CREATES

C- Concept Map

Pseudomonas aeruginosa: A strain of bacteria that is Gram-negative, facultatively aerobic, and commonly encapsulated. Characterized as an opportunistic pathogen that is resistant to antibiotics and commonly occurs alongside cystic fibrosis. Researchers from the study used this strain of bacteria in their experiment.

Bacteriophage: A type of virus that infects bacteria and reproduces within the bacteria it infects.

Bacteriophage resistance: The ability of a bacteria to counteract bacteriophage infection.

Biofilms: A collective of one or more microorganisms that clump together and grow on many different surfaces. Biofilms increase the durability and survivability of the organisms that are part of it.

Motility: How an organism moves. Various types of motility include twitching or swimming motility.

Prophages: Phages that have integrated themselves into the bacterial genome but are in an inactive state.

Lysogenic Cycle: Life cycle for phages that involve integration of itself into the host genome and remaining there until phage switches to lytic cycle.

Lytic Cycle: Life cycle for phages that infect their host and hijacks their cell machinery to produce more phages which eventually involves in lysis of host that releases more phages.

Superinfection exclusion proteins: Proteins tha t inhibit further phage infection by altering the bacterial cell envelope.

Virulence factors: Molecules synthesized by pathogens that infect or damage the host.

Morons: Prophage genes that increase bacterial fitness.

Horizontal Gene Transfer: Transfer of genetic material between organisms. Various modes of HGT involve transformation, transduction, and conjugation.

Summary: Researchers knew that there was no previous study regarding the investigation of the activities of phage morons. In particular, the study focuses on the phenotypic changes that these morons cause to the bacteria. This also sheds more light on how these prophages influence bacterial behavior and ultimately the bacteria’s behavior in regard to human disease.

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.

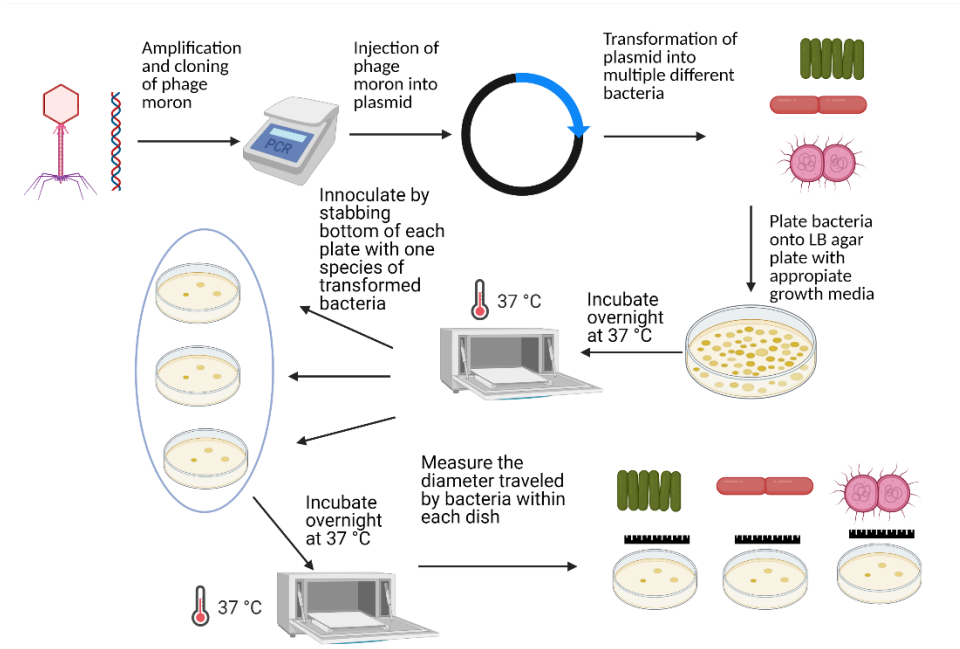
7. **Description:** Testing effects of phage moron on rhamnolipid production in bacteria
   Type: Experimental test
   Hypothesis: Since *P. aeruginosa* is reliant on overproduction of rhamnolipids and alginate virulence factors to infect the epithelial cells of cystic fibrosis patients, if phage morons 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.
10. **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*.

TE- Student Experiments

- **Problems with the Paper**
  - Some of the data did not have a control group because the data was normalized around what was presumably the control group.
    - Continuing on this point, even if that is what they did, it should be mentioned explicitly that this was done.
  - I felt that a greater diversity of morons should be used since they all affect the bacteria differently and perhaps a pattern as to how these morons affect bacteria can be discerned if the sample size were larger.
    - This could also lead to more interesting data as none of the morons had an effect on antimicrobial resistance but perhaps other morons may have had an effect.
- **Questions that were not addressed**
  - Why was PA14 the only strain they tested with the deletion mutations? Why was PAO1 not tested with PA14?
  - Along the same line, why was JBD26 the only one that went through RNA seq?
  - Why was only PAO1 used as the control for the rhamnolipid assay?
  - Why was only PA14 injected with JBD44-8?
  - Why did only PA14 infected with JBD44-8 have an effect on the survival rate of *D. melanogaster*?

- **Questions that can be answered in the future**
  - What are the specific mechanisms of these morons that cause the change in the bacteria?
  - What are the effects of these phage morons on bacterial strains that are not *P. aeruginosa*?

Using Multiple Different Strains of Bacteria



Testing Superinfection With more than 2 Phage morons