Development of Bacteriophage Therapy for Novel Treatment of Bacterial Infections

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Abstract

Bacteriophages have been used to treat bacterial infections for over 100 years in some parts of the world, but their use in the West was discontinued as antibiotics were discovered. The use of antibiotics since the 1950s has resulted in multidrug resistance genes in many bacterial pathogens. The objective of this research project is to develop novel bacteriophage therapies against human pathogenic bacteria which are becoming difficult to treat using conventional antibacterial drug regimens. Bacteriophages will be enriched from wastewater samples or environmental water samples, amplified on pathogen broth cultures, isolated by plaque assay, and individual phage plaques harvested. Phage stocks are prepared and cryopreserved. The phage stocks will be characterized by molecular techniques to determine genome type, size, and sequence. Preliminary results show that the supplemented broth medium we developed will enable us to isolate phages from environmental water obtained from the Bird Preserve located in Las Vegas, NV. We show that we were able to isolate phage specific against our chosen positive control bacteria, *Escherichia coli*. Plaque forming units (PFU) were quantified at 2.7 x 10^{10} PFU/mL in one pond location, and 4.5 x 10^{10} PFU/mL in a second pond location. A selected number of phage plaques will be harvested and enriched on the *Escherichia coli* host used in the initial amplification to use as positive controls. Such phage stocks will be useful in developing phage cocktails for treatment against other bacterial pathogens, such as *Neisseria gonorrhoeae*, *Streptococcus pneumoniae*, and *Micrococcus luteus*.

Introduction

As antibiotic resistance increases, the outlook of clinical bacteriophage therapy as an alternative to fight bacterial infections becomes more prevalent. Recent breakthroughs include an approved clinical trial where cystic fibrosis patients colonized by *Pseudomonas aeruginosa* will be treated with phage therapy to evaluate if it safely decreases the amount of *Pseudomonas aeruginosa* in their lungs (Tamma et al., 2022). However, bacteriophage studies have yet to develop a control bacteria in their protocol to compare findings to successful phage lytic results against tested bacteria. Therefore, our research aims at forming a protocol using *E.coli* as a positive control for future use in phage experiments. *E.coli* is a bacteria that has been used as a model organism in research due to its ability to be grown easily in the lab, and E. coli phages are readily isolated from most environmental water samples, which makes this bacteria and its phages an ideal control for isolating phages of other bacteria. This experiment looked into the ability to find lytic phage against *E.coli* in the water specimens we used to isolate the phages of other bacteria, the ability to get results on media growth plates, the ability to generate lytic zones in media, and the protocol to isolate the phage found in the media. Our findings indicate that *E.coli* was ideal in producing positive controls for all of the methods listed above.

Methods

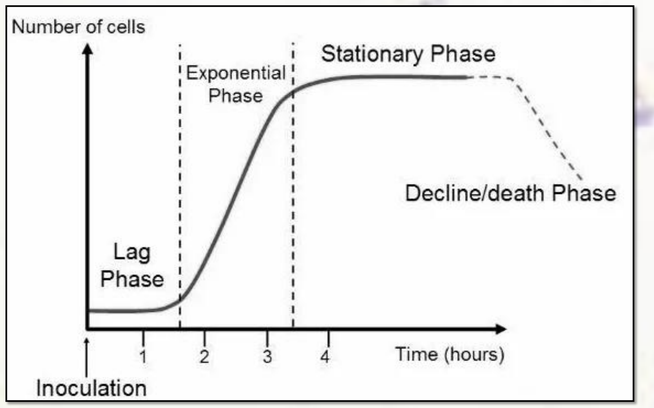
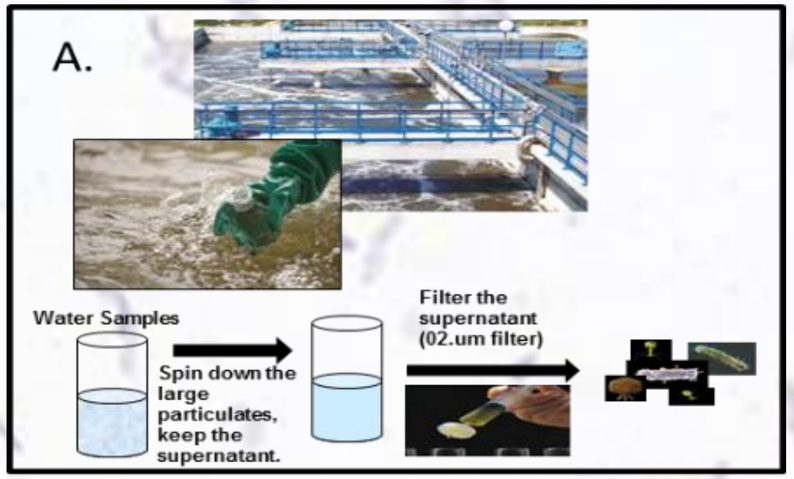


Figure 1. Growth Curves. Bacteria were cultured from frozen stocks and incubated on chocolate agar plates for two days to ensure growth. Bacteria colonies were cultured overnight in Medium C. Gram stain was performed to ensure no contamination. An OD measurement was taken of the overnight cultures. 2mL aliquots were used to inoculate each experimental culture tube. The media used for this experiment included Fastidious Broth (FB), Brain Heart Infusion (BHI), and three different supplemented BHI media we called A, B and C. Using the overnight culture bacteria, we inoculated three tubes containing 20mL for a total of 15 tubes per experiment using each of the five media we were testing. We incubated these samples throughout the day, taking OD readings every hour until lag phase to evaluate bacterial growth. OD data were used to generate growth curves using Growth rates software.

Methods - Continued



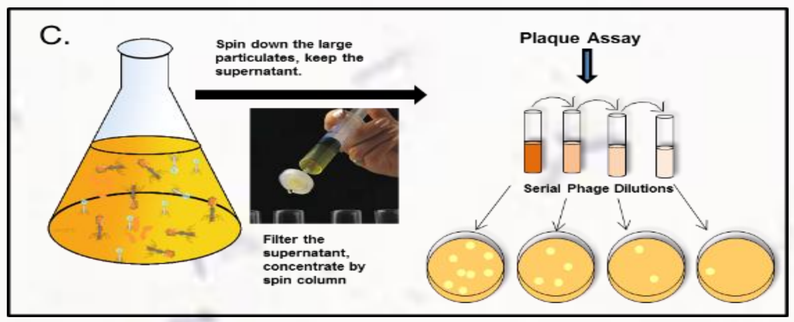


Figure 2. Filtration of wastewater. Wastewater was first centrifuged at 3610 RPM for 25 minutes in a 50ml centrifuge tube. The sample was then filtered using 0.20 syringe filters into new tubes which is shown in Figure 2A. The new filtered samples were then incubated with TSB as depicted in Figure 2B. Alongside preparing the phage prep, we grew *E. coli* samples in TSB and monitored for the beginning of log phase via OD readings (data not shown). To run the lytic plaque assay 300ml of soft agar was added to 50ml centrifuge tubes at 55 degrees C. The phage prep was split into separate tubes with different dilution via TSB. The phage prep and *E.coli* cells were added to the tube filled with the soft agar. The sample was then poured into a petri dish as shown in Figure Cand incubated overnight at 37 degrees C. 20 lytic E.coli plaques were then picked and placed in tubes filled with 200 microliter of TSB in 50ml centrifuge tubes. The plagues were broken down manually using sterile plastic loops. Another 1 ml of TSB was added and placed in a minishaker for 1 hour. After an hour and getting the bacteria in growth phase, the E.coli sample was placed into the plaque samples. The samples were then incubated in the shaker overnight. After 24 hours the tubes were observed for clarity as a sign of bacterial death due to phage infection and lysis.

Results

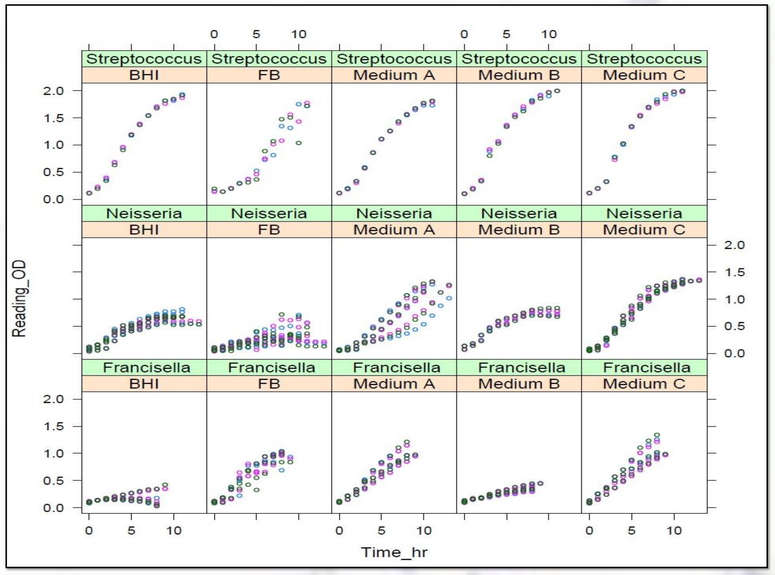


Figure 3. Best Broth for Fastidious Bacterial Pathogen Logarithmic Growth. Growth curve experiments using broth media additives were run in triplicates and repeated 3 times. The average OD readings of the triplicates for each bacterium species in each media are shown.

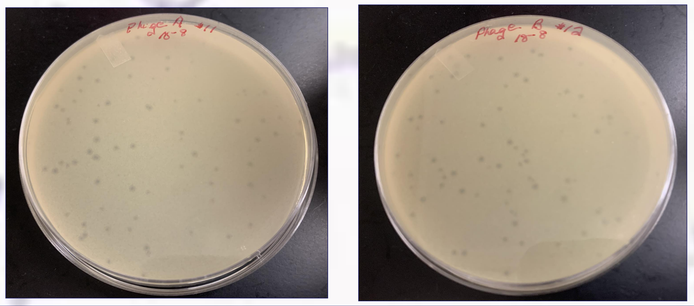


Figure 4. *E. coli* phage plaque assay. E.coli had positive results in the plaque assay for phage present. The dilution of 10^8 gave us well spaced plaques that were then isolated. Following the isolation of plaques, we were able to recreate lytic results in TSB broth after 24 hours of incubation with *E. coli.*

Conclusions

- The growth curve data in Figure 3 demonstrated that Fastidious Broth (FB) was not the best growth medium for the fastidious pathogenic bacteria, and indicated that each genus required specific culture broth medium to reach log phase within a time frame to allow for phage inoculation.
- We were able to easily grow E.coli in supplemented broth media and isolate phages. Additionally, we were able to yield lytic results following plaque enrichment of *E.Coli.* This data supports our hypothesis that *E. coli* is a viable positive control in further phage isolation

References

- Tamma, P. D., Souli, M., Billard, M., Campbell, J., Conrad, D., Ellison, D. W., & Schooley, R. T. (2022). Safety and microbiological activity of phage therapy in persons with cystic fibrosis colonized with Pseudomonas aeruginosa: study protocol for a phase 1b/2, multicenter, randomized, double-blind, placebo-controlled trial. Trials, 23(1), 1-12.

Acknowledgements

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