Isolation of Bacteriophages Affecting Enterohemorrhagic Escherichia coli O157:H7

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ABSTRACT

Background: Escherichia coli is an important pathogen and microorganism of the normal intestinal flora of humans and animals. One of the important serotypes of E. coli is O157: H7. Because of the excessive and arbitrary use of antibiotics, multiple drug resistance has increased against these organisms. The main problem in treating infections caused by E. coli is its dependence on the administration of a large number of common antibiotics and the resistance of some strains to antibiotics. Phage therapy refers to the therapeutic use of phages to eliminate bacterial infections. In the first step, it is necessary to separate and identify bacteriophages that affect the target bacteria. Therefore, the present study was performed to isolate the phage that was effective on enterohemorrhagic E. coli isolates.

Materials & Methods: In this study, after collection of sewage samples, bacteriophages were isolated by filtration and enrichment in an enterohemorrhagic E. coli overnight culture. The presence of bacteriophage was detected by plaque observation in a double layer agar and confirmed by TEM electron microscopy.

Results and Conclusion: The results of the observation with electron microscopy revealed the presence of bacteriophage with the appearance of the Cystoviridae, Myoviridae and Podoviridae families. Unfortunately, although the titration of phages and molecular study were not performed in the current study due to the lack of budget, we found antibacterial activity of isolated phages using plaque formation observation, and the presence of phages belonging to Cystoviridae, Myoviridae and Podoviridae families was confirmed by TEM microscopy. Therefore, the effective phage against O157: H7 was successfully identified, isolated, and purified.

Keywords: Escherichia coli, bacteriophage, enterohaemorrhagic, treatment

Introduction

Escherichia coli O157: H7 (E. coli) is an important dietary pathogen that causes dysentery and sometimes hemolytic-uremic syndrome (HUS) (1). This human-animal common human pathogen can remain in food and environments and produce biofilms. Consumption of contaminated foods such as beef, dairy products, ready-made salad, vegetables, and fruits are some of the ways this pathogen is transmitted (3-7). Cow is the primary reservoir of O157 and is a seemingly healthy carrier of this
bacterium in the gastrointestinal tract. Cow excretes O157 from its feces, which is the most important risk factor for the contamination of the carcass of this animal (6, 8). Bacteriophage is a type of virus hosted by bacteria. Twort in 1915 and d’Herelle in 1917 discovered bacteriophage and so it has been an option used to control and eliminate bacteria ever since (9). Due to public health concerns about the emergence and increase in antibiotic-resistant bacteria, the use of bacteriophages in the food production and processing industry and medicine has been very welcomed (9-12). Given the high cost of the pharmaceutical industry from discovering new antibiotics, it is essential to develop an alternative treatment regimen that is easy, inexpensive, affordable, robust, and with few side effects to reduce infectious diseases (13, 14).

Studies have shown that phage therapy is successful in the biological control of pathogenic strains of E. coli (18-15) and is superior to other methods because of reproducibility. Unlike antibiotics, when the number of pathogens increases, their specificity, and function can improve. Admittedly, the disadvantage of phage therapy for controlling intracellular endotoxin-producing bacteria is that we may experience an excessive secretion of toxins after the bacteria have died and their walls have been ruptured (17).

Bacteriophages are a new and suitable option to reduce the level of E. coli O157: H7. A review of previous works has shown that E. coli O157: H7 levels decreased in tomatoes, spinach, beef, and meat surfaces after phage therapy (1, 19). Another study has shown that bacteriophage has a reducing effect on the amount of E. coli O157: H7 in cooked and raw beef (20, 21). It is noteworthy that the results of another study identified podophage CA933P as a suitable solution for the removal of enterohemorrhagic E. coli (22).

Therefore, in the current research, we tried to isolate of E. coli O157:H7 bacteriophages from sewage. The results, presenting the isolated phage, can be utilized in developing biocontrol agents against E. coli contamination.

Materials and Methods

Bacteria

E. coli O157:H7 (ATCC: 35218) prepared from microbial collection of Pasteur Institute of Iran and kept in the microbiology laboratory. It was confirmed as E. coli O157:H7 by PCR assay for stx2 O157:H7 detection. Genomic confirmation was performed using the primers listed in Table 1 (23).

<table>
<thead>
<tr>
<th>Target gene</th>
<th>Primer sequence</th>
<th>Amplicon Size</th>
<th>Reference</th>
</tr>
</thead>
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<tr>
<td>stx2</td>
<td>Forward: TTA ACC ACA CCC CAC CGG GCA GT Reverse: GGA TAT TCT TCT CCC CAC TCT GAC ACC</td>
<td>524</td>
<td>Pollard, Johnson, Tyler, and Rozee (1990) (24)</td>
</tr>
</tbody>
</table>

Sampling and Preparation of Sewage

The sampling was conducted via a sterile glass bottle; and sewage samples were taken from a refinery in Chaharmahal and Bakhtiari province. Then the samples were transferred to the laboratory and centrifuged at 8000g for 10 minutes. Finally the supernatant was filtered by sterile 0.2 μm Minisart filters (Sigma- Aldrich, Cat. No.: 16534K).

Preparation of Bacteria and Adding Sewage

At first, one milliliter of overnight bacterial culture medium was added to 20 milliliters of BHI liquid culture medium, and the suspension was incubated for 3 hours at 37°C. Then, 20 milliliters of filtered sewage were added to this suspension and incubated at 37°C for 24 hours. Afterwards, it was centrifuged for 10 minutes at 8000 g. Finally, the supernatant was filtered through a 0.45 μm sterile syringe filter (Sigma- Aldrich, Cat. No.: CLS431225) (24).

Phage Isolation

Bacteria were grown in 20 mL of BHI medium and incubated for 4h at 37°C. Then, 20 mL of the filtered supernatant of the sewage culture in BHI whose preparation steps were added to the bacteria culture and incubated for 24 h at 37°C. After Centrifuge for 10 minutes at 8000 g, the supernatant were filtered through a 0.2 μm syringe filters (Sigma- Aldrich, Cat. No.: CLS431229). Phage isolation was performed using the double agar method. First, 9 mL semi-solid BHI (containing 0.7% agar) was placed into tubes and sterilized. When the temperature of the semisolid medium reached to about 45°C, 0.1 mL of overnight cultured and filtered bacteria was added to it and spread throughout the culture medium. This culture medium was then added to a solid and sterile BHI medium (containing 1.5% agar) to form a two-layer culture. When the agar was tightened, 20 μL of the filtered sewage was placed in the center of the culture medium and incubated for 24 hours at 37°C (24).

Electron Microscopy

The phage suspension was centrifuged for 90 minutes at 20000g. The supernatant was then slowly withdrawn from the tube and the pellet adhered to the tube wall was dissolved in 50 mL of Phage buffer and again centrifuged as described above. After centrifuge, the supernatant...
solution was removed and the pellet was dissolved in 25 mL of Phage buffer. For the coloring of the phages, 10 μL of the suspension was transferred to a carbon-treated copper grid (400 Mesh) and placed for 210 seconds in this mode. Then, the grid was then placed in the room temperature for 20 seconds. After that, 20 μL of uranium acetate was poured onto the grid and after 160 seconds. Eventually, the excess uranium acetate was removed gently using a drying paper, and the grid was left in room temperature for 30 minutes to be completely dried (25).

Results and Discussion

In the current study, for the confirmation of O157:H7, the presence of stx2 fragment of O157:H7 was detected using PCR test. Positive PCR product and positive control sample were in the size of 524 bp, while no bands were detected for negative control after electrophoresis in 1% agarose gel (Figure 1).

To detect the plaque formation, lytic bacteriophages were isolated after the inoculation of the sewage into double layer agar, and the phage plaques were completely formed in the plates. These plaques indicated that these phages had lytic effects on E. coli O157: H7 (Figure 2).

Therefore, in the current study, using TEM microscopy observation, phages against E. coli O157:H7 belonging to the Podoviridae, Myoviridae and Cystoviridae families isolated from the liquid sewage samples were taken from a refinery in Chaharmahal and Bakhtiar province, Iran. The basis of the diagnosis in the present study was the shape and symmetry of the phages, which, of course, must be confirmed by other molecular methods. In the previous studies, E. coli phages have been isolated from different sources. For example, Jurczak-Kurek et al. (2016) detected 60 infecting E. coli bacteriophages from urban sewage. They found the phages belonging to Siphoviridae and Podoviridae families, using virion and plaque morphology..
evaluation, propagation temperature range and thermal inactivation conditions and the effects of the osmotic shock, high and low pH and detergent or organic solvents, and finally genomic analyses (25). In the present study, due to lack of budget, we only performed virion and plaque morphology evaluation that should be confirmed with more tests.

In another study, Askora et al. (2015) identified an effective lytic bacteriophage against a number of enthaemorrhagic and enteropathogenic Escherichia coli strains. They named the isolated phage as øZE1. It was confirmed that the phage belonged to family Siphoviridae using electron microscopy and produced lysis on four E. coli strains. Also, the resistance to pH, heat, and chloroform was evaluated, and genomic analysis of ØZE1 phage was performed (26). In the present study, similar to the study of Askora et al., the initial basis for the diagnosis of phage was based on the electron microscopy.

Jamal et al. (2015) isolated and characterized a phage belonging to Myoviridae that was effective on antibiotic resistant Escherichia coli strains. In their study, the phage morphology was identified using TE microscopy the same as what we performed in the current research. Furthermore, they described high levels of resistance of MJ1 to heat and pH change (27).

The sufficient therapeutic effect of phages has been established in many studies. For example, Periasamy and Sundaram (2013) applied bacteriophages for pathogen removal from wastewater. In their study, E. coli specific phage was isolated, and its effective titer was standardized. (24). Although, the titration of phages were not performed in the current study, we found antibacterial activity of isolated phages using plaque formation observation.

In another study, Sadekuzzaman et al. (2017) reduced E. coli O157:H7 in biofilms by bacteriophage BPECO 19. The phage treatment performed by Sadekuzzaman et al. decreased E. coli viability (2). Phage treatment, which was used in this study, reduced the survival of O157: h7. With plaque formation test, we found antibacterial activity of isolated phages against E. coli O157:H7.

Arthur et al. (2017) used bacteriophage to reduce the bacterial population in the skin and carcasses of cows found in beef processing plants. The results showed that phage did not significantly reduce E. coli O157: H7 during processing (28). In another work, Seo et al. (2016) inhibited the growth of O157:H7 in beef, pork, and chicken meat by BPECO19 phage (29).

Overall, due to the increasing threat imposed by multidrug resistance, it is necessary to search for novel antimicrobials. There is a growing need for alternative agents of antibiotics and conventional drugs for the prevention and treatment in humans and animals. Exploring bacteriophages as biological control agents can help control antibiotic resistant pathogens. In addition, bacteriophage may apply in pathogen detection and biopreservation. In the present study, E. coli O157: H7 infecting phages were isolated and were available for complementary studies assaying the biological aspects of isolated phages and their in vivo and clinical applications. In addition, further studies can be helpful to use the potential for therapeutic potential and evaluate isolated phages for virulence factors and their ability to transmit genes.

**Conclusion**

Overall, in the current study, E. coli O157:H7 phages belonging to the Podoviridae, Myoviridae and Cystoviridae families were isolated from liquid sewage samples and are available for the future studies.

**Acknowledgment**

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چکیده

جوادی جداسازی باکتریفازه‌ای موثری به‌عنوان کنترل/ اشراپیکی همواره‌ی H7

فروش مختاري

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اطلاعات مقاله

نوبت‌سند انتشار: 1399/10/21

موضوع و ترجمه: ویروس شناسی پزشکی

روشکا: نوبتهای جاذبیت فاقد و ارزش‌یافته O157:H7

مقدمه

باکتریفازه‌ای ورودی‌های هسته‌ای که باعث باکتری‌های میزبان d’Herelle و Twort در 1912 به وسیله ناودانی و آزمایش‌های آزمایشگاهی از توانایی کنترل/ اشراپیکی H7 استفاده شدند. این مناسب‌گرایی باکتری‌های مورد توجه باید شرایطی عمومی را در مورد باکتری‌های مقاومن به آنتی‌بیوتیک استفاده از باکتریفازه‌ای کنترل باکتری‌های کنترل فوق‌العاده برای درمان O157:H7 در طبیعت و پزشکی را شامل شود. این مناسب‌گرایی ممکن است باعث ایجاد حمایت زیادی در طول پیک‌سازی در این نوشتار باشد.

جداول

1. گروه تایپولوژی، دانشکده دامپزشکی، دانشگاه شهرکرد، شهرکرد، ایران

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Majallah-1 mirkub shinash-1 pizishki-i Iran.
DNA اوری از باکتری E. coli (ATCC: 35218) با حاوی O157: H7 در روش آگاربای از سایت Aldrich، باید در دمای 37 درجه و در مدت ۸۰۰۰ ساعت آماده شود.

**جدول ۱.** منشأ ورودی مورد استفاده برای تایید زنومی اشتهاییکی (ATCC: 35218)

<table>
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<th>زن هدف</th>
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<tbody>
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<td>۵۲۴</td>
<td>Pollard, Johnson, Tyler, and Rozee (1990) (24)</td>
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<tr>
<td></td>
<td>Reverse: GGA TAT TCT CCC CAC TCT GAC ACC</td>
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**نمونه برداری و آماده سازی فضایل**

نمونه‌گیری با استفاده از یک بطری با نام E. coli O157: H7 Goz مورد بررسی قرار گرفت. برای تایید زنومی اشتهاییکی (یا یکی از آنها) در سایت Aldrich، باید در دمای ۸۰۰۰ ساعت آماده شود.

**بهبود باکتری و افزودن فضایل**

در ابتدا، یک میلی‌لیتر محلول مایع کشت باکتریایی شبانه به ۲۰ میلی‌لیتر BHI مایع اضافه شد. سپس، نمونه‌های فضایل E. coli O157: H7 Goz در دمای ۸۰۰۰ ساعت آماده شود، سپس باید در روش آگاربای از سایت Aldrich، باید در دمای ۸۰۰۰ ساعت آماده شود.
روی و ﻫﻤﮑﺎران.فروغ باکتریوفازهای موثر عليه باکتری/شیمیایی کلی همراه باکتری coli O157:H7

میکروسکوپ الکترونی

بعضی از باکتری‌ها و عواملی که آنها را ویژه‌سازی می‌کنند ممکن است در سطح الکترون تصفیه شوند. E. coli O157:H7 با یک ساختار استحکام‌دهنده شامل چندین نی‌شاخه و توده‌ای به شکل اپی‌پدیا می‌باشد که به عنوان یک ناحیه همبستگی در بین باکتری‌ها در یک مجموعه فازی جداگانه حضور دارد.

نتایج و بحث

در مطالعه حاضر، برای تایید H7، PCR و وجود قطعه STX2 از O157: H7 با استفاده از تست PCR و به داشت، محققی انجام شد. PCR H7 تشخیص داده شد. محققی از این که بعد از کنترل منفی تشخیص داده شد (شکل 1).

1. نمونه مثبت برای STX2 (5/4 جفت بار) 1: از 100 جفت بار، 3: کنترل منفی
2. برای تشخیص تشکیل پلاک، یک از الکترن فلزی به آگار و دو لایه باکتریوفازی کردند. یک رونده پلاک فازی و یک روند پلاک فازی کاملاً در پلاک باشد، این نشان می‌دهد که این باکتری‌ها تاثر کاملی بر

Shod (25).

روی E. coli O157: H7 دارند (شکل 2).

شکل 2. یک بلاریک در محیط کشت در تصاویر گرفته شده توسط میکروسکوپ الکترونیTEM به دلیل ویژگی‌های مورفولوژیکی، به نظر می‌رسید فازهای جدا شده به خانواده Cystoviridae، Podoviridae و Myoviridae با نمای های-5، (شکل 3-5) انقباض است. اعضای خانواده میوویریده بررسی کردند. در حالی که اعضای سیستومیوییریده دارای سر کروی و دم انقباضی هستند.

شکل 3. تصویر میکروسکوپ الکترونیکی فاز متعادل به

شکل 4. تصویر میکروسکوپ الکترونیکی فاز متعادل به

H7 PCR E. coli O157:
در تحقیق دیگری اثر مشابه اثرِ ساختاری (۲۰۱۶) در بیافلام با استفاده از باکتری‌فاز Myoviridae که در غرب ایالات فرآوری شده‌اند. نتایج به‌دلیل اینکه در ابزار اشکالی که در امکان پذیرفته ندارند، باعث می‌شود که فعالیت‌های تحقیقاتی و تحقیقاتی در این زمینه بهتر و بهتر انجام شود.

در مطالعه‌ای دیگری استفاده یافت که با استفاده از باکتری‌فاز Myoviridae اثر انگشتی که در غرب ایالات متحده انجام شده‌است. نتایج به‌دلیل اینکه در ابزار اشکالی که در امکان پذیرفته ندارند، باعث می‌شود که فعالیت‌های تحقیقاتی و تحقیقاتی در این زمینه بهتر و بهتر انجام شود.

در مطالعه‌ای دیگری که با استفاده از باکتری‌فاز Myoviridae اثر انگشتی که در غرب ایالات متحده انجام شده‌است. نتایج به‌دلیل اینکه در ابزار اشکالی که در امکان پذیرفته ندارند، باعث می‌شود که فعالیت‌های تحقیقاتی و تحقیقاتی در این زمینه بهتر و بهتر انجام شود.
سپاسگزاری

(شماره گرنت: 88:GRD30M1801)

تغییرات در منافع

میان نوسان‌های هیج گونه تغییرات در منافع گزارش نشده است.

جدای شده و برای مطالعات تکمیلی ارزیابی جنبه‌های بیولوژیکی
فاوئیاژه حیاژه‌ای در vivo در دسترس هستند. علاوه بر این، مطالعات بی‌شماری برای استفاده از
پتانس بالقوه درمانی و ارزیابی چنین جدایی شده برای فاکتورهای
حدت و توابع آنها در انتقال و مفید باشد.

نتیجه‌گیری

بطورکلی، در مطالعه حاضر، Haصل E. coli O157: H7 Cystoviridae و Myoviridae Podoviridae
متعلق به خانواده‌های

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