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Characterization of Immunity Systems in A Cluster Phages

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Superinfection Immunity in Cluster A Mycobacteriophages

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Introduction

Bacteriophages are viruses that infect bacteria. Bacteriophages may use two different cycles to infect their hosts. During the lytic cycle, a phage acts like a virus by hijacking the host cell and using its resources to make new phages. This process ultimately results in the host cell lysing, or bursting, which kills the host cell. During the lysogenic cycle, the phage genome integrates into the bacteria chromosome and becomes a part of the host. The phage can survive within the host for a longer period during the lysogenic cycle than the lytic cycle because the lysogenic cycle does not kill the host. Phages can also belong to a variety of different clusters. Different cluster phages, and even different subcluster phages, have different properties. While the lysogenic cycle may be evolutionarily beneficial, it does have some problems. If a phage remains in the lysogenic cycle, the host is susceptible to infection from other bacteriophages that are competing for the same resources. The process where a host cell that has been previously infected by one phage gets coinfected by another phage is called superinfection. Because of the possibility of superinfection, the phage must evolve mechanisms to control lysogeny while also defending against other superinfecting phages. This is the phage's immunity system which consists of different repressor proteins. These repressors determine if a superinfecting phage can infect the original phage's lysogen. Different subcluster phages have different repressors. If the repressors are identical, no superinfection occurs. This is known as homoimmunity. Alternatively, if the resident phage and challenging phage contain unrelated and unsimilar repressors, the challenging phage would completely infect the resident phage's lysogen. This is known as heteroimmunity. However, there is another immunity system known as mesoimmunity that results in an incomplete immunity or partial infection. This occurs when the two phages have repressors that are similar but not identical. It is possible that one phage could infect a similar phage's lysogen, but the similar phage would not be able to infect the original phage's lysogen.

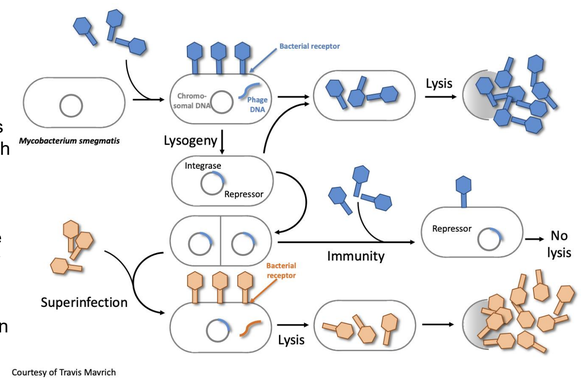
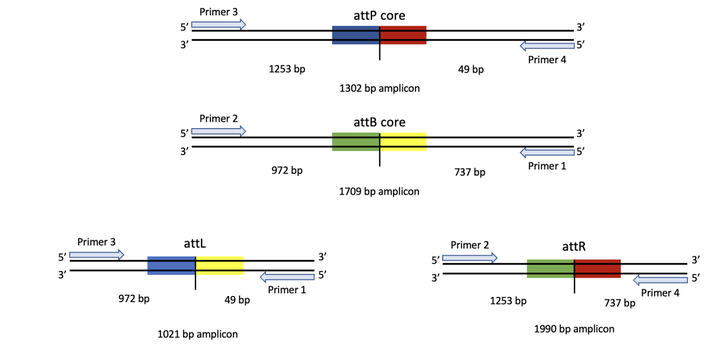


Figure 1: Lytic and lysogenic phage lifestyles.

PCR Results for Zolita Lysogen



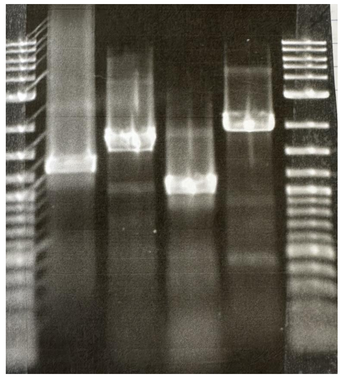
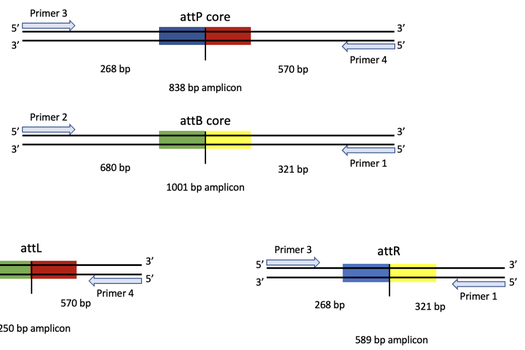


Figure 2: PCR results to verify the presence of the Zolita lysogen. The lysogen is formed when the phage integrates into the bacterial chromosome. Phage integrases carry out recombination between attachment sites on the phage and bacterial genomes, known as the attP and attB sites. The PCR amplifies the regions of DNA over the attP core in the phage lysate and attB core in the bacterial culture. In the potential lysogen, the PCR amplifies the region where the bacteria and phage genomes integrate. The amplicon bands were expected to be 1302 bp, 1709 bp, 1021 bp and 1990 bp (from left to light). A 1 kb plus ladder was used.

PCR Results for Aglet/Milton Lysogens



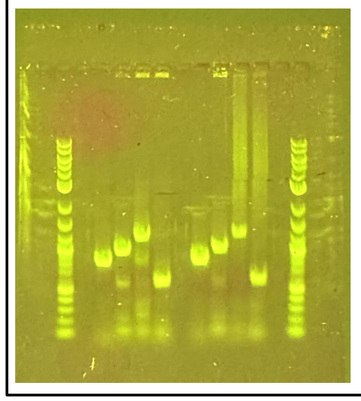


Figure 7: PCR results to verify the presence of Aglet and Milton lysogens. I designed the primers to amplify the attP, attB, attL, and attR regions for Aglet and Milton and the expected amplicon sizes were 838 bp, 1001 bp, 1250 bp, 589 bp. In lane 1 is the 1 kb plus ladder, followed by the four Aglet samples and then the four Milton samples, and finally an additional 1 kb plus ladder. The bands on the gel matched the expected amplicon sizes, which indicated that both the Aglet and Milton samples contained a purified lysogen. The gels were stained using SYBR Safe and were visualized on the transilluminator.

Immunity Assays

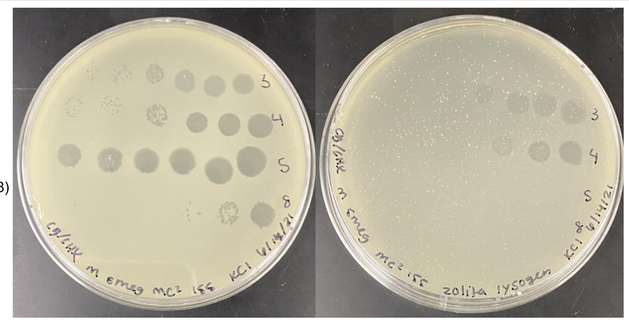


Figure 3: Left: All lysates infect *Mycobacterium smegmatis* mc^2 155. **Right:** A3 cluster phage, Aglet, A4 cluster phage, Milton, and A8 cluster phage, Saintus, can infect the Zolita lysogen. However, the lysate of A5 cluster phage, Zolita, does not infect the Zolita lysogen, suggesting that phages cannot infect their own lysogens.

Figure 4: Left: All lysates infect *Mycobacterium smegmatis* mc^2 155. **Right:** A7, A12, and A14 cluster phages can also infect the Zolita lysogen. However, Swirley, another A5 cluster phage, cannot infect the Zolita lysogen. This suggests that the phages of the same subcluster have similar repressor proteins which prevent superinfection from occurring.

Preparation of a Purified Lysogen

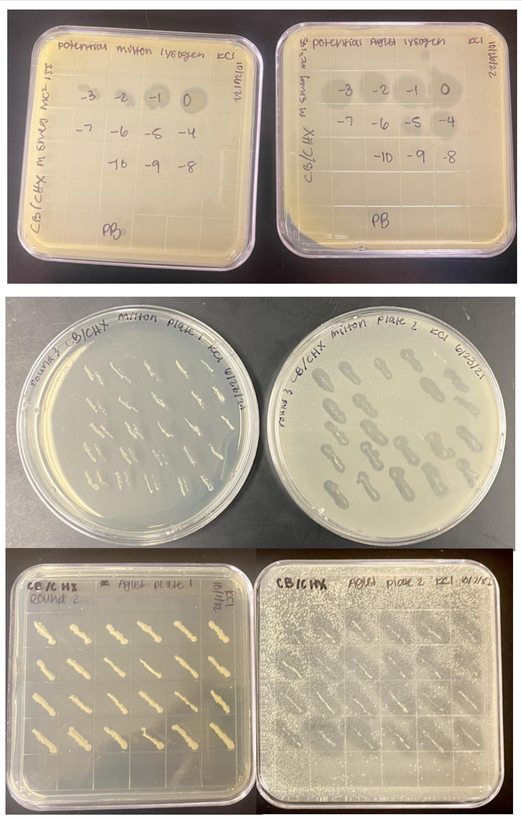


Figure 5: Phage liquid release assay. Serial dilutions of Milton (left) and Aglet (right) lysate were spotted onto plates with *Mycobacterium smegmatis*. The plates were allowed to incubate for several days until a bacterial mesa formed in the center of the clearings. A sample from the mesa was then streaked onto an agar plate and was allowed to incubate until colonies formed. Many colonies were then used in a patch test to check for the presence of a lysogen and to purify potential lysogens.

Figure 6: Preparation of purified lysogens of Aglet and Milton using patch tests. Plate 1 on the left just contains an agar base and plate 2 on the right also contains top agar with m. smegmatis. Plate 2 is made to show which phage colonies can infect the bacteria. Samples of the phage lysogen are then taken from plate 1 to continue the purification process. Three rounds of patch tests were performed to ensure the lysogen was purified and from a single colony. The above plates are from the second round of purification, which is why some of the potential lysogen colonies do not infect the bacteria. The bottom plates are from the third and final round of purification. All the potential lysogen colonies are able to infect the bacteria, indicating that the lysogen is pure. Following the third round of purification, PCR can be performed to verify the presence of the lysogen.

Conclusion

Phage lysates from the same cluster as A5 cluster phage Zolita were able to infect the Zolita lysogen. However, phage lysates from the same subcluster, such as A5 cluster phage, Swirley, were not able to infect the Zolita lysogen. The Zolita lysate also was unable to infect the Zolita lysogen. This suggests that while phages in the same cluster may have distinct repressor proteins, phages in the same subcluster have repressor proteins that are too similar for superinfection to occur. Therefore, identical phages and phages within the same subcluster appear to exhibit homoimmunity. It is unclear from the immunity assays whether phages of the same cluster, but not the same subcluster, exhibit heteroimmunity or mesoimmunity. The phage lysates appear to infect the *Mycobacterium smegmatis* more completely than the Zolita lysogen, which would suggest mesoimmunity, but further experiments would need to be done to verify this. The PCR results revealed the presence of purified Zolita, Aglet, and Milton lysogens. The next step in this research would be to perform immunity assays with the Aglet and Milton lysogens and the Zolita lysate to see if it is possible for Zolita to superinfect an A3 of A4 phage lysogen. These results would reveal more details about the repressor proteins of the phages and would further indicate whether the phages exhibit heteroimmunity or mesoimmunity.

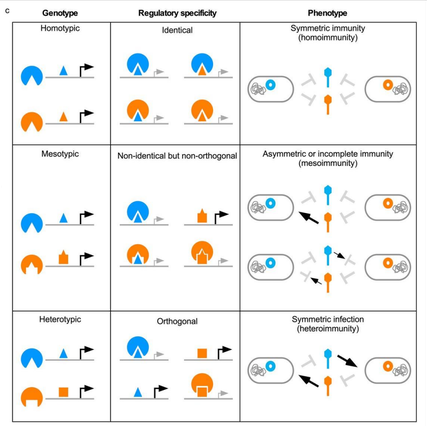


Figure 8: Homotypic, mesotypic and heterotypic immunity arise from interactions between resident and superinfecting phage repressor proteins.

References:
Mavrich TN, Hatfull GF. 2019. Evolution of superinfection immunity in cluster A mycobacteriophages. mBio 10:e00971-19

Jordan TC, Hatfull GF, et al. 2014. A broadly implementable research course in phage discovery and genomics for first-year undergraduate students. *mBio* 5:e01051-13.