E: Elucidate the Hypothesis

Overall Research Question:

Can coevolution mechanisms between bacteriophages and their bacterial hosts be identified and attributed to specific causes/mutations, specifically between *Bacillus* strains and their phages?

Experiments:

1. Plaque assay to screen for phage-resistant bacterial mutants
    a. Experimental Test
2. Plaque assay to screen for regained-infectivity phage mutants
    a. Experimental Test
3. Mobility assay to analyze replication rate of control and mutant bacterial strains
    a. Experimental Test
4. Storage stability analysis of wild-type and mutant phages to determine their infectivity against control and mutant bacterial strains
    a. Experimental Test
5. Binding ability (absorption) analysis of wild-type and mutant phages to control and mutant bacterial strains
    a. Experimental Test
6. Genome sequencing of phage-resistant bacterial mutants
    a. Descriptive Study
7. Genome sequencing of phage mutants showing conserved mutation sites to observe polymorphisms and nucleotide compositions
    a. Descriptive Study
8. Bioinformatic analysis and structural modeling of wild-type and mutant proteins
    a. Descriptive Study

Hypotheses:

1. Since plaque assays are performed to determine if phages can infect a particular bacterial host and mutations may occur to allow bacteria to increase their fitness, if a bacterial clone is persistently resistant to a phage, then virtually no plaques will be present on the agar plate after multiple inoculations.
2. Since plaque assays are performed to determine if phages can infect a particular bacterial host and mutations may occur to allow phages to better infect their host, if a phage can reinfect a bacterial mutant, then plaques will be present on the agar plate after multiple tests.
3. Since motility assays can be used to determine the replication/growth rate of a bacterial strain and phenotypic changes are expected between bacteria and their mutants, if a bacterial mutant has a different replication rate than the original bacterial strain, then the clone of the bacterial mutant will be significantly different than the wildtype clone when plated on the agar plate.
4. Since previous studies have shown that stability of virion particles are largely dependent on time and changes in stability may occur in mutant phages, if regained-infectivity

phage mutants are less stable than their wild-type phages, then the phage titer for the phage mutants when added to their bacterial hosts should decrease over time.

5. Since the loss of binding ability, or adsorption, of a phage to its host bacteria plays a large role in phage resistance and may determine whether or not a phage can infect its host, if regained-infectivity phage mutants bind more readily to bacterial strains, then there will be a higher phage titer for the mutant phage when added to the bacterial strains.
6. Since host resistance mechanisms arise due to mutations in the bacteria's genome, if different phage resistant bacterial strains have the same resistance mechanisms, then conserved mutations sites should be present among the different bacteria genomes.
7. Since phage infectivity mechanisms arise due to mutations in the phage's genome, if the phage mutants have the same resistance mechanisms, then conserved mutations sites should be present among the different phage genomes.
8. Since three protein in the phage's genome were shown to be mutated among the phage mutant strains, if a mutation in the residues contribute to an acquired function in the phage, then the secondary structure of the mutated protein should be different compared to the protein in the wild-type phage.