Effects of Phage Cocktail Compared to Antibiotic Treatments on Host Bacterial Cell (*Pseudomonas Chlororaphis*)

Minjin Lee | Department of Biology | Advisor: Dr. Stephanie Strand
The College of Wooster | Wooster, Ohio 44691

INTRODUCTION

A phage cocktail is combining various phages to target one bacterial strain of interest, often used to treat antibiotic-resistant bacteria. A narrow host range of phage cocktail, allowing it to focus on infecting specific species, prevents phages from infecting other microorganisms within the interior of multicellular organism [1]. The current research specifically focuses on how the phage cocktail is more efficient than the antibiotics: how fast the bacteria gain resistance on each treatment, and how different drugs–antibiotics and phage cocktails–can be used together to increase efficiency in treating the bacterial host.

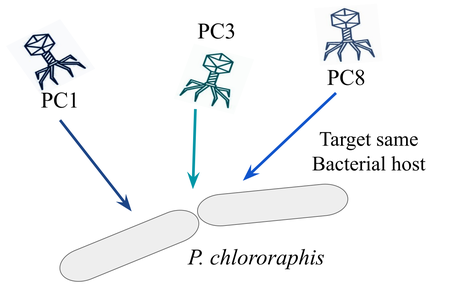
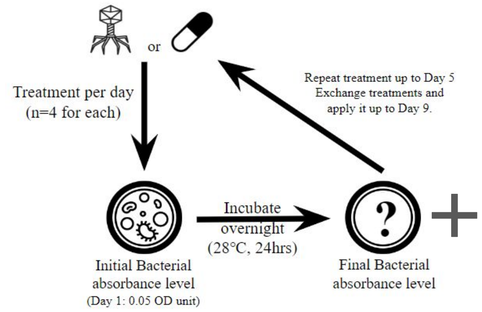


Figure 1. Designed phage cocktail *(1:1:1 ratio)* [1, 2]

METHODS



I. Find minimum inhibitory concentrations (MIC) of antibiotics and calculate multiplicity of infection (MOI) of the phage cocktail to create treatments. [3]

II. Add treatment (0.1 MOI phage cocktail, 0.01mg/mL antibiotics (gentamicin) every day, measure Optical Density (OD) units of the sample each day (OD 600), and streak 3- 10 fold diluted samples on LB media agar plates.

III. Record absorbance levels (OD unit), compare the number of colonies grown on LB media agar plates, including time that the treatment has been applied.

RESULTS

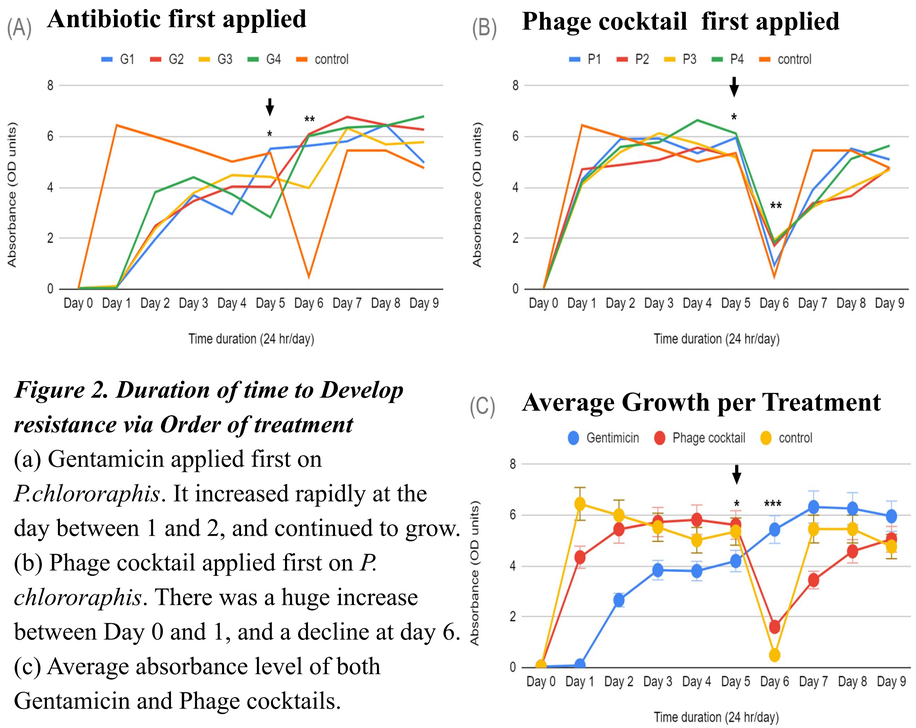


Figure 2. Duration of time to Develop resistance via Order of treatment
(a) Gentamicin applied first on *P.chlororaphis*. It increased rapidly at the day between 1 and 2, and continued to grow.
(b) Phage cocktail applied first on *P. chlororaphis*. There was a huge increase between Day 0 and 1, and a decline at day 6.
(c) Average absorbance level of both Gentamicin and Phage cocktails.

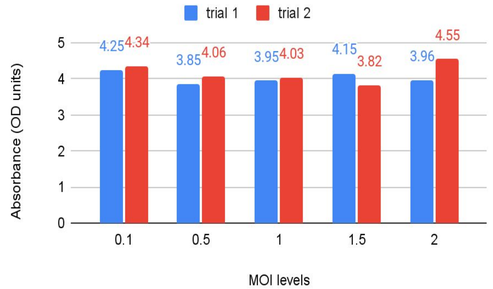


Figure 3. Bacteria cell growth in different MOI levels. Regardless of the different MOI–0.1, 0.5, 1.0, 1.5, 2.0–levels of absorbance were same.

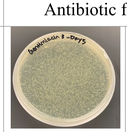
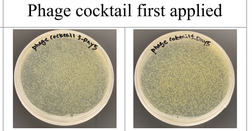
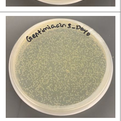
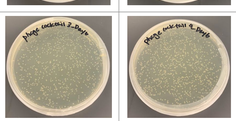
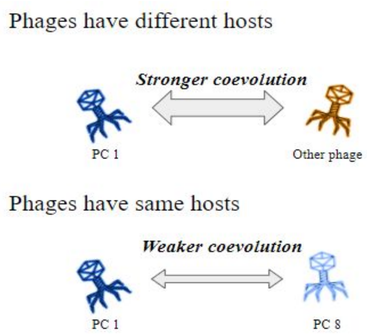
	Antibiotic first applied	Phage cocktail first applied
Day 5* changed treatment		
Day 6 Results of changed treatments		

Figure 4. Changes in the number of bacterial colonies present on the plate. Intensity of the antibiotic first applied did not decrease, but the phage cocktail first applied decreased dramatically.

DISCUSSION

Evolution of the resistance strain of the host bacteria once treated with phage cocktail occurred faster than when treated with antibiotics.

- MOI of phage cocktail that suppresses the growth of host cell was not determined.
- Antibiotic was able to suppress growth of both non-resistant and phage-cocktail-resistant hosts.
- Phage cocktail was less likely to suppress growth of both non resistant and antibiotic resistant hosts.



Limitations might be present due to COEVOLUTION.
→ Bacteriophage-bacteriophage coevolution: increase rate of infection by transferring DNA horizontally, suited to specific bacterial cell receptors, etc. [4]

REFERENCE

[1] Abedon, S. T., Danis-Wlodarczyk, K. M., & Wozniak, D. J. (2021), *Pharmaceuticals*, *14*(10), 1019.
[2] Liu, H., Li, H., Liang, Y., Du, X., Yang, C., Yang, L., ... & Song, H. (2020), *Theranostics*, *10*(14), 6310.
[3] Green, K. J., Dods, K., & Hammer, K. A. (2020), *PLoS One*, *15*(12), e0243246.
[4] Karaolis, D. K., Somara, S., Maneval, D. R., Johnson, J. A., & Kaper, J. B. (1999). A bacteriophage encoding a pathogenicity island, a type-IV pilus and a phage receptor in cholera bacteria. *Nature*, *399*(6734), 375-379.