Exploring How *Pseudomonas chlororaphis* 14B11 Bacteria Responds to Simultaneous Bacteriophage-Antibiotic Therapy—the Evolution of Double Resistant Strains

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ABSTRACT

Antibiotics have long been used to clinically treat bacterial infections, but overuse has led to the emergence of antibiotic-resistant bacteria. Although bacteriophage therapy was put forward as a potential alternative, it can lead to resistance in bacteria. With limited research on bacterial susceptibility to simultaneous bacteriophage-antibiotic treatment, this project investigates the emergence of double (phage-antibiotic) resistant bacteria strains, the presence of mutations in target gene and the mechanism of resistance. We grew *Pseudomonas chlororaphis* 14B11 under simultaneous exposure to bacteriophage & antibiotic, and we tested the strains for resistance. gDNA of select mutants were amplified and sequenced to identify oprM gene mutations. Only phage-antibiotic resistant strains grew at concentrations above MIC, and they showed no OprM mutations, suggesting that double resistance may arise through intrinsic mechanisms. Overall, our findings indicate the contribution of other genes to the evolution of phage-antibiotic resistant strains.

BACKGROUND

- The overuse or misuse of antibiotics leads to the emergence of antibiotic-resistant bacteria strains[1], that are responsible for 2.8 million infections in the U.S. annually. Bacteriophage therapy was introduced as an alternative treatment for multidrug-resistant infections[2]. However, there’s little evidence to support the ability of phage therapy to eliminate multi-bacterial species infection[3].
- Phage-Antibiotic Synergy is the sequential combination of phage and antibiotics, working together to effectively reduce bacterial growth[4]. However, the ability to prevent the emergence of multidrug-resistant strains remains unknown. Phage-antibiotic can also produce synergistic effects when bacteria is exposed to both antibacterials simultaneously[5]. (Figure 1).

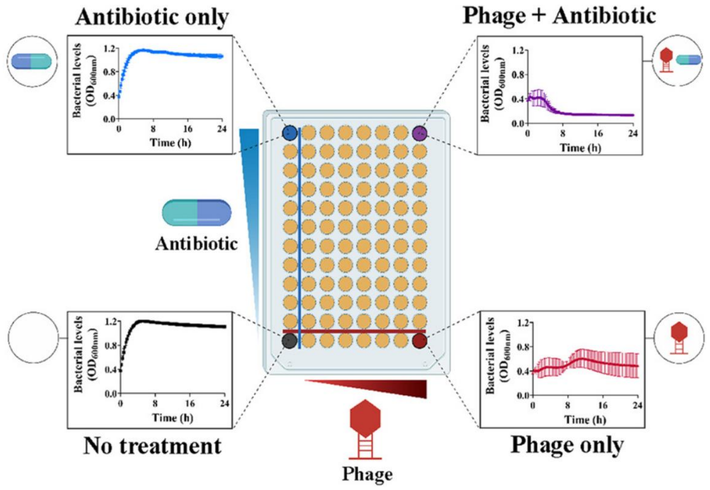

Figure 1. Phage-antibiotic treatment work synergistically to decrease bacterial growth over time compared to antibiotic only and phage only treatment. Blue – bacterial growth in antibiotic treatment, red – bacterial growth in phage treatment, Black – control; bacterial growth in the absence of phage and antibiotic, Purple – bacterial growth at the highest concentration of phage and antibiotic.

HYPOTHESIS & RESEARCH OBJECTIVES

This project aims to:

1. **evolve *P. chlororaphis* strains that are resistant to simultaneous phage-antibiotic treatment**
2. **identify and compare mutations of target gene—*oprM*– in wildtype and mutant strains.**

METHODS

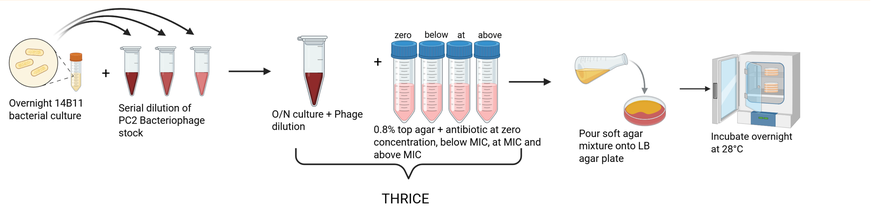

Figure 2. Overview of phage-antibiotic simultaneous treatment on *P. chlororaphis* to evolve mutant strains. Created in BioRender.

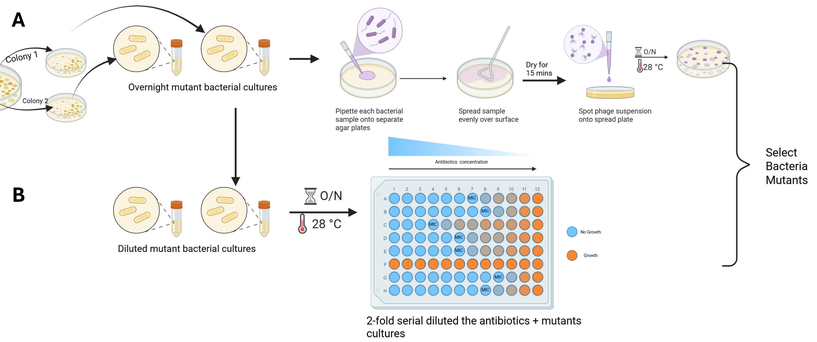

Figure 3. Overview of Resistance tests conducted on evolved *P. chlororaphis mu*tant strains. (A) Spot test to determine phage resistance (B) MIC assay to determine MIC changes relative to 14B11 wild-type (WT). Created in BioRender.

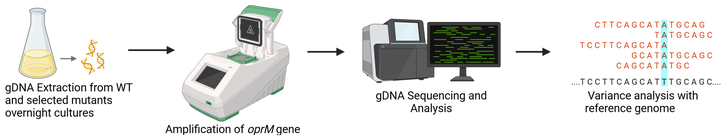

Figure 4. Overview of the genomic sequencing and analysis process for 14B11 WT and mutant samples. Created in BioRender.

RESULTS

Table 1: Resistance of Selected Mutant Strains. Table showing phage resistance and changes in the MIC of antibiotics (G418 and TET) for 14B11 WT and its selected mutants.

Sample name	Phage Resistant (PR)	G418 MIC	TET MIC
14B11 WT	No	32 µg/mL	4 µg/mL
G32-1	Yes	64 µg/mL	——
G32-2	Yes	32 µg/mL	——
10^-1/T8-2*	No	——	4 µg/mL
10^-2/T2-1	Yes	——	8 µg/mL
10^-3/T2-1	Yes	——	8 µg/mL
10^-3/T2-2	Yes	——	4 µg/mL

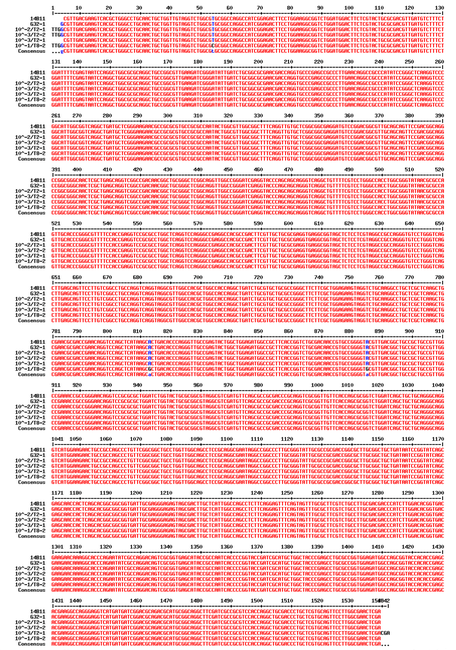

Figure 5. Multiple Sequence alignment of 14B11, G32-1, 10^{-2}/T2-1, 10^{-3}/T2-1, 10^{-3}/T2-2 and 10^{-1}/T8-2 amplified DNA samples in order. It shows the mutation that occurs in each nucleotide sequence relative to other sequences.

CONCLUSIONS

- Double resistant strains emerged from phage-antibiotic simultaneous treatment. Absence of mutations point to intrinsic mechanism of resistance.
- Double susceptible mutant was evolved and had a C-terminus alanine insertion likely due to point mutation in its coding sequence

FUTURE DIRECTIONS

- Whole-genome sequencing will be instrumental in identifying mutations in other genes.
- Evolving double resistant strains mutants *in vivo* could help determine how host conditions influence the emergence of double resistance.

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