

**E: Elucidate the Hypothesis for Each Experiment \*pathogen = *R. solanacearum*\***

**Identify the Overall Research Question:**

What are the effects of phage therapy in the tomato plant rhizosphere against *Ralstonia solanacearum*, and how does phage therapy influence phage-resistance and the diversity and composition of the wider rhizosphere microbiome?

**List of Experiments (Based on Figures)**

- Description:** In a greenhouse, researchers introduced either one, two, or three phage types to the rhizosphere that had soil with *R. solanacearum*. The control included no added phages. After this, researchers recorded bacterial wilt disease incidence as well as pathogen and phage densities in accordance with the treatment condition given.

**Type:** Experimental Test (Figure 1a, b)
- Description:** Researchers performed this experiment in an existing field that naturally contained *R. solanacearum*. When tomato plants were planted, researchers either added no phages (control), or a single phage type. Another experimental condition included the addition of all four phage types to the rhizosphere of the tomato plant, tracking disease index after subsequent growth.

**Type:** Experimental Test (Figure 1c)
- Description:** Researchers exposed evolved pathogens from the greenhouse experiment (treated with one, two, or three phage types) and recorded resistance to ancestral phages as well as coevolved phages. Experimental control included a pathogen that evolved without the presence of a phage.

**Type:** Experimental Test (Figure 2a, b)
- Description:** Researchers used evolved pathogens from the greenhouse experiment once again and calculated the pathogen carrying capacity in the absence of phages, organized by how many phage types the pathogen was exposed to during tomato plant growth. The control was an ancestral pathogen unexposed to phages. Also created a figure that looks at correlation between pathogen carrying capacity and mean resistance to ancestral phages to establish fitness tradeoffs.

**Type:** Experimental Test (Figure 2c, d)
- Description:** Researchers looked at the rhizosphere communities from the initial greenhouse experiment and measured rhizosphere composition and diversity. The control for these tests included the pathogen of interest without any phage treatment. These figures were made using a variety of analytical tools from the DNA captured from the microbiome samples, arranged based on the type of phage therapy received (1, 2, 3 types of phage). Also, looked at relative densities of the pathogen and other phyla given the treatment

**Type:** Experimental Test (Figure 3a, b, c)

6. **Description:** Researchers used data collected on OTUs for single-phage and three-phage treated communities from the greenhouse experiment and used Netshift to identify possible driver taxa within the microbiome. These driver taxa potentially played roles in the changing network structure of the community.  
**Type:** Partly Experimental/Observational (Figure 3d)
7. **Description:** Researchers used a lab experiment to test whether phages directly change the rhizosphere microbiome community. Included looking at composition and diversity of a community (control), with three-phage combination, with pathogen, and with both phage and pathogen.  
**Type:** Experimental Test (Figure 4a, b)
8. **Description:** Researchers randomly chose 400 bacteria from rhizosphere soil used in greenhouse experiment, created phylogenetic tree based on these, and measured their effects on the growth of the ancestral pathogen.  
**Type:** Experimental Test (Figure 4c, d)
9. **Description:** Researchers created a pathway schematic that attempts to highlight phage therapy and its ecological and evolutionary mechanisms against the pathogen and disease incidence. This was done using a PLS-SEM analysis.  
**Type:** Analytical Model (Figure 5)

### Identifying a Hypothesis for Each Experiment

1. **Overall Study**  
Since *R. solanacearum* is a causative agent of bacterial wilt and is found in the tomato plant rhizosphere, if phage therapy can effectively inhibit bacterial wilt without negatively affecting the rhizosphere microbiome, then the usage of phage therapy will reduce *R. solanacearum* population and virulence without directly introducing drastic changes to the rest of the microbial community.
2. **Experiment 1** (Figure 1a, b)  
Since bacterial wilt disease is caused by *R. solanacearum*, if combination phage therapy effectively targets *R. solanacearum*, then there will be a decrease in disease incidence and pathogen density when implementing combination phage therapy.
3. **Experiment 2** (Figure 1c)  
Since increasing the number of phages in combination is expected to limit pathogen density by increasing efficiency of infection, if implementing combination phage therapy can effectively infect *R. solanacearum*, then bacterial wilt disease severity will be lower in plants treated with multiple types of phages rather than a single type of phage.
4. **Experiment 3** (Figure 2a, b)  
Since hosts are known to coevolve with phages in an evolutionary arms race, if *R. solanacearum* coevolves with phages in combination phage therapy, then *R.*

*solanacearum* samples that were treated and grown with phages will show increased resistance when exposed to phages that target it.

5. **Experiment 4** (Figure 2c, d)

Since fitness trade-offs in *R. solanacearum* for phage resistance has been observed in previous studies, if these trade-offs weaken *R. solanacearum*'s ability to cause disease, then coevolved *R. solanacearum* will be limited to lower carrying capacities with negative correlation toward increased resistance to phages.

6. **Experiment 5** (Figure 3a, b, c)

Since phages operate within the microbiome and can alter populations of targeted bacteria such as *R. solanacearum*, if phage therapy alters bacterial composition of the tomato plant rhizosphere, then there will be a measurable change in the microbiome of the sampled rhizosphere when exposed to phage therapy.

7. **Experiment 6** (Figure 3d)

Since driver taxa drive bacterial community composition within a microbiome, if phage therapy contributes to the alteration of the bacterial community, then driver taxa networks will be observable in the community according to the phage therapy given.

8. **Experiment 7** (Figure 4a, b)

Since phages are known for host specificity and precision, if phage therapy only indirectly affects the microbiome community through its interactions with *R. solanacearum*, then communities with only the phage present will have similar composition to just the community while the community with the pathogen will have a drastically different composition.

9. **Experiment 8** (Figure 4c, d)

Since a reduction in pathogen density by phages can increase niche space for competing native bacteria, if combined phage therapy increases bacteria that compete and antagonize *R. solanacearum*, then competition co-culture experiments will reveal decreased growth of *R. solanacearum* when placed with these strains of bacteria.

10. **Experiment 9** (Figure 5)

Since a PLS-SEM (partial least-squares structural equation model analysis) estimates and quantifies complex cause and effect relationship models, if phage combination therapies effectively reduce bacterial wilt disease incidence, then a pathway produced by PLS-SEM using experimental results will highlight a relationship that shows phage therapy reduces disease incidence.