Investigating the Efficiency of Bacteriophages to Combat Antimicrobial Resistance in *Pseudomonas chlororaphis* 14B11

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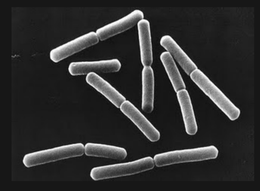

ABSTRACT

Despite antibiotics being used to treat bacterial infections in the United States for roughly a century, antimicrobial resistance is on the rise due to misuse and overuse of antibiotics. Therefore, new methods are needed to treat antimicrobial resistant (AMR) bacteria. One method is the use of bacteriophages, which are viruses that infect bacteria. Phages can potentially provide therapeutic properties as they can infect bacteria in vivo. One specific type of phage therapy is utilizing a bacterium's evolution of phage resistance as a mechanism for decreasing its antibiotic resistance. Once a bacterium becomes resistant to phages, it could lead to increased antibiotic susceptibility and would allow antibiotics to be more effective. In this study, the antibiotic susceptibility of *Pseudomonas chlororaphis* 14B11 was tested before and after phage infection. Phage-resistant strains treated with ampicillin showed a ~4-fold increase in antibiotic susceptibility, indicating there was a mutation made in the bacterial genome. Candidate genes for further study were identified, which were *mexA*, *mexB*, and *oprM*, as these may play a significant role in phage binding and adsorption. This study is important to gain more knowledge about bacteriophage therapy and the impact bacteriophages have on different species of bacteria.

INTRODUCTION

- Rise of AMR bacteria due to misuse, overuse, and limited available treatment options (1)
- Bacteriophage therapy: Use of phages as an alternative to or in addition with antibiotics (2)
- Phage-antibiotic synergism to promote fitness trade offs:
  - The use of phages and antibiotics can have a greater outcome than the use of either one individually (3)
  - A decrease in phage susceptibility will result in an increase in antibiotic sensitivity (2)
- *P. chlororaphis* is an under investigated species
- Efflux pumps are used to transport substances outside the cell (like antibiotics) (4)
  - Can be used as phage receptors
  - MexAB-OprM: Most common efflux pump contributing to antibiotic resistance in *P. aeruginosa*

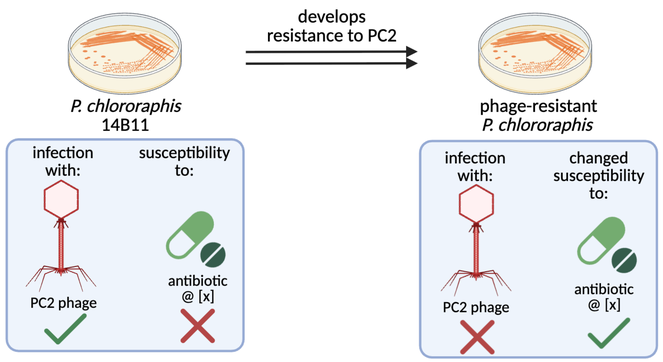

Figure 1. Proposed research question to investigate if there is an increase in antibiotic susceptibility after *P. chlororaphis* 14B11 is infected with the phage PC2. Before phage infection, the parent 14B11 strain is susceptible to the PC2 bacteriophage and resistant to antibiotics at a given concentration. The phage-resistant strains would be resistant to the PC2 phage and might be susceptible to antibiotics at the same concentration.

METHODS

Hypothesis: Development of phage-resistant strains from *P. chlororaphis* 14B11 will lead to increased susceptibility to ampicillin and/or erythromycin. This may occur due to mutations associated with phage resistance in one or more genes that code for the MexAB-OprM efflux pump.

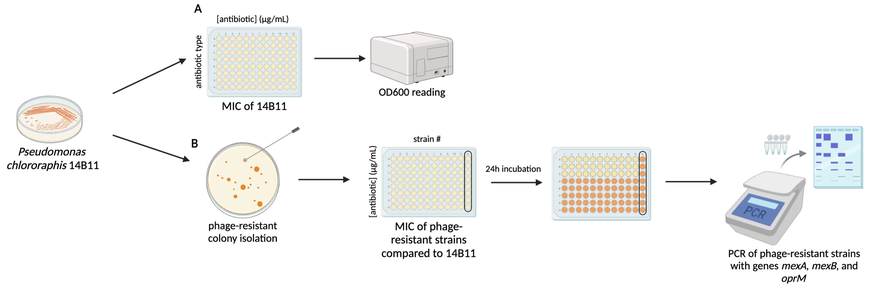

Figure 2. Experimental design. (A) Testing antibiotic susceptibility of 14B11 parent strain via the minimum inhibitory concentration (MIC) to determine the minimum antibiotic concentration that inhibits bacterial growth. (B) Isolating phage resistant strains to test MIC, where those with an increase in antibiotic susceptibility were used for PCR with the *mexA*, *mexB*, and *oprM* genes. Orange shows bacterial growth while yellow is no growth. The column that is circled in black is the 14B11 parent strain, so it can be compared to phage resistant strains.

RESULTS

- ~4-fold increase in antibiotic susceptibility when treated with ampicillin (Figure 3A)
- ~2-fold increase in antibiotic susceptibility when treated with erythromycin for phage-resistant strains #1 and #8 (Figure 3B)
- *mexA*, *mexB*, and *oprM* genes were successfully amplified in strains 14B11, #7, #8, and #9 (Figure 4)

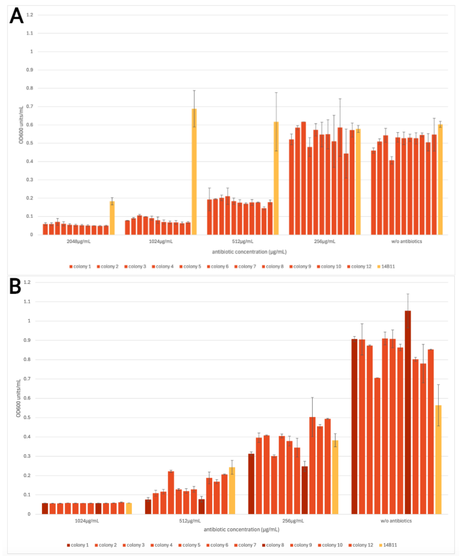

Figure 3. Optical density at 600nm of evolved phage-resistant strains treated with antibiotics at varying concentrations compared to a control without antibiotics. The antibiotics used were (A) ampicillin and (B) erythromycin. The optical density was taken at a wavelength of 600nm after 24 hours of incubation at 28°C. The OD values are the average from two trials, where the error bars are the standard deviation of the two trials. These OD values are being compared to the parent strain (yellow) and the phage-resistant colonies treated without antibiotics.

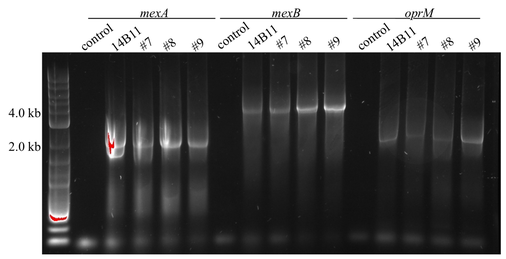

Figure 4. Agarose gel electrophoresis (1% agarose) of PCR products amplified using primers specific for the *mexA*, *mexB*, and *oprM* genes. A 1% agarose gel stained with gel red was used. Each gene used strains 14B11 and #7 through #9. Quick Load 1 kb Plus DNA Ladder from New England Bio Labs is shown in the first lane.

CONCLUSION

- Increase in antibiotic sensitivity after treatment with ampicillin and erythromycin
- Provides a deeper exploration of phage-antibiotic synergism + insights on therapeutic properties of phages
- Limitation: Development of phage-resistant strains can differ in vitro vs. in vivo

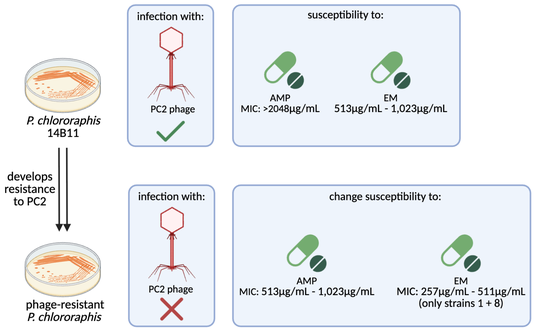

Figure 5. Results concluded phage-resistant strains having an increase in antibiotic susceptibility when treated with ampicillin and erythromycin.

FUTURE WORKS

Are there mutations in other regions of the genome?

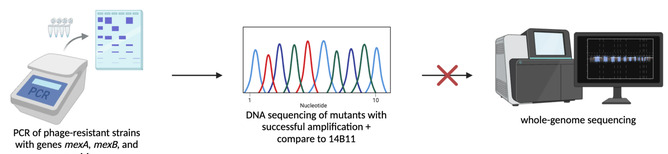

Figure 6. Whole genome sequencing of phage-resistant strains to determine if there are any mutations in the rest of the genome. PCR would be done on the *mexA*, *mexB*, and *oprM* genes and DNA sequencing will be done if there is successful amplification of these genes. If there are no mutations present in these genes, WGS will be done.

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