

Introduction & Background

Bacteriophages (phages) are viruses that specifically infect bacteria. MaGuCo and Chickaboom are recently identified phages that were isolated on host Arthobacter globiformis and genetically sequenced. According to their sequencing, they are both temperate/lysogenic phages¹.

Temperate phages incorporate their DNA into the genome of their host as a prophage and are replicated within the host genome each time the host cell replicates. The bacteria with the phage incorporated is then called a lysogen. When the host cell is stressed, the prophage can revert to the lytic cycle where it uses the host cell mechanisms to produce and assemble phage particles before lysing the cell and releasing new phages (Fig 1). Evidence of phages can be seen as clearings in a bacterial lawn (plaques) where the phages have killed the bacteria.



Fig. 1: Life cycles of lytic and lysogenic phages

While incorporated with the host cell, temperate phages use various mechanisms to impart immunity to their host against other phage attacks, particularly from genetically similar phages².

This study set out to investigate whether Chickaboom could infect lysogens containing MaGuCo prophages and vice versa.

Hypothesis

Given that MaGuCo and Chickaboom are not genetically similar, it is anticipated that lysates of each phage will be able to infect lysogens containing prophages of the other.

Acknowledgements

Thanks to NHTI's Dr Benjamin Moyer and Karel Pluhar for guidance with initial phage isolation and advice, UNH Manchester's Dr Kyle MacLea for lab resources and advice, NH-INBRE for funding and SEA-PHAGES for provision of the protocols involved in this project.

Arthrobacter globiformis Temperate Phages: Chickaboom Infects MaGuCo Lysogens

Rachel Pitt, Beth Wilkes

Department of Natural Sciences

Experimental Workflow



Experimental Challenges



Fig. 3: Chickaboom – no mesas

Fig. 2: Chickaboom - contaminated









Fig. 4: Liquid phage release - contaminated



Fig. 5: Lysogen 6 - contaminated

3a.Patch Assay (Lysogen Verification Test)

3b.Liquid Phage Release (Lysogen Verification Test)

•	A
•	A
	E
	(
•	A
	-

<u>cycle</u>



Experimental Results

1. Lysogen Production

- From 10 plates per phage lysate:
- MaGuCo yielded ~60 mesas (potential lysogens). Chickaboom first appeared contaminated (Fig. 2)
- and subsequently failed to yield mesas (Fig. 3).

2. Lysogen Isolation

- 20 MaGuCo mesas were sampled and streaked (2 from each plate):
- From these, 5 streak plates yielded individual colonies.
- 10 samples from these colonies were initially tested:
- 7 samples passed patch assay 1.
- These 7 samples were purified and retested.
- 6 samples passed patch assay 2.
- Attempt 1: All plates appeared contaminated (Fig. 4). Attempt 2 - Samples 1, 2a:
- Both plates showed small clearings to high dilutions (10⁻⁸), indicating confirmed lysogens.
- Attempt 3 Samples 3a, 6, 8a, 9:
- Plates showed same pattern of small clearings out to high dilutions, but the control section of plates that should have been clean also showed clearings so lysogens were not fully confirmed.

4. Sensitivity Assay

- Lysogen 6 plate was contaminated throughout, so no results were obtained for this sample (Fig. 5).
- All other plates had clean control sections.
- Chickaboom lysate produced clearings up to dilutions of 10⁻⁶ on all plates and up to 10⁻⁸ in some.
- MaGuCo lysate produced clearings up to 10⁻⁴ on most plates but these were smaller than the Chickaboom clearings.

Conclusion

Although it was not possible to test lysates on Chickaboom lysogens, the hypothesis that Chickaboom would be able to infect MaGuCo lysogens was supported.

References

- 1 Russell, D.A. & Hatfull, G.F. (2017). PhagesDB: the actinobacteriophage database. Bioinformatics, 33(5), 784-786. https://doi.org/10.1093/bioinformatics/btw711 2 Bondy-Denomy, J., Qian, J., Westra, E. R., Buckling, A., Guttman, D. S., Davidson, A.R., & Maxwell, K. L. (2016). Prophages mediate defense against phage infection through diverse mechanisms. International Journal for Microbial Ecology, 10(12), 2854-2866. https://doi.org/10.1038/ismej.2016.79
- Fig 1. Emerson, J. B. (2021). The virus as a concept Fundamentals of virology. *Encyclopedia of Virology (4th edition).*
 - https://www.sciencedirect.com/topics/immunology-and-microbiology/lysogenic-