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A Dive into Pseudomonas “PC” Series Phages; The Good, The Bad, The Virulent

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Abstract

Antibiotic resistance in pathogenic bacteria has been an increasingly problematic threat to humanity with an expected death toll of 10 million by 2050. Bacteriophage (phage) therapy is a promising solution that utilizes viruses to eliminate bacteria in place of antibiotics. Bacteriophages are highly specific to their respective bacterial targets and are often specific to a particular bacterial genus or even species. Because of the specificity phages have between hosts, finding and characterizing phages with specific traits suited to particular pathogens is a current bottleneck to the phage research process. In this study, using traditional spot testing procedures, I characterized the host range of phage PC7 , determining its ability to infect a broad number of strains within various species within the genus *Pseudomonas.* To determine genes required for the entry and replication of phage PC7 inside a host, I developed a novel screening procedure. The procedure was capable of screening up to 96 *P. clororaphis* transposon mutants per 12 hours utilizing 96 well plates to identify phage resistant mutants for further characterization. Using this screening procedure, phage PC7 was discovered to be unable to efficiently propagate inside transposon mutant DD3, suggesting an alteration or loss of a gene encoding a protein required for efficient phage propagation. This research is designed to be repeatable for phages more relevant to clinical research enabling quick identification and characterization of phages effective against pathogenic bacteria.

Research Objectives

- Analyze the host range of phages targeting of a set of bacteria in the family *Pseudomonas* ✔
- Develop a screening procedure utilizing transposon mutants to identify phage resistant mutants ✔

Bacteriophage Morphology and Host Specificity

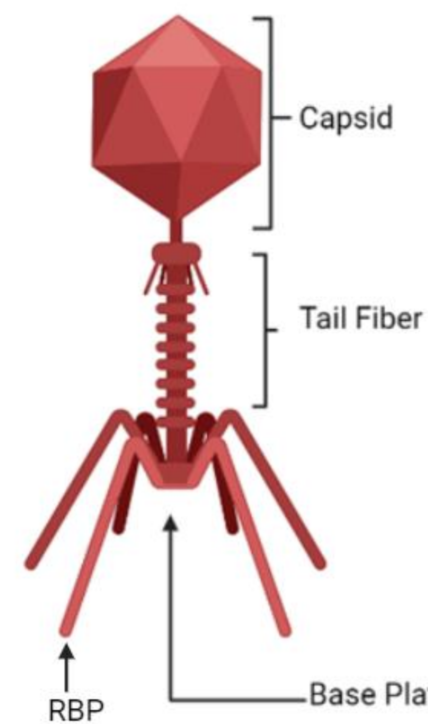

Figure 1: **Bacteriophage Morphology.** Bacteriophages are a subset of viruses that contain 4 main regions, a capsid containing genetic information, the tail fiber, the receptor binding protein, responsible for initial host recognition. And the base plate, which allows for DNA adsorption

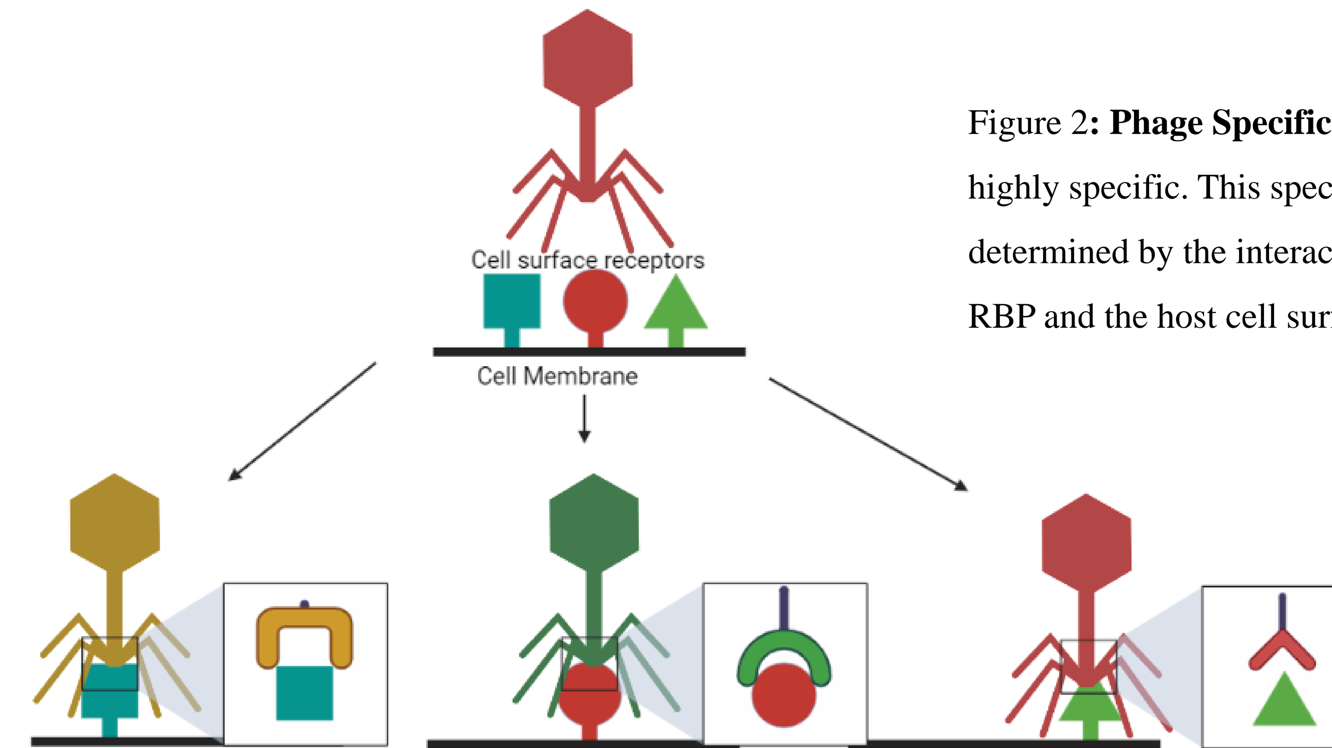

Figure 2**: Phage Specificity**. Phages are highly specific. This specificity is determined by the interaction between their RBP and the host cell surface protein.

Utilizing Host Range Specificity Testing, “PC-” Series Phages PC2 and PC7 Both Found To Have Notable Host Ranges

Figure 3: **Bacteriophages PC1, PC2, PC6, PC7, and PF7 Infection Range Against 11 Different Strains of *Pseudomonas* of Various Families.** Phages were exposed to various strains of pseudomonas via simple plaque assay procedure, which involves exposing phage to a bacterial lawn**.** Table 1 displays the overall infection range of each of the bacteriophages. The key designates the different patterns of infection along with pictured examples. Large defined plaque for a + infection, a few small plaques for a +/- infection, and a lawn of bacteria with no visible plaques for a – infection. Phages PC 2 and PC7’s host ranges were notable for their unique aspects. PC2 infected *P. Brassicacaerum* strain 93F8, if not poorly, and PC7 infected all strains within the species it interacted with. Ultimately PC7 was chosen for further research.

Bacterial Strains			Viral Strains				
			PC1	PC2	PC6	PC7	PF7
	P. Clororaphis	14B11	+	+	+	+	+
		48B8	+	+	-	+	+
	P. Protegens	14B2	-	-	-	-	-
		15G2	-	-	-	-	-
	P. Brassicacaerum	36D4	-	-	-	-	-
		93F8	-	±	-	-	-
	P. Fluorescens	36F3	+	+	+	+	+
		89F1	-	-	+	+	-
		28B5	+	±	-	+	+
	P. Fredrickbergergenses	39A2	+	+	±	+	+/±
	P. Aerugenosa	PA01	-	-	-	-	-

KEY	
+	Infection displayed
-	Infection not displayed
±	Partial/Low infection displayed

Phage PC7 Lysis Host Cell in 120 – 200 Minutes

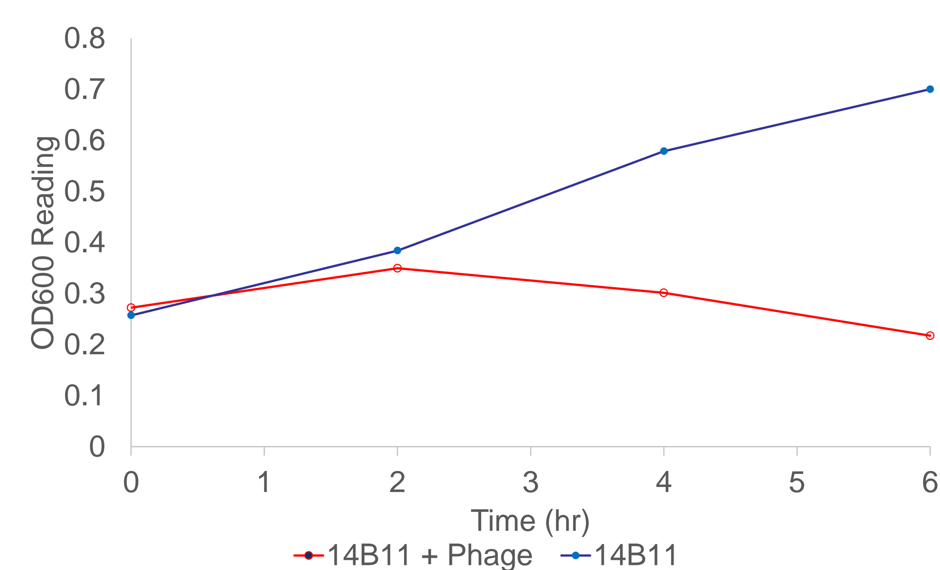

Figure 4: **Growth Curve of WT 14B11 During Phage PC7 Exposure.** P. clororaphis strain 14B11 introduced into 200 µL of LB media on a 96 well plate and grown for 12 hours before being exposed to 10 µL phage PC7 for a period of 6 hours.

Identifying DD3 As a PC7 Resistant *P. Clororaphis* 14B11 Transposon Mutants

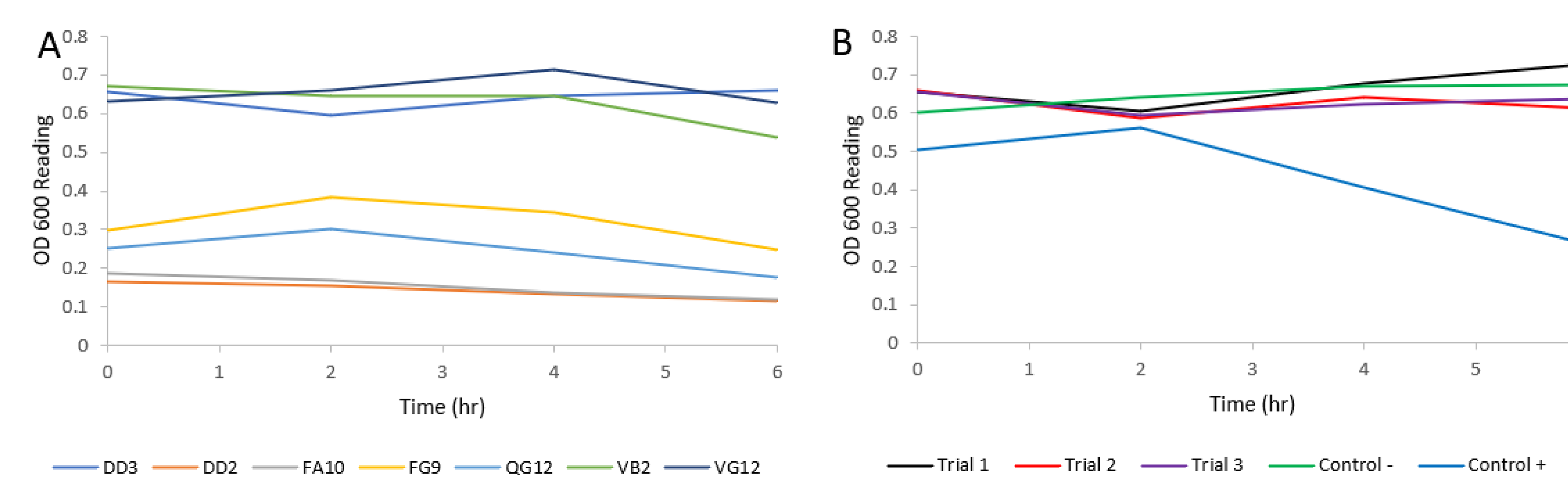

Figure 5: **Screening of Potentially Phage PC7 Resistant 14B11 Transposon Mutants.** After screening the 14B11 transposon mutant library, 7 mutants showed potentially interesting growth patterns. These 7 were grown and exposed to phage for a further 3 trials. Finally, all three trials were averaged, and a growth curve was generated of the bacteria inoculated with phage PC7 (A). DD3 was found to grow after phage PC7 inoculation in all three trials (B). Positive control is WT 14B11 inoculated with phage PC7 and negative control is WT 14B11 without inoculation of phage. DD3 was chosen over other strains such as VG12 and VB2 as it was the only strain to show consistent growth after the 2 hours mark

What Makes a “Good” Phage “Good”?

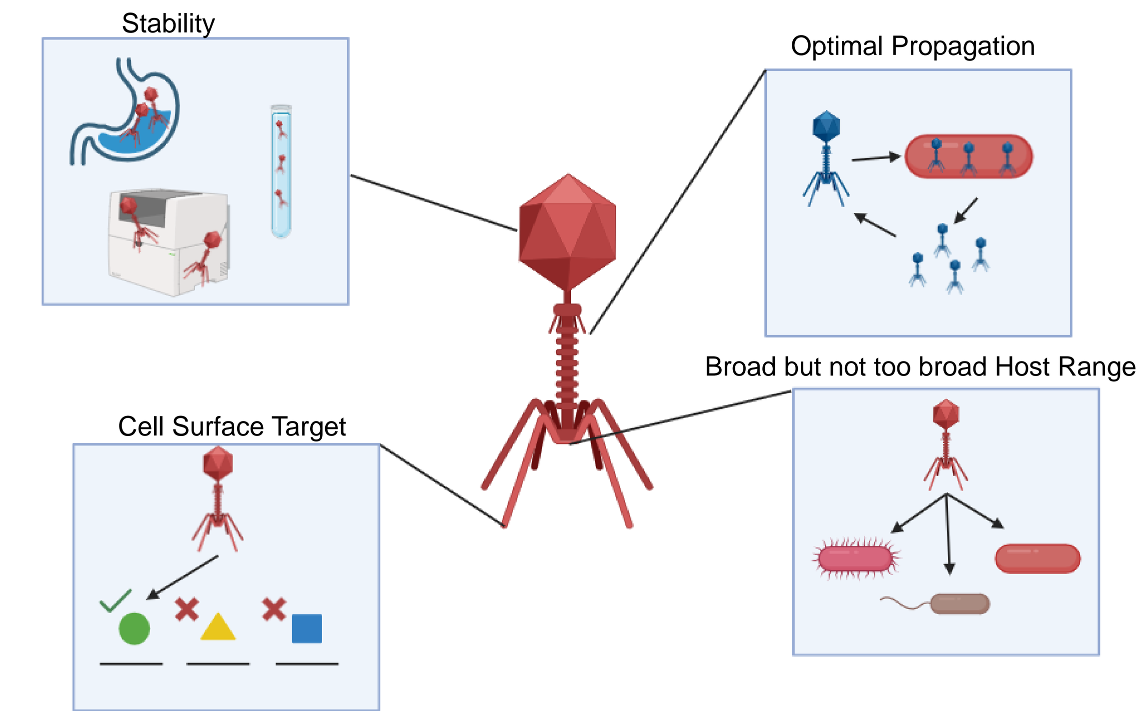

Figure 6: **Qualities of a “Good” Phage**. Phages that are useful in clinical or commercial applications typically have a few shared traits: Stable in desired conditions, efficient propagation, a conserved host cell surface target, and a host range that is broad but not too broad.

Potential phage discovery Pipeline

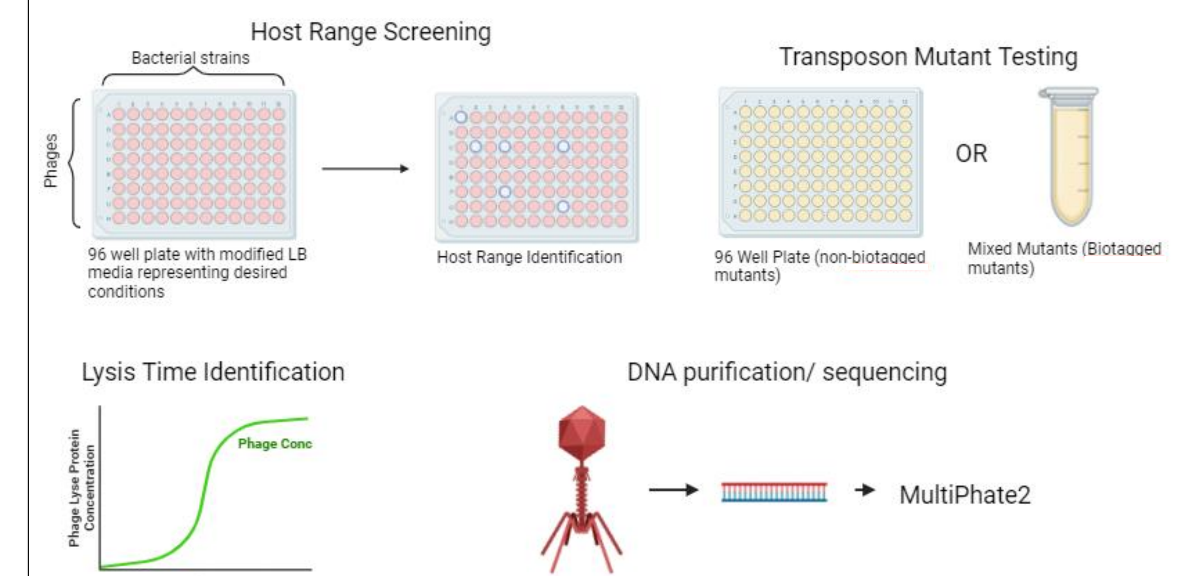

Figure 6: **Proposed Phage Discovery Pipeline**. To uncover phages with useful traits for clinical or commercial applications, I have put together a potential series of tests utilizing all I have learned throughout this project.

Future Research

- Complete the identification of PC7’s cell surface receptor target
- Sequence and annotate the genome of PC7
- Test the proposed phage pipeline

Acknowledgements

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Citations