E- Elucidate

Overall Research Question: Since phage morons encode genes that are evolutionarily advantageous for bacteria, if these morons are providing an evolutionary advantage through changing the bacteria's phenotype, then there should be a difference between normal bacteria and phage infected bacteria within the motility, biofilm forming capabilities, virulence factor production, killing capabilities, and resistance to superinfection.

Experiments

1. **Description:** Testing effects of phage moron on bacterial growth **Type:** Descriptive Study

Hypothesis: Since phage morons alter *P. aeruginosa* phenotype through integrating their own genome into the bacteria's, if these phage morons alter bacterial growth, then when measuring the growth rates of these infected bacteria, the bacteria infected with the morons should have differing growth rates from the normal bacteria.

2. **Description:** Testing effects of phage moron on twitching motility in bacteria **Type:** Experimental test

Hypothesis: Since phage morons alter *P. aeruginosa* phenotype through integrating their own genome into the bacteria's, if these phage morons affect the bacteria's pili, then there should be an observed difference in the distance traveled between the normal and infected bacteria.

3. **Description:** Testing effects of phage moron on swimming motility in bacteria **Type:** Experimental test

Hypothesis: Since phage morons alter *P. aeruginosa* phenotype through integrating their own genome into the bacteria's, if these phage morons affect the bacteria's flagella, then there should be an observed difference in the distance traveled between the normal and infected bacteria.

4. **Description:** Testing effects of phage moron on biofilm formation in bacteria **Type:** Experimental test

Hypothesis: Since prophage acquisition has been associated with changes in bacterial virulence and formation of biofilms typically increase virulence, if these moron genes increase virulence through formation of biofilms, then bacteria infected with these phages should have a corresponding higher OD595 than regular bacterial biofilms.

5. **Description:** Testing effects of phage moron on superinfection in bacteria **Type:** Free Response

Hypothesis: Since phages infect bacteria and utilize their cellular machinery to reproduce, it would be harmful to the prophage if another phage infected the same bacteria. As a result of this phages can carry genes that confer resistance to subsequent infections (superinfections). If these phage morons provide genes that benefit the bacteria through resistance to superinfections, then phages that carry these morons should be more resistant to subsequent phage infections.

6. **Description:** Testing effects of specific deletion mutations within the phage moron on superinfection in bacteria

Type: Experimental test

Hypothesis: Since prophages have multiple genes that confer resistance to superinfections, if a knockout mutation of genes JBD26-31 and JBD26-61 were performed, then both strains of *P. aeruginosa* should have increased susceptibility to superinfection.

7. **Description:** Testing effects of phage moron on rhamnolipid production in bacteria **Type:** Experimental test

Hypothesis: Since *P. aeruginosa* is reliant on overproduction of rhamnolipids and alginate virulence factors to infect the epithelial cells of cystic fibrosis patients, if phage morons change the output of virulence factors, then infected bacteria should produce different amounts of rhamnolipids compared to uninfected bacteria.

8. **Description:** Testing effects of phage moron on the lipid polysaccharide, specifically the O antigen in bacteria

Type: Experimental test

Hypothesis: Since the O antigen is one of the ways the immune system detects the presence of an infection and alteration to the O antigen helps bacteria evade detection, if phage morons increase bacterial survival through modifications to their O antigen, then infected strains should have their O antigens modified.

9. **Description:** Testing effects of phage moron on elastase production in bacteria **Type:** Experimental test

Hypothesis: Since *P. aeruginosa* is reliant on overproduction of rhamnolipids and alginate virulence factors to infect the epithelial cells of cystic fibrosis patients, if phage morons change the output of virulence factors, then infected bacteria should produce different amounts of elastase compared to uninfected bacteria.

 Description: Testing effects of phage moron on bacteria in vivo in bacteria Type: Experimental test

Hypothesis: Since the previous experiments demonstrated these altered virulence phenotypes in vivo, if these altered virulence phenotypes affect infections in vitro, then infections of *Drosophilia melanogaster* with both infected and uninfected *P. aeruginosa* should result in different survival rates of *D. melanogaster*.