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Isolating Bacteriophage for Potential Treatment of Chronic Multi-Drug Resistant Escherichia coli Infections

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Abstract

The misuse and overuse of antibiotics has led to the intense rise in antibiotic resistance. As society transitions into the post antibiotic era, there will be a great need for new therapeutic strategies to address multiple drug resistant bacterial infections. One such method, called bacteriophage therapy, allows for specific targeting of certain pathogenic bacteria through the use of viruses that attack bacteria; termed "bacteriophage" or simply "phage". Urinary tract infections are among the most common pathological human infections that rely heavily on the use of antibiotics, the major cause of which is the bacterium *Escherichia coli*. During the Spring 2021-Spring 2022 semesters, I evaluated an existing set of *E. coli*-specific phage isolated by the Larsen lab from environmental sources and also isolated new phage that target various strains of wild-type and laboratory-adapted *E. coli*. These isolates were also screened against the uropathogenic *E. coli* (UPEC) strain, CFT073, a well-established model research organism. None of the phage isolates were determined to inhibit the growth of the CFT073 strain. I also wrote a book during the Fall 2022 semester, aimed at a third to fourth grade audience. Through this study and future publication of my children's book, I hope to add to the plethora of literature surrounding phage therapy in order to ensure the protection of public health despite the upsurge in antibiotic resistant bacteria.

Introduction

Since the humble beginnings of Alexander Flemming's discovery of penicillin in 1928 and the mass production of the drug initiated by the work of Ernst Chain and Howard Florey in the 1940s (Gaynes, 2017), widespread antibiotic use has allowed for a significant decline in deaths from bacterial infection as well as the introduction of a multitude of surgical procedures - including joint replacements and organ transplants (*Peak Antibiotics*, 2019). However, the misuse and overuse of antibiotics has led to the rise in a pandemic more deadly than Covid-19: antibiotic resistance (*Peak* Antibiotics, 2019). It is predicted that within ten years, most, if not all, of the antibiotics currently in use will be essentially obsolete due to the upsurge in multiple drug resistant (MDR) bacterial strains.

As society transitions into the post antibiotic era, there will be a great need for new therapeutic strategies to address MDR bacterial infections. One such method, called bacteriophage therapy, allows for specific targeting of certain pathogenic bacteria through the use of viruses that attack bacteria; termed "bacteriophage" or simply "phage" (Summers, 2004). Due to their high specificity, a broad pool of phage must be identified to provide an expansive database and phage repository to identify and tailor phage sets to treat specific MDR bacterial infections.

Urinary tract infections are among the most common pathological human infections that rely heavily on the use of antibiotics, the major cause of which is the bacterium *Escherichia coli* (Morrill et al., 2017). During the Spring and Fall 2021 semesters, I evaluated an existing set of *E. coli*-specific phage isolated by the Larsen lab from environmental sources and also isolated new phage that target various strains of wild-type and laboratory-adapted *E. coli.* These isolates

were screened against the uropathogenic *E. coli* (UPEC) strain CFT073, a well-established model research organism (Chockalingam et al., 2019) with a solved genome (Luo et al., 2009).

During the Spring 2022 semester, I had intentions of sequencing an existing *E. coli*-specific phage, HR6B, and several freshly isolated phage from a variety of municipal sewage treatment plants in the greater northern Ohio area. Originally isolated by the Larsen lab from horse feces, the HR6B phage was found capable of infecting the CFT073 strain during the Spring 2021 semester. During the Fall 2021 semester, several other phage - including B/BG, B/PB-2, B/PC-1, B/PC-2, B/PC-3, B/SB, B/US-1, B/US-3. W/AB-1, W/AB-2, W/PB-2, W/PC-2, W/PC-3, W/SB-2, W/SB-3. W/US-2, and W/US-3 - were also found capable of inhibiting CFT073 growth. In addition to potentially sequencing these genomes, I continued to isolate new phage from agricultural sources and municipal sewage systems that target various strains of *E. coli.* These new isolates were screened against the UPEC strain, CFT073.

To communicate the importance of my study with a broader audience, I authored a children's book during the Fall 2022 semester, aimed at a third to fourth grade audience. With the intention of publishing my work, I hope to alert younger science-interested minds about the dangers of MDR infections such that they are inspired to enact change.

Methods

Spring 2021

The UPEC strain, CFT073, was first tested for β-lactam resistance. This was achieved through the lawn growth of the host in standard T-top medium on T-agar plates (Miller, 1972) with each β-lactam placed onto the host and incubated at 37˚C overnight. Following incubation, plates were scored for β-lactam activity, evident as zones of growth inhibition. These zones

were measured and evaluated to determine the host's susceptibility/resistance to the drugs in a clinical setting (Chockalingam et. al., 2019).

Samples were collected from several sewage treatment plants across northern Ohio locations - Archbold, Perrysburg, Upper Sandusky - as well as Windsor, Canada. Initial phage isolation and maintenance of phage relied on the laboratory-adapted *E. coli* K12 strain W3110 (Hill & Harnish, 1981). This strain has a reduced amount of surface-expressed polysaccharide and is thus more susceptible to phage than wild-type *E. coli*. Due to its higher susceptibility to phage, the W3110 strain is consequently more efficient at propagating a wide range of *E. coli*-specific phage.

Once isolated, individual phage were tested for their ability to infect and inhibit the UPEC strain, CFT073. To determine susceptibility to phage, lawn growth of the CFT073 host was completed in standard T-top medium on T-agar plates (Miller, 1972) with each phage pipetted onto the host and incubated at 37˚C overnight. Following incubation, plates were scored for the presence of phage, evident as zones of growth inhibition. Once such zones were observed, the phage which produced these zones underwent serial 10-fold dilutions through the inoculation of these phage with overnight cultures in either W3110. The cultures which achieved a higher growth rate before collapse into a decline in cell density (as determined by spectroscopy) ultimately yielded the highest production of phage. These cultures were then treated with chloroform to kill any remaining cells and the remaining product was titrated to determine phage concentration.

Fall 2021

Samples were collected from several sewage treatment plants across northern Ohio locations - Archbold, Perrysburg, Upper Sandusky, Sandusky Bay, Port Clinton, and Bowling Green - as well as from feces gathered from 21 horses at Beethoven Farms in Perrysburg, OH.

Initial phage isolation and maintenance of phage again relied on the laboratory-adapted *E. coli* K12 strain W3110 (Hill & Harnish, 1981) but also the laboratory-adapted *E. coli* B strain, ECB (Jeong et. al., 2009). Due to its higher susceptibility to phage, the W3110 strain is consequently more efficient at propagating a wide range of *E. coli*-specific phage. ECB is also used for phage isolation as it is deficient in proteases, has a low acetate production at a high level of glucose, and an enhanced membrane permeability due to its simplicity. The main difference between B and K12 strains lies in the absence of flagellar component genes, the DNA cytosine methylase (dcm), and an additional type II secretion system in B strains that are not found in K12 (Jeong et. al., 2009). The ECB strain was used for this semester's experimentation as it more closely resembled that of the uropathogenic CFT073 strain as they are both B strains, while W3110 is a K12 strain. Once isolated, individual phage were tested for their ability to infect and inhibit the UPEC strain, CFT073, following the same procedure as described during the Spring 2021 semester.

Spring 2022

Initial phage isolation and maintenance of phage only relied on the laboratory-adapted *E. coli* K12 strain W3110 (Hill & Harnish, 1981). This strain demonstrated a greater efficiency at propagating a wider range of *E. coli*-specific phage than the ECB strain used in the Fall 2022 semester. Samples were collected from several domestic farm animal droppings in Wood County and other Ohio locations. The Larsen lab also had access to samples from a variety of municipal sewage treatment plants collected by the Davis laboratory in an ongoing study of local

water quality. These samples were also used to isolate new *E. coli* specific phage. Isolated individual phage were tested for their ability to infect and inhibit the UPEC strain, CFT073, once again.

Fall 2022

Over the course of about a month, a book, titled "The Defeat of the Not-So-Superbug", was authored and illustrated. The text was written first, based upon the format of a variety of children's books - including, but not limited to: *Diary of a Wimpy Kid* series, *The Magic Treehouse* series, *Junie B. Jones* series, and others. Following the text, free images were selected from Adobe stock and/or drawn by myself to represent characters or concepts with which intended readers are not familiar. Finally, the images were compiled with the text in a chosen handwritten font - from dafont.com - to synthesize a pdf file, with the intention of publishing the work for third/fourth graders to read.

Results

Spring 2021

The CFT073 strain demonstrated susceptibility to all of the β-lactams tested. A set of 10 phages from various samples which demonstrated the ability to inhibit the growth of W3110 were isolated. However, these same phages lacked the ability to inhibit the growth of the CFT073 strain. Only one isolated phage from the set of pre-existing *E. coli* specific phage from the Larsen lab was capable of inhibiting CFT073 growth. This phage, HR6B, was originally isolated from horse feces - an agricultural source, rather than a human source.

Fall 2021

Four sets of phage - totaling 73 phages - from various sewage and horse samples which demonstrated the ability to inhibit the growth of W3110 and/or ECB were isolated. However, 56 out of the 73 new phage isolates could not inhibit CFT073 growth. Only 17 isolated phages from one of the human sewage sample sets were capable of inhibiting CFT073 growth.

Spring 2022

A total of 30 phages from various samples which demonstrated the ability to inhibit the growth of W3110 were isolated. However, these same phages lacked the ability to inhibit the growth of CFT073. Only one isolated phage, B/PB-2, appeared capable of inhibiting CFT073 growth after various verification processes including spotting the phage onto lawn growth of the pathogen. However, upon performing a 10-fold serial dilution scheme out to 10^{-4}, this phage demonstrated an inability to inhibit CFT073 and most likely was able to inhibit the pathogen's growth due to lysis from without.

Fall 2022

The book was completed and edited thoroughly by my secondary Honor's project advisor. The book will be published following the end of the semester.

Discussion

In future studies, it may prove beneficial to work with *E. coli* that are absolutely resistant to β-lactam antibiotics as well as antibiotics of other classes to truly address the issue of multi-drug resistance. Based upon the findings from the Spring 2021 semester, samples from agricultural sources were thought to be more favorable than those gathered from human sewage as the HR6B phage was isolated from horse feces. However, the existence of the phages isolated

during the Fall 2021 semester demonstrated that sewage samples should not be disregarded as potential sources for phage isolation.

DNA sequencing of the phages found to inhibit CFT073 growth was initially the direction for the Spring 2022 semester. Unfortunately, once these phages were tested once again against the CFT073 strain, none of them proved capable of inhibiting its growth. The introduction of another uropathogen would prove interesting to determine if any of the current phage isolates can inhibit this new pathogen's growth. DNA sequencing of any phage capable of inhibiting CFT073 and/or the new uropathogen should then be conducted.

As noted previously, the ultimate outcome of this study is a stepping stone to accomplishing a much larger goal of protecting public health. Through the isolation of phage which can attack the lab adapted *E. coli* W3110 and the UPEC strain, CFT073, further research can be launched to discover other phages which inhibit the growth of various UPEC strains. In this era of skyrocketing instances of antibiotic resistance, there is a desperate need for a new way to combat bacterial infection. Phage therapy offers the possibility for a new treatment method but can only be acquired through intensive research. I am hoping to continue to add to the plethora of literature surrounding this topic through my own research in order to ensure the protection of public health despite the upsurge in antibiotic resistant bacteria.

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