

CREATES Analysis Template

Experimental Test

Figure or Table Number:

1

“Official” title for this figure or table (from the caption):

Phage combinations and incidence of disease

My (simplified, decoded, in regular language) title for this figure or table:

Phage combination therapy decreases disease incidence and pathogen density

The controls in this experiment are:

- No phage treatment during greenhouse growth
- No phage added to soil in field experiment

They are represented (in which part of the chart or graph, or what figure panels?)

- Figures 1 a-b: as a red dotted line
- Figure 1c: the leftmost values on the plot, labeled “Control”

The experimental conditions are:

Greenhouse: Tomato plant rhizospheres treated with 1, 2, or 3 types of phages

Field: Single phage treatment or 4-phage treatment

They are represented as:

Figures 1 a-c highlight the results from each experimental condition. (plots)

We need to compare the controls in Figure 1a and 1b with the experimentals in

Figure 1a and 1b to find out:

Whether disease incidence changed based on the addition of phages and whether pathogen and phage density changed based on the addition of phages

We need to compare the controls in Figure 1c with the experimentals in

Figure 1c to find out:

Whether single phage or 4-phage (multitype-phage) treatment is more effective at lowering plant disease severity.

When we make these comparisons, we conclude from this figure:

In Figure 1a, the disease incidence of bacterial wilt disease decreases as the number of phage types used in treatment increases. This points to combination phage therapy increasing effectiveness at preventing disease.

In Figure 1b, both pathogen and phage density decrease as the number of phage types used in treatment increases. This points to a possible connection between phage density and pathogen density as well.

In Figure 1c, the 4-phages treatment method is more effective at decreasing disease index than single phage treatment methods. The distribution in this data shows using 4 phage types had the best biocontrol efficacy.

Was the hypothesis supported? Why or why not?

The hypothesis was supported because it shows that phage combination therapy effectively decreases disease incidence as it pertains to the pathogen. It also shows that phage therapy decreases pathogen density, supporting the hypothesis that predicted the phages would be able to target the pathogen.

Also, the hypothesis predicted that phage combination therapy would help increase host infectivity, and this is supported based on the results from Figure 1c.

The following issues are ones of concern to me (these can be things you don't understand, or criticisms of the method, questions for the authors, or anything else that comes to mind):

The violin plot in Figure 1c can be a bit confusing to decipher based on all the dots that seem to be outliers. Also, the pathogen and phage density labeling on Figure 1b can be confusing because some may take phage density as contradictory to the x-axis which lists "number of phages." To prevent this confusion, number of phages could be replaced with phage types.

CREATES Analysis Template

Experimental Test

Figure or Table Number:

2

"Official" title for this figure or table (from the caption):

Resistance evolution to ancestral and coevolved phages

My (simplified, decoded, in regular language) title for this figure or table:

Coevolved pathogens show increased resistance to phages with fitness trade-offs (carrying capacity)

The controls in this experiment are:

They are represented (in which part of the chart or graph, or what figure panels?)

- Pathogen evolved in absence of phages (Figure 2a)
- Growth of ancestral pathogen in absence of phages (Figure 2c)

- In both Figure 2a and 2c, was a red dotted line within the plot

The experimental conditions are:

Figure 2a: number of phages pathogen was coevolved with in greenhouse experiment

Figure 2b: same as 2a, but resistance taken against both ancestral and coevolved phages

Figure 2c: same as 2a, but dependent variable was carrying capacity

Figure 2d: correlation plot with x-axis displaying mean resistance to ancestral phages and y-axis displaying carrying capacity; also marks phage treatment (0, 1, 2, 3 phage types)

They are represented as:

Figures 2a and 2c use box plots to highlight means and data points.

Figure 2b shows means as well to indicate resistance to both ancestral and coevolved phages.

Figure 2d is a scatterplot with a parsimonious line through the plot to indicate general trend and model

We need to compare the controls in **with the experimentals in**

to find out:

If mean resistance to ancestral phages changes based on how many phage types the soil was treated with

We need to compare the controls in **with the experimentals in**

to find out:

If pathogen carrying capacity of *R. solanacearum* in the absence of phages changes depending on the type of phage treatment it underwent in the greenhouse

When we make these comparisons, we conclude from this figure:

An increased number of phage types in treatment of the pathogen results in selection for a more phage-resistant pathogen population. This is seen in resistance against both ancestral and coevolved phages. However, increased phage resistance is also seen to correlate with decreased pathogen carrying capacity.

Was the hypothesis supported? Why or why not?

The hypothesis is supported because it shows that increasing exposure of the pathogen to phages does select for more phage resistance. The hypothesis is also supported because it was inferred that fitness trade-offs would also occur in the pathogen due to this resistance. In this figure, the trade-off is quantified with a decreased pathogen carrying capacity in more resistant pathogens.

The following issues are ones of concern to me (these can be things you don't understand, or criticisms of the method, questions for the authors, or anything else that comes to mind):

The plot in Figure 2b seems a bit redundant, and could have been merged with Figure 2a to prevent confusion. Resistance to both ancestral and coevolved phages shows that the pathogens are more widely resistant to phages, but it was not the best looking figure to highlight this.

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Experimental Test

Figure or Table Number:

“Official” title for this figure or table (from the caption):

Effects of phages on rhizosphere communities

My (simplified, decoded, in regular language) title for this figure or table:

Phage therapy associated with changes in diversity and composition of rhizosphere

The controls in this experiment are:

- No phages added (Figure 3a-c)

They are represented (in which part of the chart or graph, or what figure panels?)

- In Figure 3a: represented by red area, denoted N0
- In Figure 3b: denoted as red dotted line on plot
- In Figure 3c: bar shown to the very left of plot, with 0 on x-axis

The experimental conditions are:

- For Figures 3a-c, the experimental conditions include the type (amount of types) of phage treatment received

They are represented as:

- Figure 3a: labeled areas denoting microbiome composition
- Figure 3b: distribution dots based on OTUs
- Figure 3c: bars with appropriately labeled treatment on x-axis

We need to compare the controls in

with the experimentals in

to find out:

How the composition of the microbiome, diversity of the microbiome, and abundance of different bacteria are altered based off what phage treatment is performed.

When we make these comparisons, we conclude from this figure:

From all these figures, we can conclude that phages are associated with changes in the rhizosphere communities. These figures and experiments cannot show that phages are directly responsible for this change, but they do point to driver taxa influencing these community changes (Figure 3d) which are associated with single or three-phage treatment.

Was the hypothesis supported? Why or why not?

The hypothesis is supported because it was known that phages interact with the bacterial target, which is the pathogen *R. solanacearum* in this case. The phage treatment does seem to be associated with these rhizosphere community changes, but it is unclear if it is direct because these experiments look at the rhizosphere after treatment with phages and the targeted pathogen.

The following issues are ones of concern to me (these can be things you don't understand, or criticisms of the method, questions for the authors, or anything else that comes to mind):

Figure 3d is a bit confusing because of all the lines that are presented. It is a network figure so this does make sense, but the researchers could have made a figure that was a bit easier to digest, highlighting the driver taxa relationships between different phage therapies that they spotlighted in the text.

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Experimental Test

Figure or Table Number:

“Official” title for this figure or table (from the caption):

Phage specificity and effects on the suppression of rhizosphere microbiota

My (simplified, decoded, in regular language) title for this figure or table:

Phages indirectly change the rhizosphere microbiome

The controls in this experiment are:

- Community analysis without added phage or pathogen (Figure 4a-b)
- Taxa unaffected by number of phages (Figure 4d)

They are represented (in which part of the chart or graph, or what figure panels?)

- In Figures 4a-b, the control is clearly labeled as “Community.”
- In Figure 4d, a grey dotted line is denoted on the plot as “taxa unaffected by number of phages”

The experimental conditions are:

- Community + Phage, Community + Pathogen, Community + Pathogen + Phage (Figures 4a-b)
- Taxa reduced with increasing number of phage and taxa enriched with increasing number of phage (Figure 4d)

They are represented as:

- Clearly marked in Figures 4a-b
- Denoted as blue dotted line and orange dotted line respectively in Figure 4d

We need to compare the controls in

with the experimentals in

to find out:

How the community rhizosphere microbiome is affected when adding only the phage pathogen, or both to them

We need to compare the controls in 4d with the experimentals in

4d

to find out:

The effect that these taxa may have on the growth of the pathogen, *R. solanacearum*

When we make these comparisons, we conclude from this figure:

We can conclude that the phage alone with the community is very similar to just the community by itself. It is shown that the pathogen itself is what has a great effect on both the diversity and composition of the community rhizosphere. Also, it is shown that taxa enriched by increased phages seem to negatively affect pathogen growth as well.

Was the hypothesis supported? Why or why not?

The hypothesis is supported because, since it is based on phages having precise host specificity, its associations to a changing rhizosphere microbiome are due to indirect feedback rather than direct interactions. Also, the reduction of pathogens may support the enrichment of antagonistic and competing bacterial species that further alter the microbiome and limit pathogen growth.

The following issues are ones of concern to me (these can be things you don't understand, or criticisms of the method, questions for the authors, or anything else that comes to mind):

The phylogenetic tree shown in Figure 4c indicates the bacteria randomly chosen for the analysis in Figure 4d, but I believe this could have been replaced by a table, with clear results indicating that the phages used were unable to infect these chosen bacteria.

CREATES Analysis Template

Free Response

Figure or Table Number:

“Official” title for this figure or table (from the caption):

Mechanisms underlying phage-mediated effects on bacterial wilt disease

My (simplified, decoded, in regular language) title for this figure or table:

Schematic connecting phage therapy and its effects to reducing bacterial wilt disease

Analysis of the figure or table:

This figure was obtained by plugging in experimental results into a PLS-SES analysis. Through this analysis, cause and effect relationships were able to be hypothesized, and these are shown with this schematic. Some clear relationships, such as phage therapy increasing phage resistance and decreasing pathogen carrying capacity are highlighted. Some interesting takeaways include the nonsignificance of microbiome diversity to disease incidence.

When we analyze the figure or table, we conclude:

Phage therapy addresses bacterial wilt disease in different ways, from both ecological and evolutionary standpoints. This figure includes the concepts of fitness trade-offs, effects of pathogen density, and more.

Was the hypothesis supported? Why or why not?

The overall hypothesis of the study is supported. This schematic shows that phage combination therapy can be a way in which bacterial wilt disease is prevented. Its clear negative effects on pathogen carrying capacity, which, when positive, in the schematic leads to disease incidence is a sign that it can be explored more as a form of agricultural biocontrol.

The following issues are ones of concern to me (these can be things you don't understand, or criticisms of the method, questions for the authors, or anything else that comes to mind):

The schematic seems a bit simplified, and I wish the authors gave a bit more analysis of the figure in the relevant results section. The numbers above the arrows also denote magnitude, but a one sentence qualification of this magnitude in the results would help increase the significance of the figure.