.coli* strains (19).In other study, Lin *et al.* indicated phage AB2 was not able to lyse neither of 10 *E.coli* (20).

Lytic Bacteriophages affecting *Escherichia coli* have different lytic potential. In conclusion Our study indicated the siphophage had lytic potential on the EPEC strain. We suggest this phage be used for further studies on clinical EPECs and other strains..

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Ethics Approval

All procedures of the study were approved by the Ethics Committee of Tarbiat Modares University (grant no. IR.MODARES.REC.1398.008). No experiments were carried out on humans or animals.

Declarations

Conflict of Interest

The authors declare no conflict of interest

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