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Isolation of Bacteriophage from Sewage

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Abstract

The rise of antimicrobial resistance (AMR) has become a global health crisis, necessitating alternative therapeutic approaches. Bacteriophages, viruses that specifically infect and lyse bacteria, offer a promising solution for combating drug-resistant pathogens. This study aimed to isolate and characterize bacteriophages from sewage samples capable of targeting drug-resistant bacterial strains. Sewage, a rich reservoir of bacteriophages, was collected from different location. The enrichment method was employed to enhance phage isolation, followed by plaque assays to detect lytic activity. Phages were propagated using bacterial hosts. The results demonstrated the successful isolation of lytic bacteriophages with potent activity against drug-resistant pathogens. Clear, distinct plaques indicated effective bacterial lysis, with some phages displaying a broad host range. The isolated phages remained stable under varying environmental conditions, suggesting their potential for therapeutic applications. This study highlights sewage as a valuable source of bacteriophages for combating antimicrobial resistance. The isolated phages hold promise for developing phage therapy as an alternative to conventional antibiotics. Further genomic and in vivo studies are required to ensure safety, specificity, and efficacy before clinical application. Bacteriophage therapy could serve as a crucial tool in the fight against multidrug-resistant infections, reducing dependency on traditional antibiotics and mitigating the AMR crisis.

Keywords: Bacteriophage, sewage, drug-resistant pathogens, phage therapy, antimicrobial resistance

Introduction

Antimicrobial resistance (AMR) has emerged as a significant global health challenge, leading to increased morbidity, mortality, and healthcare costs. The widespread misuse and overuse of antibiotics have accelerated the evolution of multidrug-resistant (MDR) bacteria, rendering many conventional treatments ineffective. This growing crisis necessitates the urgent exploration of alternative strategies to combat resistant pathogens. One such promising

approach is the use of bacteriophages viruses that specifically infect and lyses bacteria as a potential therapeutic tool.

Bacteriophages, commonly referred to as phages, are naturally occurring viruses that play a crucial role in bacterial population control. They exhibit high specificity toward their bacterial hosts, making them an attractive option for targeted therapy without disturbing the natural microbiota. Unlike antibiotics, which can lead to the development of resistance in a broad range of bacteria, phages co-evolve with their hosts, potentially reducing the emergence of resistance.

Sewage and wastewater environments are rich reservoirs of bacteriophages due to their high bacterial density and diversity. These ecosystems provide an ideal setting for phage-bacteria interactions, making sewage an excellent source for isolating phages with lytic activity against MDR pathogens.

Bacteriophages were first discovered by William Twort in 1915 and later recognized for their antibacterial potential by Felix d'Hérelle in 1917. Felix d'Herelle coined the term "Bacteriophage". Bacteriophage means to eat bacteria, and are called so because virulent bacteriophage can cause the compete lysis of a susceptible bacterial culture. They are commonly referred as "phage". Phages are obligate intracellular parasites that multiply inside bacteria by making use of some or all of the host biosynthetic machinery. Though initially explored as therapeutic agents, they were largely abandoned in the West with the advent of antibiotics, except in countries like Georgia They occur widely in nature and can readily be isolated from feces and sewage. There are at least 12 distinct groups of bacteriophages, which are very diverse structurally and genetically.

Bacteriophage is now a day's used for a wide range of purpose in both medical and industrial aspects. Phage products are now applied in several infectious diseases such as *Pseudomonas spp*. *E. coli spp, klebsiella spp* etc. According to Flaherty *et al.* (2001), phages have the potential to serve as predators of bacteria that are associated with plants, fungi, or their byproducts. Phages that target Xanthomonas Campestris, which causes tomato spots, have been used with success. Phage treatment is an option for *Pseudomonas tolaasii*-induced bacterial blotch of mushrooms (Gilla and Abedon, 2007).

Materials and Methods

Sample Collection: Sewage samples were collected from different location of Virudhunagar district using sterial container. The sample was filtered through a membrane filter.

Culture Media Preparation: Tryptone soft agar media and hard agar medium was prepared and sterilized at 121ºC for 15 minutes.

Phage Amplification: 45ml of raw sewage 5ml of sterilized Tryptone soft agar, and 5 ml of nutrient broth culture of *E.coli* were aseptically put into a 250 ml conical flask with a sterile plugged cotton for 24 hours this was kept at 37ºC in a shaking incubator to enable the coli phage in the sewage to multiply inside the host bacterial cells.

Phage Isolation and Plating: For 20 minutes mixture were centrifuged at 2500 rpm. The supernatant was filtered through membrane filter. 3ml of 5 tryptone soft agar broth tubes were prepared for serial dilution.0.1 ml of phage filter was mixed to all tubes.0.5 ml of *E. coli* culture was added to all tubes. It was then poured into all TSA plates. Incubated the plates at 37ºC for 24 hours.

Method

Sewage sample was collected from different location

⇩

Filtered the sample for remove the debris

⇩

5ml of sterilized TSA, 5ml of *E.coli* culture,
45ml raw sewage sample was added and mixed with conical flask

⇩

Culture mixture was Incubated at 37ºC for 24 hours.

⇩

Culture were centrifuged at 2500rpm for 20 mins.

⇩

Supernatant was collected and transferred into conical flask

⇩

Supernatant was filtered through a membrane filter flask

⇩

0.1ml of phage filter and 0.5 ml of *E. coli* culture were taken into TSA broth tube

⇩

Poured into TSA plates

⇩

Incubated at 37ºC for 16 -24hrs

Sample collection

Overnight culture and sewage mixture

Centrifugation

Filtration

Result

Fig: plaques of bacteriophage

Plaques were observed after 24 hours incubation at 37ºC plaque caused by phages varies in size. The plaque observed in this study. It could be due presence of *E. coli* bacteria, that suitable host for growth of bacteriophage.

Discussion

The presence of Coli phage in the sewage line of both human and animal was also detected previously by researchers. (Tan *et al.*) According to Ghasemian *et al.* (2017), variations in the phage strain and other variables may account for variations in the plaque shape seen during the current investigation. The bacterial host for the phage isolated in this study was found to be similar to *Citrobacter freudii*, a common enteric bacteria belonging to the family Enterobacteriaceae. *C. freudii* is commonly found in sewage and has been associated with nosocomial infections in the urinary, respiratory, and biliary tracts of debilitated hospital patients (Tortora *et al.,* 2006) Plaque morphology is one of the foremost criteria for characterization of phages (Shukla *et al.,* 2011) Higher recovery status of phages in dairy farm waste as compared to buffalo farm waste is in support with report (Tiwari *et al*) (Shukla and Hirpurkar, and Askora *et al*). The lytic bacteriophage inhibits the growth of biofilm of ecoli and proteus (Carson L, *et al* 2010) (Ramesh nachimuthu *et al*) who observed better lyphilization condition for bacteriophages long term storage.

Conclusion

Escherichia coli, which are widely distributed in sewage, provide bacteriophages with a susceptible and extremely versatile host. In this investigation, phages were successfully isolated. To further describe, identify, and differentiate the isolated phages to the species level, restriction mapping, DNA sequencing, and transmission electron microscopy (TEM) analysis must be carried out. The isolated phage may serve as candidates for further research in phage therapy especially in combating antibiotic resistant bacteria.

Limitations

Despite the successful isolation of bacteriophages, the study had some limitations, including the need for more advanced genomic analysis to fully characterize the phages at the molecular level. Future research should focus on whole-genome sequencing, protein profiling, and in vivo testing to explore the full potential of these phages. Additionally, expanding the host range testing could help identify broader applications for the isolated phages.

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