Directed Evolution of T4 Bacteriophage

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Antibiotic-resistant bacteria threaten a future where even minor infections may become untreatable. This growing concern has renewed interest in an old alternative—phage therapy. Phage therapy uses bacteriophages, or phages, which are viruses that infect and kill bacteria. While promising, the approach is limited by phages' typically narrow host ranges. This study aimed to test the adaptability of the T4 phage's host range through directed evolution. Inspired by the Appelmans protocol, which uses phage cocktails, this experiment instead used a single phage type. T4 phage was exposed to four new E. coli strains using mixed and sequential presentation methods. The phage and bacteria were incubated in nutrient broth, and optical density was measured to track bacterial and phage activity. Significant changes in turbidity were observed in both experimental setups. To determine whether these changes were due to phage activity, plaque spot tests were conducted. These results suggest that the T4 phage evolved to infect at least one previously non-permissive E. coli strain. However, several samples also showed sharp drops in turbidity without prior exposure of the phage to those strains, suggesting that other factors, such as toxin accumulation, may have contributed to bacterial decline. Future work will involve performing additional plaque assays on current and past lysates to confirm host range expansion and rule out non-phage-related bacterial death. This research highlights the potential of directed evolution in expanding phage applicability and also the importance of distinguishing between biological mechanisms when evaluating bacterial death.

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