Name

CREATES Analysis Template

Experimental Test

Figure or Table Number: 1

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

Figure 1. Isolation and characterization of phage-resistant mutants of BMB171 and regained-infectivity phage mutants of vB_BthS_BMBphi.

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

Figure 1. Isolation of resistant bacteria against vB_BthS_BMBphi infection and mobility changes in the resistant bacteria.

The controls in this experiment are:

Wild type B. thuringiensis BMB171 strain, which acts as a positive control that always shows plaque formation.

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

The control strain is represented in Figure 1A at the bottom, which is the only agar plate with plaque formation.

The experimental conditions are:

The mutations in the bacterial genome due to antagonistic coevolution between B. thuringiensis BMB171 strain and its wild type phage vB_BthS_BMBphi.

They are represented as:

Either plaque formation or non-plaque formation. The plates that display no plaque formation are considered as phage resistant bacterial strains, or PRBs.

We need to compare the controls in 1A **with the experimentals in** 1B **to find out:**

Name

The isolation of phages that have regained infectivity via mutations.

We need to compare the controls in 1C **with the experimentals in** 1D **to find out:**

Quantitative evaluation of clone size difference between the wild type strain of B. thuringiensis and other PRBs.

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

The phage-resistant bacterial mutants have gained mutations that allows for protection against the wild type phage vB_BthS_BMBphi, while also driving subsequent antagonistic evolution of phages that have obtained enough mutations to regain infectivity to the first-generation phage resistant bacterial hosts.

Was the hypothesis supported? Why or why not?

Yes, the hypothesis was supported by other subsequent experiments which identified the regions of genomic mutations that are conserved in between all phage-resistant bacteria, which was the flagellum gene FlhA.

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):

I don't understand why PSB-5, unlike other phage-resistant mutants, did not gain increase in its mobility, which was the phenotypic change observed in other phage-resistant mutants.

Name

CREATES Analysis Template

Experimental Test

Figure or Table Number: 2

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

Figure 2. Storage stability of the phage vB_BthS_BMBphi and vB_BthS_BMBphi-M1. The infectivity of the two phages was tested against the strains BMB171 and PRB-4, respectively, after storage for 24, 48, and 72h.

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

Figure 2. Loss of infectivity and stability of regained-infectivity phage over the course of time inside a stored agar plate.

The controls in this experiment are:

Wild type phage vB_BthS_BMBphi infecting wild type B. thuringiensis BMB171 strain.

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

The left bar graph in Figure 2.

The experimental conditions are:

With all other variables controlled, the only experimental condition applied to this agar plate was the infecting phage and the bacterial host that is being infected.

They are represented as:

The control variable is represented as the title of the bar graph, which indicates that the phage is a wild type phage.

We need to compare the controls in 2 **with the experimentals in**

Name

2 **to find out:**

Phages that have undergone mutations and regained infectivity experience decrease in storage stability, which is indicated by the lower phage titer obtained from the supernatant.

We need to compare the controls in 2 **with the experimentals in**

3 **to find out:**

Decrease in storage stability is tied to an increase in adsorption rate of these phages.

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

Although regained-infectivity phage mutants do gain higher binding affinity, which is logical since these mutants regain access inside the bacterial hosts, these mutants experience lower storage stability due to the alterations in their genes.

Was the hypothesis supported? Why or why not?

Yes, the hypothesis was supported by the data, because the mutant phages did show faster decrease in phage titer during the storage period.

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):

While the phage titer decreased drastically in the phage-resistant strain PRB-4, the phage titer remained almost identical in the WT strain when the mutant phage was co-present with the phage-resistant bacterial strain for about 24 hours. Are there any specific details of the mutant phage that allows for the phage to remain active for longer time compared to when it infects the mutant bacterial strain?

Name

CREATES Analysis Template

Experimental Test

Figure or Table Number: 3

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

Figure 3. Adsorption of phage vB_BthS_BMBphi (A) and vB_BthS_BMBphi_M1 (B) to strain BMB171 and four phage resistant mutants.

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

Figure 3. Better binding affinity observed in regained-infectivity phage mutants compared to the wild type phage.

The controls in this experiment are:

Wild type phage vB_BthS_BMBphi displaying great adsorption to the wild type B. thuringiensis BMB171 strain, but being unable to adsorb to any other PRBs.

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

The control strain, which is the wild type strain, is placed on the graph in panel A (black line).

The experimental conditions are:

Either wild type strain vB_BthS_BMBphi or the mutant strain vB_BthS_BMBphi-M1 being exposed to 4 picked PRBs separately, each of them interacting with 1 PRB at a time.

They are represented as:

Each line with different color represents different bacterial host co-cultured together with either the wild type phage or the mutant phage (M1).

We need to compare the controls in 3A **with the experimentals in** 3B **to find out:**

Name

Only the mutant phage was able to adsorb to all bacterial hosts, while the wild type phage could not bind to any bacterial hosts with phage-resistant mutations.

We need to compare the controls in 3B **with the experimentals in** 2 **to find out:**

Comparison of these two figures show how these phage mutants have selected maintenance of their race over their stability.

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

Although regained-infectivity phage mutants do gain higher binding affinity, which is logical since these mutants regain access inside the bacterial hosts, these mutants experience lower storage stability due to the alterations in their genes.

Was the hypothesis supported? Why or why not?

Yes, the hypothesis was supported by the data, because the mutant phages did show higher binding affinity and in the subsequent experiments it was shown that the mutations in the baseplate protein is what actually confers these phages to have better binding affinity.

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):

I need clarifications on why in the phage adsorption graph the wild type phage titer decreases slightly in other host bacterial strains also.

Tae Yoon Juen

CREATES Analysis Template

Descriptive Study

Figure or Table Number: 4

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

Figure 4. Analysis of the mutations of the phage-resistant bacterial mutants. (A) The mutant sites in four phage-resistant bacterial mutants. The functions and locations of the cell surface proteins that mutated in all the four mutants are indicated. The secondary structures of the protein are indicated. (C) Transmembrane domain of protein FlhA from strain MBM171 and PRB-1.

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

Figure 4. Genemone analysis of the conserved sites of mutation in all phage-resistant bacterial strains reveal that they all have deletion mutation in the gene that encodes for flagellum.

If we compare panel(s)/column(s) 4A **and** 4B **, we learn about:**

We can determine that the conserved pattern in mutation that is observed in FlhA gene is a deletion mutation universally shared between phage-resistant bacteria.

If we compare panel(s)/column(s) 4B **and** 4C **, we learn about:**

We know that the deletion mutation has led to deletion of one hydrophobic region in the flagellum protein that enters the membrane.

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

The flagellum protein goes through deletion mutation in all phage resistant bacterial hosts.

Was the hypothesis supported? Why or why not?

Contrary to what was expected, the phage-resistant bacterial hosts' mobilities were not compromised due to the mutation in the flagellum protein.

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):

I did not understand why the patterns observed in PRB-5 was inconsistent with rest of the phage resistant bacterial hosts if they all underwent the same type of deletion mutation.

Tae Yoon Juen

CREATES Analysis Template

Descriptive Study

Figure or Table Number: 5

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

Figure 5. Polymorphism of the mutant nucleotides in phage genome. The ratio of the nucleotide compositions of the three mutant nucleotides in four phage genomes is shown. The nucleotide composition was obtained by analyzing the raw reads archived by genome sequencing and the sites of each mutant nucleotide were shown.

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

Figure 5. Single nucleotide polymorphism in the genes encoding for baseplate protein in regained-infectivity phage mutants.

If we compare panel(s)/column(s) 5 **and** 5 **, we learn about:**

There were mutations in 3 different locations of the gene associated with the formation of the phage baseplate.

If we compare panel(s)/column(s) 5 **and** 5 **, we learn about:**

The synergium mutation of nucleotides from three different phage tail proteins are responsible for the regained infectivity of the phage.

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

The flagellum protein goes through deletion mutation in all phage resistant bacterial hosts.

Was the hypothesis supported? Why or why not?

Yes, the hypothesis was supported, because it was change in the baseplate structure that conferred regained-infectivity of these phages, specifically the single nucleotide polymorphisms.

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):

How was this ratio of nucleotides obtained?