Anthony Trieu

CREATES Analysis

Experimental Test - Screening for Phage-Resistant Bacteria

Figure or Table Number: 1A

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

Phage-resistant mutants of BMB171 isolated in this study

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

Six of nine bacterial clones shown to be persistently resistant to phage infective after more than 5 inoculations

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The controls in this experiment are:

-bacteria strain BMB171 (non-resistant strain)

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

The phage-resistant strains are represented by the top 6 agar plates in Figure 1A.

The experimental conditions are:

-bacterial clones that were repeatedly exposed to phage and shown to be resistant

They are represented as:

The phage resistant bacterial strains are represented as the top 6 agar plates in Figure 1A.

We need to compare the controls in Figure 1A **with the experimentals in**

Figure 1A **to find out:**

Which bacterial strains are resistant to phage infection.

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

6 of the 9 bacterial clones which were tested were shown to be resistant to the phage. This is demonstrated by the absence of plaques on the plates labeled PRB-1, PRB-2, PRB-4, PRB-5, PRB-6, and PRB-8. We can confirm this by comparing the experimental plates to the control plate. On the plate labeled BMB171, where the original bacterial strain was inoculated, there are clear plaques.

Was the hypothesis supported? Why or why not?

The hypothesis was: since plaque assays are performed to determine if phages can infect a particular bacterial host and mutations may occur to allow bacteria to increase their fitness, if a bacterial clone is persistently resistant to a phage, then virtually no plaques will be present on the agar plate after multiple inoculations.

The hypothesis was supported because based on the results, there were bacterial strains that were shown to be resistant to the phage. This was demonstrated by the absence of plaques on the plates labeled PRB-1, PRB-2, PRB-4, PRB-5, PRB-6, and PRB-8.

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 figure only provides the agar plates of 6 of the 9 bacterial clones that were tested with the phage. It would have been helpful to display the other bacterial clones that were non-resistant so that we could compare them to the ones that were deemed resistant.

-The methods were a little bit hard to follow. I think they were too concise and it seemed like the authors tried to consolidate everything into a few sentences. I had a hard time visualizing the different steps. I think providing a flow chart would have helped my understanding a lot, especially since there were so many steps involved. For example, there was not a lot of information regarding how these bacterial clones were created.

Anthony Trieu

CREATES Analysis

Experimental Test - Screening for Regained-Infectivity Phage Mutants

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Figure or Table Number: 1B

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

Infectivity of regained-infectivity phage mutants vm_BthS_BMBphi-M1 to the phage-resistant strains and BMB171

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

Co-cultivation of phage and phage-resistant bacterial yielded 6 phage mutants that could reinfect the bacterial mutant strains

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The controls in this experiment are:

-plate with bacteria strain BMB171 (non-resistant strain)

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

The middle agar plate labeled BMB171 in Figure 1B

The experimental conditions are:

-plates inoculated with both the bacterial mutant and the co-cultivated phage

They are represented as:

-the agar plate surrounding the middle agar plate (plates are labeled PRB-1, PRB-2, PRB-4, PRB-5, PRB-6, and PRB-8)

We need to compare the controls in Figure 1B **with the experimentals in**

Figure 1B **to find out:**

Which bacterial mutant strains the mutant phages can reinfect.

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

All phage isolated could infect the six phage-resistant mutants. This was demonstrated by the appearance of plaques on each agar plate. Although these plaques are not as noticeable as in the control group, they are clearly there.

Was the hypothesis supported? Why or why not?

The hypothesis was: Since plaque assays are performed to determine if phages can infect a particular bacterial host and mutations may occur to allow phages to better infect their host, if a phage can reinfect a bacterial mutant, then plaques will be present on the agar plate after multiple tests.

The hypothesis was supported since the plates showed that the phage could infect all the bacteria mutants. This was supported by the presence of plaques on the agar plates.

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 methods section was hard to follow. There were some terms that I had to look up, but even then I was still confused about some of the steps. They were hard to visualize and seemed to be too condensed. I will recommend expanding on the methods a little more so that another person can replicate the steps more easily.

-There was not a concrete hypothesis outlined in the results section. The author simply stated the results without much background information or reasoning.

Anthony Trieu

CREATES Analysis Template

Experimental Test - Mobility of Bacteria

Figure or Table Number: 1C and 1D

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

The mobility of the strains BMB171 and the phage-resistant mutants

Comparison of the bacterial clone size of strain BMB171 and the phage-resistant mutants

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

Mobility assay showed faster replication rate for all but one phage-resistant bacterial mutant

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The controls in this experiment are:

-the BMB171 bacterial strain

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

-the top most clone in the mobility assay in Figure 1C
-the first bar in the bar graph in Figure D

The experimental conditions are:

-the six phage-resistant bacterial mutants

They are represented as:

-clones on the mobility assay (6 bottom ones) in Figure 1C
-the five right most bars in the bar graph in Figure D

We need to compare the controls in 1C **with the experimentals in**

1C **to find out:**

The overall replication rate of phage-resistant bacterial mutant strains relative to the control strain

We need to compare the controls in 1D **with the experimentals in**

1D **to find out:**

The size of the clones of the phage-resistant bacterial mutant strains relative to the control strain

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

Strains BMB171 and PRB-5 formed the smallest clones on the plate. The other five mutants showed bigger clones, suggesting faster replication rates

Was the hypothesis supported? Why or why not?

Since mobility assays can be used to determine the replication/growth rate of a bacterial strain and phenotypic changes are expected between bacteria and their mutants, if a bacterial mutant has a different replication rate than the original bacterial strain, then the clone of the bacterial mutant will be significantly different than the wildtype clone when plated on the agar plate.

The hypothesis was not refuted, though not completely accepted as well. Although most of the resistant bacterial strains had larger clones, there was one clone that was still relatively small. This may have been an anomaly, but it certainly showed that not all resistant bacteria were significantly different from their original strain.

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):

Anthony Trieu

-What were the conditions of this mobility assay? The author only stated that an agar concentration of 0.3% was used. How exactly did they test this?

-How were the clones created? Under what conditions?

-What did they use to measure the diameter of the bacterial clones? ImageJ?

-Overall, I think the methods could have been more detailed. For example, the author only said a mobility assay was carried out, but they could have gone into depth about what the conditions were and how exactly they selected these clones.

Anthony Trieu

CREATES Analysis

Experimental Test - Storage Stability of Phage

Figure or Table Number: 2

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

Storage stability of the phage vB_BthS_BMBphi and vB_BthS_BMBphi-M1.

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

Storage stability analysis of wild-type and mutant phage show instability of mutant phage albeit an ability to infect resistant bacteria

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The controls in this experiment are:

-the non-resistant bacterial strain BMB171
-the wild-type phage

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

-First set of bars in each bar graph in FIgure 2
-The wildtype phage is represented by the left bar graph in Figure 2

The experimental conditions are:

-the phage resistant bacterial mutant PRB-4
-the regained infectivity phage mutant

They are represented as:

-Second set of bars in each bar graph in FIgure 2
-The mutant phage is represented by the right bar graph in Figure 2

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

The stability of the wildtype and mutant phages when tested against the non-resistant and resistant (PRB-4) bacterial strains

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

The wild-type phage infects the non-resistant bacterial strain consistently at all time intervals. However, it cannot infect the resistant bacterial strain.

The mutant phage is unstable because infectivity against the non-resistant bacterial strain decreases as the time interval increases. The same holds true when it is tested against the resistant strain. Although the mutant phage can infect the resistant bacteria, it does so at decreasing levels at higher time intervals.

Was the hypothesis supported? Why or why not?

The hypothesis was: Since previous studies have shown that the stability of virion particles are largely dependent on time and changes in stability may be observed in mutant phages, if regained-infectivity phage mutants are less stable than their wild-type phages, then the phage titer for the phage mutants when added to their bacterial hosts should decrease over time.

The hypothesis was supported. The mutant phage was shown to be unstable because its infectivity decreased at higher time intervals. This suggests that there is a mechanism involved that causes the mutant phage to die when it fails to infect the bacteria.

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 exactly was this storage analysis conducted? The paper did not discuss this. What is included in this analysis?

-Why was the phage only tested against PRB-4? Was there a specific reason for choosing PRB-4? Why not the other resistant strains?

-The author could be more descriptive in terms of why they chose to test certain strains and chose to omit others. I think this would help clarify things as well as address disparities in other strains.

Anthony Trieu

CREATES Analysis Template

Experimental Test - Adsorption Analysis

Figure or Table Number: 3

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

Absorption 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:

Wild-type phage demonstrates binding ability to only non-resistant bacterial strain while phage demonstrates binding ability to both control and mutant bacterial strains

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The controls in this experiment are:

-wild-type phage
-non-resistant bacterial strain

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

-wild type phage is represented by panel A
-nonresistant bacterial strain is represented by black line in each graph

The experimental conditions are:

-mutant phage
-phage-resistant bacterial strains (PRB-1, PRB-4, PRB-5, PRB-8)

They are represented as:

-the mutant phage is represented by panel B
-phage-resistant bacterial strains are represented by colored lines in each graph (red, blue, green, pink)

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

The binding ability of wild-type and mutant phages to resistant and non-resistant bacterial strains.

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

The wildtype phage demonstrates binding ability to only the non-resistant bacterial strain

The mutant phage demonstrates binding ability to both the non-resistant and the resistant strains. It binds to the control strain the best, then PRB-5.

Was the hypothesis supported? Why or why not?

Since the loss of binding ability, or adsorption, of a phage to its host bacteria plays a large role in phage resistance and may determine whether or not a phage can infect its host, if regained-infectivity phage mutants bind more readily to bacterial strains, then there will be a higher phage titer for the mutant phage when added to the bacterial strains.

The hypothesis was supported since the mutant phages showed higher binding ability to resistant bacterial strains. This was consistent across the mutant phages, as the phage titers were all higher against each bacterial strain.

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):

-Why were only 5 strains tested? What about the other phage-resistant bacterial strains? I think the authors should be a little more clear about why they choose to test certain strains and leave others out.

I think it would also be helpful to include images to help with visualization. The steps in the methods section were a little hard to follow along. I think they could have been more comprehensive.

Anthony Trieu

CREATES Analysis

Descriptive Study - Genome Analysis of Phage-Resistant Bacterial Mutants

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Figure or Table Number: 4

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

Analysis of the mutations of the phage-resistant bacterial mutants

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

Mutation sites and location calls between wildtype and mutant bacterial strain, as well as alignment of protein FlhA and visualization of transmembrane domains

---

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

The mutation sites between wildtype and mutant bacterial strains. Figure A shows the cell surface protein that mutated in the phage-resistant strains. The FlhA is highlighted in red in Figure A, and Figure B studies this protein more closely.

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

The transmembrane domain that is deleted in the bacterial mutant strains. Figure B shows a region in protein FlhA that is deleted in all the mutant strains, and Figure C shows the transmembrane domain that is omitted as a result.

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

The first transmembrane alpha-helix domain in the N-terminal of the bacterial flagellar biosynthesis protein was deleted in the four bacterial mutants that were studied. Protein FlhA plays a role in flagellum synthesis. A mutation in this protection may cause the phage resistance of BMB171 since the flagellum has been proven to be the phage-binding receptor of some phages. Further analysis is required.

Was the hypothesis supported? Why or why not?

Since host resistance mechanisms arise due to mutations in the bacteria's genome, if different phage resistant bacterial strains have the same resistance mechanisms, then conserved mutation sites should be present among the different bacteria genomes.

The hypothesis was supported because they were able to identify a conserved mutation that was shared between the bacterial mutants. All mutants showed a deleted protein in the Flha protein and a consequent loss of transmembrane domain. However, further analysis is required to better understand this mutation.

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 was confused by parts of the results section. It seemed like the authors were going back and forth between different techniques, which made it difficult for me to follow along. For example, I was confused as to whether they were comparing PRB-5 to other PRB-strains.

-They mentioned how the flagellum was proven to be the phage-binding receptor of some phages. I was curious as to what phages these are. Do they belong to the same family as the phage they were studying? How was this proven? Did they use microscopy?

-I am curious as to what programs/software they used to visualize the transmembrane domains. I don't believe they mentioned this in the methods section.

Anthony Trieu

CREATES Analysis

Descriptive Study - Genomic Analysis of Mutant Phages

Figure or Table Number: 5

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

Polymorphism of the mutant nucleotides in phage genome

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

Three nucleotide sites in the phage genome showed conserved mutations among the phage mutants

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

The three mutant nucleotides that show polymorphisms in the phage genome. In position 24,500nt, there is a shift from C to T. In position 26,344, there is a shift from G to A. Lastly, at position 29,139, there is a shift from position G to A. This is demonstrated by the different colors.

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

By analyzing the raw reads, we can see there is a conserved mutation at three different sites in the mutant phage genomes. The results also suggest that the individual mutant existed before the screening of the phage, but was higher when the phage acquired regained infectivity after co-cultivation.

Was the hypothesis supported? Why or why not?

Since phage infectivity mechanisms arise due to mutations in the phage's genome, if the phage mutants have the same resistance mechanisms, then conserved mutation sites

should be present among the different phage genomes.

The hypothesis was supported because there were three conserved mutations shown in the mutant phage genomes. They are visibly conserved as shown by the clear shift in nucleotides at these specific locations.

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 am still a little confused about the functional analysis they mentioned regarding the three different proteins. I am also still a little unclear about how they tested for polymorphisms. Are they using a specific program/software that is aligning these reads?

Anthony Trieu

CREATES Analysis

Descriptive Study - Structural Modeling

Figure or Table Number: 6

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

Predicted structure of the regions in the mutant proteins from the phage genomes

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

Structure modeling of 3 proteins in phages and mutant phages that were shown to be mutated

---

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

The predicted structure of baseplate protein Gp44 in the wild-type and the mutant phage

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

The predicted structure of tail endopeptidase protein Gp46 in the wild-type and mutant phage

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

The predicted structure of the distal tail protein Gp47 in the wild-type and mutant phage

---

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

Within each mutant phage, the mutation in each protein induces changes in the secondary structure of the protein.

These mutations are required in the ability of a phage to reinfect a resistant bacterial mutant.

Was the hypothesis supported? Why or why not?

The hypothesis was: since three protein in the phage's genome were shown to be mutated among the phage mutant strains, if a mutation in the residues contribute to an acquired function in the phage, then the secondary structure of the mutated protein should be different compared to the protein in the wild-type phage.

The hypothesis was supported since the mutations in each specific site seemed to induce changes in the secondary structure of the protein. This is demonstrated in the diagrams, where a comparison between the protein in wild-type phage and in the mutant phage are noticeably different.

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 author repeatedly mentioned function analysis of these three proteins as well as bioinformatic analysis of these individual proteins, but did not specify what these analyses were. What did they use to carry out these analyses? What programs?

-How did they create the predicted structures for the protein and the mutated proteins?