Our Phage Phriends: Isolation & Characterization of Audrick & Porcelain

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Abstract

As part of HHMI's SEA-PHAGES (Science Education Alliance - Phage Hunters Advancing Genomics and Evolutionary Science) program, USciences students were able to isolate, characterize, and annotate two novel bacteriophages this year, Audrick and Porcelain. The phages both infected *Mycobacterium smegmatis* mc^2155, but while Audrick was isolated using the enrichment protocol, Porcelain was isolated using a direct plating method. These phages were further characterized through the annotations of their genomes using DNAMaster, Starterator, Phamerator, and HHPred. It was revealed that Audrick is a C1 cluster, lytic phage that is part of the Myoviridae family with a genome length of 155,205 bp[1], and that Porcelain is a J cluster, lysogenic phage that is part of the Siphoviridae family with a genome length of 109,575 bp[2]. Further observations with electron microscopy, and additional experiments such as purification through ultracentrifugation using a CsCl gradient, lysogeny tests, and cloning of a putative repressor were conducted to obtain a greater understanding of these phages and the similarities and differences between them. For Audrick, an interesting phenomenon that occurred during ultracentrifugation was the presence of three Schlieren lines; we expected only one band of extremely pure phage. After analyzing the different bands with electron microscopy, we visualized empty heads in the top band, intact phage particles in the middle band, and an unidentified substance speculated to be ribosomes in the bottom band. Images from the top and middle bands also showed evidence of tail activation similar to that observed in phage T4. For Porcelain, lysogeny tests were conducted using MiaZeal, a highly similar J cluster phage that was previously isolated and annotated at Cabrini College. Surprisingly, Porcelain infected MiaZeal's lysogen. This result was intriguing because we had expected same-cluster phages to be homoimmune. Lastly, Porcelain's putative CI repressor was successfully amplified through PCR and was transformed into *E. coli* MACH cells. Altogether, these results reveal novel and interesting phenomena related to each of these phage "phriends" that warrant further investigation.

Introduction

- Porcelain and Audrick are currently 2 of 1513 novel Actinobacteriophages that have been sequenced.
- Audrick, a lytic, C1 cluster phage, was found in Potomac, Maryland.
- Porcelain, a temperate, J cluster phage, was found on USciences' campus in Philadelphia.
- Due to difficulty imaging Porcelain with electron microscopy (EM) , phages were purified through ultracentrifugation in order to test osmotic susceptibility.

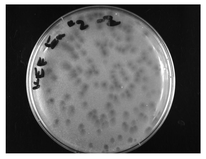

Fig. 1: Porcelain's serial dilution shows turbid plaque morphology

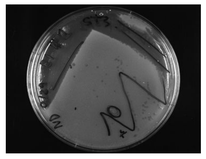

Fig. 2: Audrick's streak plate shows clear plaque morphology

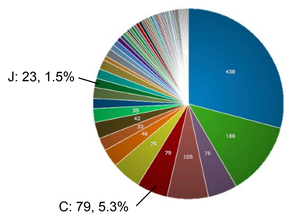

Fig.3: Cluster C and J phages are rare

Materials and Methods

Purification of Audrick

- CsCl gradient of 2.4, 4, and 5.6 M[3, 4]
- Ultracentrifugation for 2.5 hours at 24,000 RPM
- Components of Audrick's HTL were separated into Schlieren lines based upon density

Electron Microscopy

- Audrick's isolated Schlieren lines were placed on separate EM grids
- Stained for electron microscopy using 2% ammonium molybdate

The Cloning of Porcelain's Putative CI Repressor[5]

- Bioinformatics to determine the repressor sequence and putative promoter regions
- Temperature & $MgCl_2$ gradient PCR to determine ideal conditions for amplification
- Restriction digest to insert repressor sequence into vector & gel purification of product
- Ligation of plasmid with repressor sequence, and transformation of plasmid into *E.coli* MACH cells.

Homoimmunity Tests[6]

- Bacterial streaks of lysogen containing MiaZeal's prophage (a J-cluster phage isolated from Cabrini College[7]) were collected and plated
- Spot test of serially diluted Porcelain onto Miazeal's lysogen lawn

References

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[5]Brown, K., Sarkis, G.J, Wadsworth, C., Hatfull, G.F. (1997). Transcriptional silencing by the mycobacteriophage L5 repressor. *The EMBO Journal, 16(19),* 5914-5921.
[6]Lysogeny Experiments in *Phagehunting Protocols*. (2013) Retrieved from phagesdb.org/media/workflow/protocols/pdfs/LysogenyProtocol_3.19.13.pdf
[7]Mycobacterium phage MiaZeal. (2015). Retrieved from http://phagesdb.org/phages/MiaZeal/.
[8]Furukawa, H., Kuroiwa, T., Mizushima, S. (1983). DNA injection during bacteriophage T4 infection of Escherichia coli. *Journal of Bacteriology*, *154*(2), 938–945.

Audrick Results

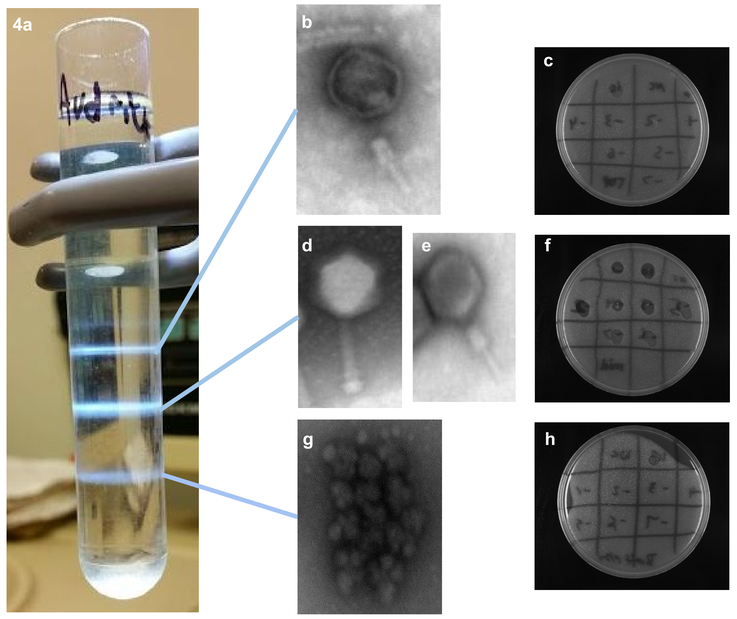

Fig. 4: Ultracentrifugation Results from Audrick: a. Schlieren lines showing phage seperation based on density b. EM of top band at 75000x, revealing empty capsids and contracted tail sheaths. c. Spot test of top band showing no plaques at any dilution. Tiny plaques were only seen at full concentration. d. EM of middle band at 120000x, showing intact phage. e. Another EM of middle band at 120000x showing Audrick's tail sheath contracted. f. Spot test of middle band reveals plaques at all dilutions. g. EM of bottom band at 75000x reveals a material that resembles ribosomes h. Spot test of bottom band showing tiny plaques only at highest concentration.

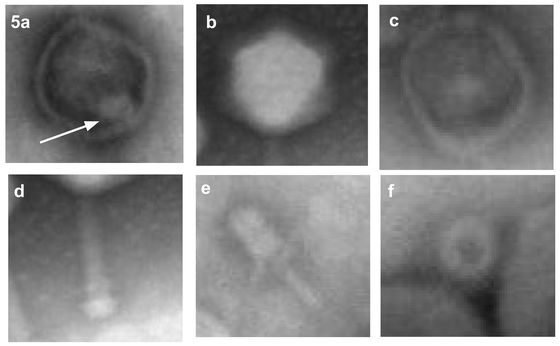

Fig. 5: EM pictures representing Audrick's infection process: a. Location of putative riveting mechanism where a rivet-like protein binds the head and tail b. Capsid with DNA, c. Empty capsid, after DNA injection d. Tail with base plate e. Core and contracted tail sheath ready for infection f. Cross section of a hollow sheath.[8]

Conclusions

Audrick

- Ultracentrifugation of Audrick gives 3 Schlieren lines, but only the middle band contains intact, infectious phage.
- Based on the pictures obtained from EM, Audrick's infection process is likely similar to phage T4's infection process.[8]

Porcelain

- Porcelain was successful in infecting a lysogen of the same cluster.
- Putative CI repressor gene was successfully transformed into *E. coli* MACH cells.

Acknowledgements

We would like to acknowledge the HHMI SEA-PHAGES Program for providing this opportunity, Dean Suzanne Murphy and Dr. Peter Berget for their financial support, Dr. Dewight Williams and University of Pennsylvania's EM facility for creating images of Audrick and Porcelain, Dr. David Dunbar for providing us with MiaZeal and his guidance and suggestions over the past year, and University of Pittsburgh for sequencing and finishing these phages.

Porcelain Results

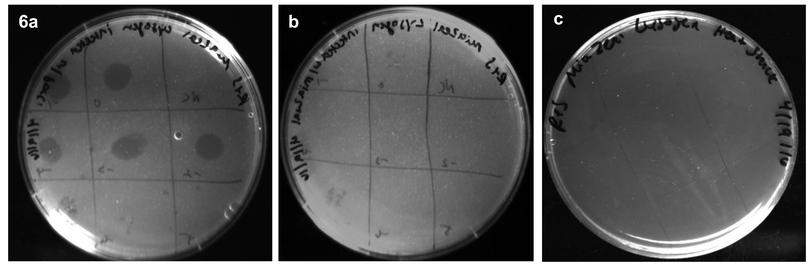

Fig. 6: Heteromoimmunity of MiaZeal and Porcelain: a. Porcelain successfully formed plaques on MiaZeal's lysogen b. MiaZeal did not produce plaques on MiaZeal's lysogen c. The complete clearing of MiaZeal's lysogen plate upon heat shock at 42ºC indicates the presence of lysogen.

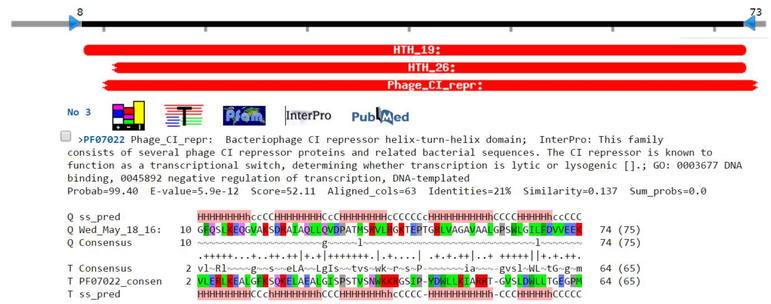

Fig. 7: Porcelain's gp93 shares homology with the lambda CI transcriptional repressor. Using HHPred with the pfamA database, the predicted structure of gp93 matched the crystal structure of the lambda CI repressor with a 99.4% probability. This was the third hit; the top two hits were also for HTH (helix-turn-helix) motifs present in lambda Cro/CI transcriptional regulators (99.6% and 99.5% probability).

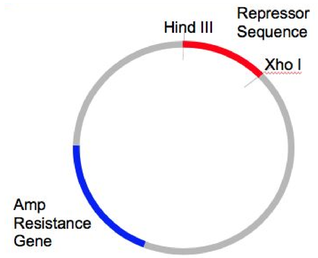

Fig. 8: Plasmid map showing ligated putative CI repressor gene in red.

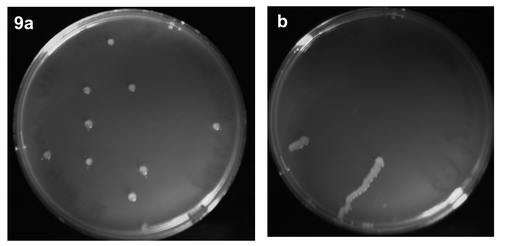

Fig. 9: Porcelain's Positive Plasmid Transformation. a. Colonies resulting from plasmid transformation containing the putative repressor. b. Negative control plate containing colonies possibly from incomplete digestion and re-ligation.

Future Experiments

Audrick

- Spectrophotometry to gain more insight into what was found in Audrick's bottom band since different molecules reflect different wavelengths of light.
- Challenge Audrick with osmotic stress prior to plating, to determine capsid fragility compared to Porcelain and other bacteriophages.

Porcelain

- Attempt to create a lysogen of Porcelain, then test if MiaZeal can infect it.
- Test Porcelain's superinfectivity by using it to infect other J cluster phage lysogens.
- Express and purify the putative repressor, then test our putative promoter sequence to determine if our putative CI repressor and promoter bind together by running a gel shift assay.
- Perform osmotic stress experiments on Porcelain and Audrick to determine if osmotic stress affects the capsids of these phages.