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Short Communication

Isolation and Screening of *Escherichia coli* O157:H7 Bacteriophage

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ABSTRACT

The severity of foodborne disease outbreaks caused by *Escherichia coli* O157:H7 has led to the search for alternative management methods, particularly phage therapy which is currently being revisited. The aim of this study was to isolate and screen lytic *E. coli* O157:H7 bacteriophages from sewage, for possible use in phage therapy. Sewage sample was collected from the Covenant University sewage treatment plant and enriched with reference *E. coli* O157:H7, ATCC 10536 for phage isolation. One lytic *E. coli* O157:H7 bacteriophage was successfully isolated. The isolated bacteriophage was screened using spot assay, to determine its susceptibility to seven previously isolated *E. coli* isolates. The seven *E. coli* isolates used in the study were pathotyped using the *E. coli* O157 latex test kit. The result obtained in this study showed that none of the other *E. coli* isolates was susceptible to the isolated phage and that none of the tested isolates belonged to the O157:H7 serogroup. This suggests that the bacteriophage isolated in this study may be specific for *E. coli* O157:H7. The observed specificity is a useful trait for its use as a potential therapeutic agent for the management of *E. coli* O157:H7 by phage therapy. Specificity is particularly important given that several harmless *E. coli* strains that play important roles in maintaining normal intestinal homeostasis, are part of the normal intestinal flora.

Keywords: Bacteriophage, *Escherichia coli* O157:H7, Foodborne disease, Phage therapy.

Introduction

Infectious diseases are one of the leading causes of death globally.[1] Several infectious diseases are contacted through the faecal-oral route, such as those caused by the enterohemorrhagic *Escherichia coli* (EHEC).[1] Although, *E. coli* forms part of the normal flora of the intestine, some strains are pathogenic causing intestinal (diarrhoea) and extraintestinal infections (urinary tract infections, sepsis meningitis) in both humans and animals.[2] The most clinically important *E. coli* serotype worldwide is *E. coli* O157:H7.[1]

Escherichia coli O157:H7, a serotype of the enterohemorrhagic *E. coli* (EHEC), causes mild diarrhoea, kidney failure and haemolytic uremic syndrome.[1] It can be transmitted through food, water and direct contact with animal manure.[3] It has been estimated globally, that *E. coli* O157:H7 causes about 2.8 million acute illnesses annually and 230 deaths.[4] Various strategies (probiotic therapy, antibiotics, organic acids) have been implemented at the pre-harvest and processing stages to control *E. coli* O157:H7 contamination in food.[5] These strategies are however limited by several factors such as the recolonization of previously treated animals by *E. coli* O157:H7, leading to food recalls and continuous foodborne disease outbreaks.[6] The detrimental effects of *E. coli* O157:H7 to humans, as well as in the food and animal industry, demand the search for alternative control methods.

Products that can contribute to the improvement of food safety along the food chain and ultimately prevent foodborne diseases are highly required. The application of bacteriophages at different stages in food production and storage, is an alternative approach for the management of *E. coli* O157:H7. Therefore, there is a need to isolate, characterize and investigate bacteriophages which have lytic activity against *E. coli* O157:H7 for their potential use in phage therapy.

Phage therapy is the use of bacteriophage for the treatment/management of bacterial infections. Bacteriophages are viruses which infect bacteria specifically. They are the most abundant microorganisms in nature and can be found in all environments, but mostly in aquatic bodies. Basically, they can be isolated from any material that supports the growth of bacteria and have also been found to coevolve with their hosts (bacteria).[7,8] Although, the use of phage therapy began and recorded some success before the antibiotic era,[9] it was not sustained nor pursued further after antibiotics were discovered. This was because of several factors including inadequate understanding of the biology of phages, poor experimental methods for adequate evaluation of phage therapy, as well as the discovery and easy use of antibiotics.[10] However, due to the rapid increase in antibiotic resistance, scientists are reconsidering the potential use of phages and their products in the management of diseases, and in the control of foodborne pathogens.[6]

In a research conducted by Raya *et al.*,[11] *E. coli* O157:H7 phages were orally administered to sheep, and successfully prevented colonization of the gut by *E. coli* O157:H7. Also, bacteriophages were successfully used to reduce *E. coli* O157:H7 counts in foods like tomato, spinach, ground beef and lettuce.[12,6] Presently, several strategies for the use of phages for disease management have been discovered. Whole-phage therapy involves the use of a whole and viable phage to infect bacteria while enzybiotics involves the use of lytic enzymes encoded by phages, which are also specific to the host.[13] In whole-phage therapy, phages can be applied in two different ways: i) using one or a small number of phage strains that have broad-spectrum activity within the genus of a bacterium or, ii) making a cocktail of phages with broad antibacterial activity against bacterial strains and species.[14] Research has shown that the use of phages in phage therapy has advantages over the use of antibiotics.[15,16,10,1] Although some disadvantages exist, phages still possess several features which makes them more effective as therapeutic agents.[16,15,9,10] The use of virulent phages may therefore provide an effective approach for the control of *E. coli* O157:H7 and other foodborne pathogens.[17-22] This research was carried out to isolate

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and characterize lytic *E. coli* O157:H7 bacteriophages from sewage samples for possible use in phage therapy.

Materials and Methods

Isolation of bacteriophage

The reference *Escherichia coli* O157:H7 ATCC 10536 used in this research was acquired from the Nigerian Institute of Medical Research (NIMR), Lagos. Sewage samples were collected from the Covenant University sewage treatment plant in dark bottles and immediately transported to the laboratory for further processing. Sample enrichment followed a previously described method with slight modifications as follows;[23] 40ml of sewage sample was mixed with 5 mL of 10x lysogeny broth (Sigma-Aldrich Corporation) and 5 mL of overnight *Escherichia coli* O157:H7 broth culture in a conical flask and incubated for 24 h with shaking at 37^{o}C. After the incubation, the mixture was centrifuged at 200 rpm for 5 min to remove debris, and the supernatant was filtered through a 0.22 µm pore membrane filter to remove bacteria from the mixture.

In order to detect and isolate phages from the samples, the Adams double layer agar method was used.[24] Firstly, a lysogeny broth agar was prepared, 200 µL of log-phase *E. coli* culture was mixed with 10µL of the enriched sewage filtrate and incubated for 10 minutes at 37^{o}C to allow for adsorption of bacteriophages to bacteria host to occur. After the incubation, 210 µL of the mixture was mixed with 3 mL of 0.7% agarose and poured onto the earlier prepared bottom lysogeny broth agar plates and swirled to produce a uniform layer at the top. The plates were incubated at 37°C for 24 h for the formation of plaques. Distinct plaques were picked from the plates and re-suspended in PBS for serial dilution and plating. This purification step was repeated till clonal population of bacteriophages were obtained and stored in PBS at 4^{o}C. The high titre lysates required for subsequent bacteriophage characterization were obtained by flooding webbed plates with 8 mL of PBS. Three hours later, a syringe was used to collect the lysate which was then filtered using a 0.22 µm membrane filter.

Isolation and identification of Escherichia coli isolates

Escherichia coli isolates were obtained from clinical and environmental samples using Nutrient agar, MacConkey agar and Eosin Methylene Blue media. Sewage sample was serially diluted up to 10^{6} before inoculation at 37°C for 24 h. The bacterial isolates were identified and characterized based on their Gram-stain reaction, morphological and biochemical characteristics.

Spot assay

The spot assay was carried out to screen the isolated bacteriophage for lytic activity against seven previously isolated *E. coli* isolates. Each isolate was sub-cultured into nutrient broth, incubated in an incubator shaker at 200 rpm, 37^{o}C overnight. A 250 µL volume of bacteria broth was mixed with 3 mL of top agar (agarose) and poured onto the bottom agar (nutrient agar) to solidify after which 10 µL of the purified phage was diluted in 90 µL of sterile PBS. The spot plate was divided into two sections (test organism section and negative control section). A 0.3 µL of the diluted phage was spotted onto the test organism section, while 0.3µL of sterile PBS was spotted onto the negative control section of the plate. The plate was left to dry, and observed for the formation of plaques after incubating overnight at 37^{o}C.

Pathotyping of Escherichia coli isolates

The seven *E. coli* isolates screened in this study were further pathotyped using the Oxoid *E. coli* O157 latex test kit (Oxoid, United Kingdom). The *E. coli* isolates previously grown in Sorbitol MacConkey Agar but stored on nutrient agar slants, were subcultured into nutrient agar plates. A drop of the test latex and a drop of saline were dispensed separately onto the reaction card. Each *E. coli* isolate was picked and emulsified in the saline drop, to obtain a smooth suspension. The test latex and suspension were then mixed and observed for agglutination after 1 min.

Results and Discussion

A lytic bacteriophage was obtained by enriching the sewage sample with *E. coli* O157:H7 ATCC 10536 strain. The bacteriophage formed tiny clear plaques on the lawn of bacterial cells (Plates 1a and 1b). The demonstration of the phage's lytic activity to *E. coli* O157:H7 is also similar to a previous report.[25] *E. coli* O157:H7 naturally inhabits the gastrointestinal tract of humans and animals, and is therefore easily transferred to sewage and waste water through faeces; making them natural members of the sewage microbial ecosystem.[25] As a result, the isolation of a lytic *E. coli* O157:H7 phage from the sewage sample in this study is considered natural, since bacteriophages are usually linked to their natural host bacteria.[26,27] Similarly, in other studies, phages were also successfully isolated from sewage water.[28-30,25]

From this study, it was observed that none of the other *E. coli* strains screened was susceptible to the isolated phage as shown in plate 2, by the absence of plaques which depicts no phage lytic activity. Furthermore, pathotyping of these non-susceptible *E. coli* isolates showed that none of the isolates was an *E. coli* O157 strain, as agglutination was not observed for any of the test isolates apart from the positive *E. coli* O157 control. This suggests high specificity of the isolated phage to *E. coli* O157:H7. Similar to the result obtained in this study, other researches have also reported the isolation of *E. coli* O157:H7 specific phages.[31,26,32,6] Contradictory to this report however, is that of,[25] who reported that *E. coli* O157:H7 phage isolated, showed lytic activity against other *E. coli* strains. This is however not unusual since some phages have broad specificity, hence the need for adequate screening prior to further investigation for therapeutic use.

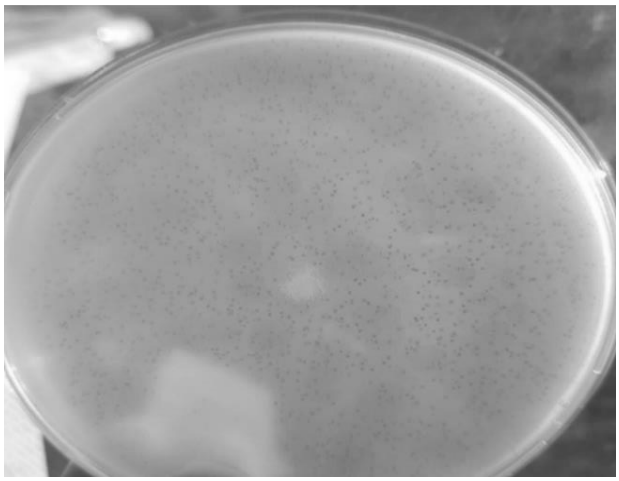

Plate 1a: Bacteriophage plaques (visible zones of lysis) indicating lytic activity against *Escherichia coli* O157:H7.

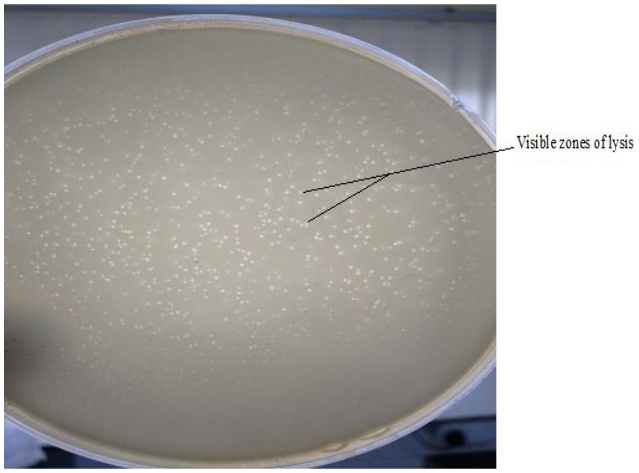

Plate 1b: Bacteriophage plaques (visible zones of lysis) indicating lytic activity against *Escherichia coli* O157:H7

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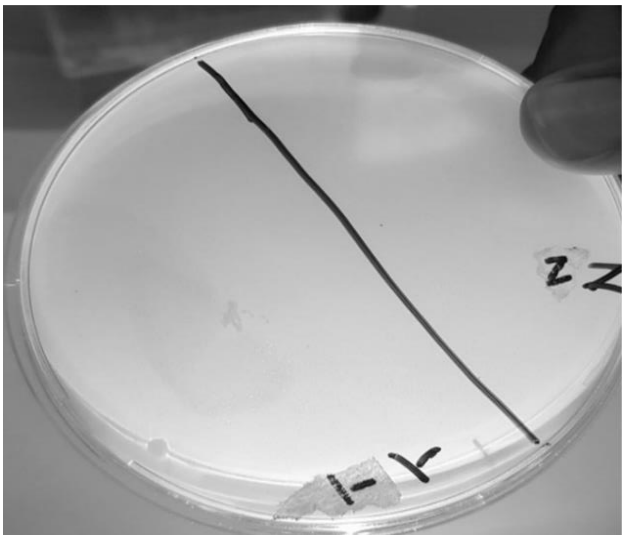

Plate 2: Negative spot assay (absence of bacteriophage plaques) result of the bacteriophage on a non-*E. coli* O157 lawn

Conclusion

The isolated lytic phage obtained in this research showed specificity to *E. coli* O157:H7 which makes it a potential therapeutic agent for the management of *E. coli* O157:H7 by phage therapy. The specificity of this isolated phage is crucial to its use in phage therapy because, other non-pathogenic and beneficial *E. coli* strains, which form part of a host's normal flora will not be harmed by it.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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