

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?

Overall Hypothesis:

Since the accumulation of mutations obtained via mechanisms of antagonistic coevolution lead to phenotypic changes in phages and their bacterial hosts, if mutations are shared within phage-resistant bacteria and regained-infectivity phage mutants, then there should be consistent patterns in the phenotypic changes and behaviors of these mutants.

List of experiments:

- 1) Description: Mobility assay
 - a. Type: Experimental
- 2) Description: Storage stability assay
 - a. Type: Experimental
- 3) Description: Binding affinity assay
 - a. Type: Experimental
- 4) Description: Genome analysis - identification of the mutant sites
 - a. Type: Descriptive
- 5) Description: Polymorphism analysis of the area of mutation in the baseplate protein of the phages
 - a. Type: Descriptive

Hypothesis:

- Experiment #1: Mobility assay
 - Since phenotypic change in bacterial mutants are expected, if wild type bacteria and first-generation phage resistant bacterial mutants exhibit different levels of mobility, then the change in the mobility is due to the genetic mutations.
- Experiment #2: Storage stability assay
 - Since mutations may affect phenotypic behaviors such as the storage stability of phages, if phage titer of phage mutants when added to bacterial hosts decreases at higher time intervals, then regained-infectivity phage mutants must be less stable than wild-type phages.
- Experiment #3: Binding affinity assay
 - Since the alterations in the phage infectivity against bacterial hosts are usually tied to the mutations in their surface receptors, if regained-infectivity phage mutants display higher adsorption rate to both the wild type host and the phage-resistant hosts, then it is safe to assume

that the mutations have occurred in the genes that encode for surface proteins.

- Experiment #4: Genome analysis - identification of the mutant sites
 - Since several other studies have indicated that the adsorption of flagellum is the first step for phage infection, if the conserved mutation between phage-resistant bacterial hosts is located in the genomic region encoding for flagellum, then the resistant hosts will experience a fitness cost due to the genomic changes in the flagellum gene.

- Experiment #5: Polymorphism analysis of the area of mutation in the baseplate protein of the phages
 - Since phage receptor binding proteins are essential during phage infection, if regained-infectivity phage mutants have gained mutations of the baseplate protein, then the mutations in the baseplate protein must be non-synonymous.