Title
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Identification of Putative Immunity System in Cluster AZ Phages

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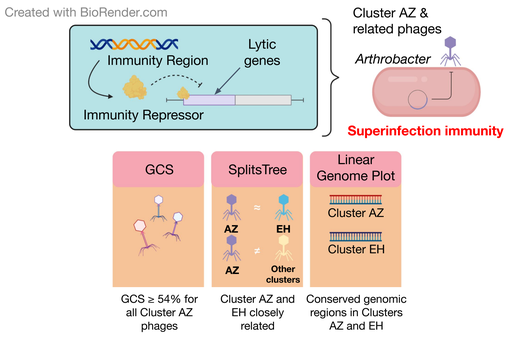
Abstract



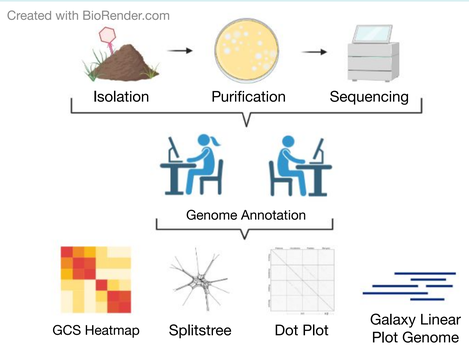
Background

- *Arthrobacter* is a common soil bacterium with robust metabolism and applications in agriculture, industry and the environment.
- Bacteriophages ("phages") are viruses infecting bacteria
- Lysogenic or temperate phages persist in the host for a period of time before transitioning to the lytic lifestyle.
- They encode immunity systems which regulate this transition and protect the prophage and its host from being infected by other phages [1,2,3].
- Immunity systems tend to be located near helix-turn-helix domains and serine integrases [1,2,3].
- Cluster AZ phages are temperate phages which infect *Arthrobacter* and exhibit immunity despite lacking an identifiable immunity repressor [4]. **This immunity system is currently unidentified.**
- Identification of the Cluster AZ immunity system will contribute to an overall understanding of *Arthrobacter*-infecting phages and provide a basis for re-engineering lysogenic phages for use in phage cocktail treatments against critical bacterial infections.

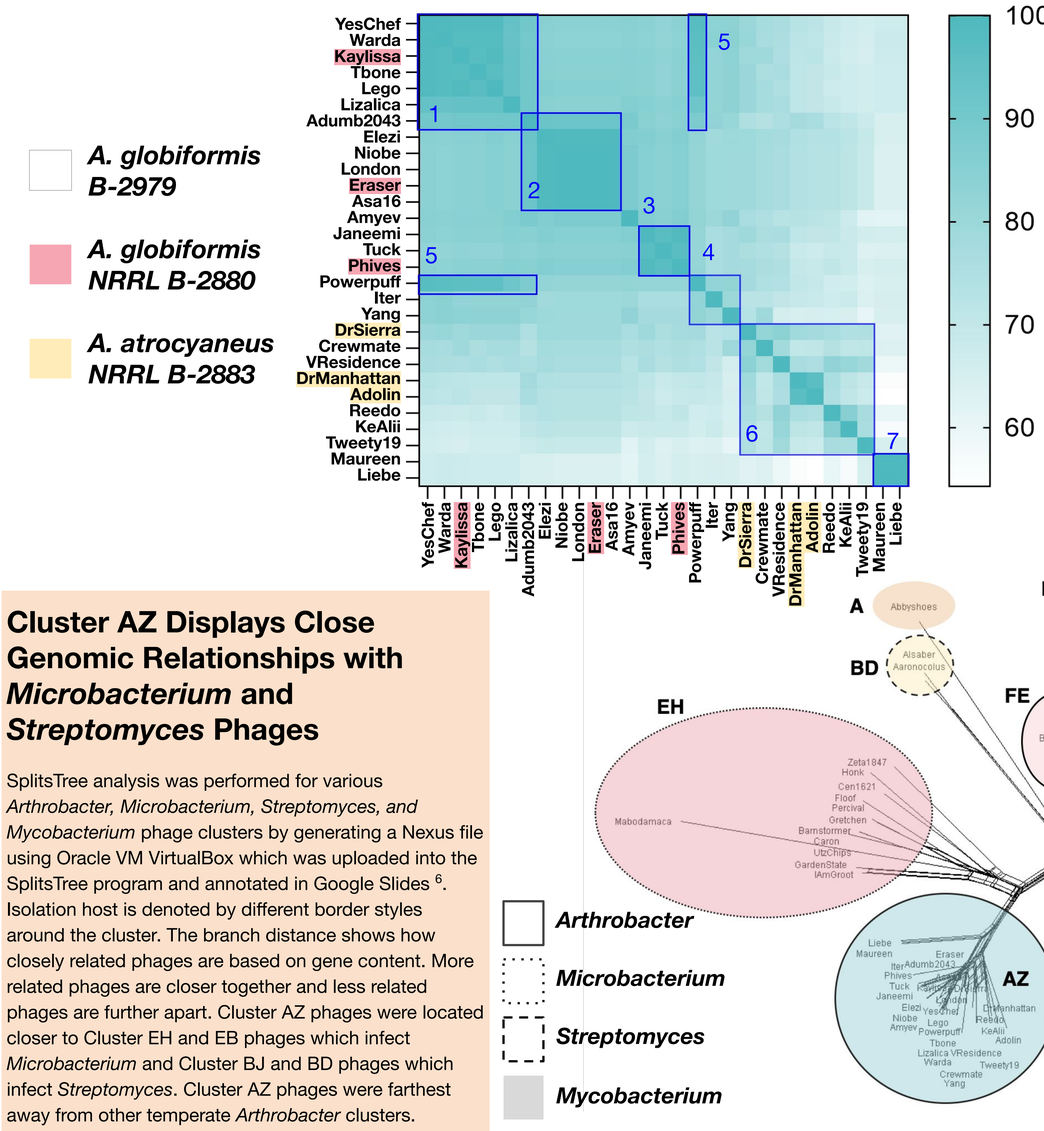
Hypothesis

If Cluster AZ phages encode an immunity system shared by related clusters, immunity genes should be observed in conserved genomic regions. Similar genome architecture and content, including synteny, would also be seen. These putative shared immunity genes should have amino acid sequence similarity.

Methods



Results

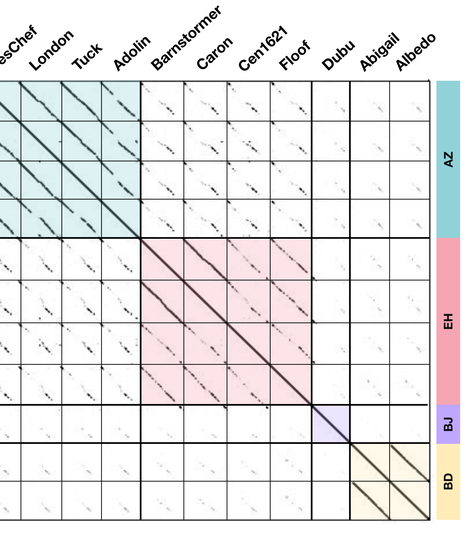


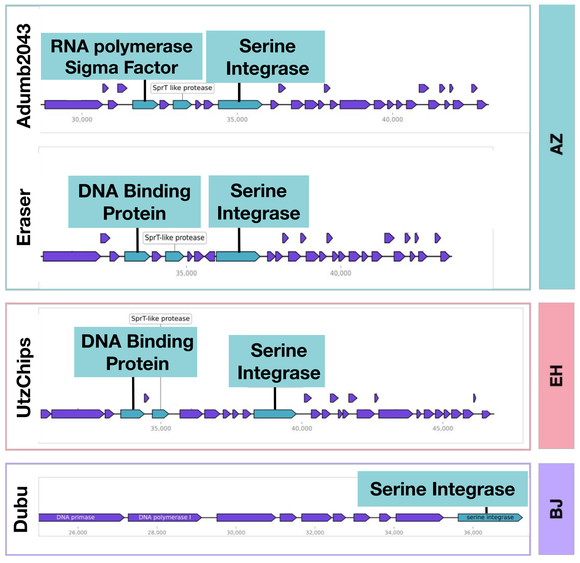
High Gene Content Similarity in Cluster AZ

Cluster AZ relatedness was determined using the PhagesDB Gene Content Similarity (GCS) Calculator [5] and visualized using a heat map generated in Prism and annotated in Google Slides. The applied heat pattern designates lower values white and higher values in teal, per the scale bar. It was found that the isolation host (denoted in white, red, or yellow) of the phage did not affect GCS. The heat map shows high overall GCS. The lowest GCS value was 54.3% and the average GCS was 78.1%. Seven unique subgroups were found to have higher GCS within each respective subgroup.

Cluster AZ Displays Close Genomic Relationships with *Microbacterium* and *Streptomyces* Phages

SplitsTree analysis was performed for various *Arthrobacter, Microbacterium, Streptomyces, and Mycobacterium* phage clusters by generating a Nexus file using Oracle VM VirtualBox which was uploaded into the SplitsTree program and annotated in Google Slides [6]. Isolation host is denoted by different border styles around the cluster. The branch distance shows how closely related phages are based on gene content. More related phages are closer together and less related phages are further apart. Cluster AZ phages were located closer to Cluster EH and EB phages which infect *Microbacterium* and Cluster BJ and BD phages which infect *Streptomyces*. Cluster AZ phages were farthest away from other temperate *Arthrobacter* clusters.





Conserved Regions within Clusters AZ and EH Were Identified

GEPARD [6] was used to create a whole genome dot plot for Cluster AZ, EH, BJ, and BD phages using a word size of 7 (left). Conservation in the right arm of the genome is observed for Cluster AZ and EH phages.

Galaxy Linear Genome Plot [7] was used to visualize annotated genomes for Cluster AZ, EH, BJ, and BD phages (right). A conserved genomic region containing three genes is observed; DNA binding protein (pham 63728), SprT-like protease, and serine integrase are present in most Cluster AZ and Cluster EH phages.

Conclusions

Cluster AZ Has High Intracluster Gene Content Similarity

- Cluster AZ phages shared > 54.3% gene content
- Hosts have no effect on gene content similarity for Cluster AZ phages, supporting previous literature [4]
- Several subgroups within Cluster AZ are more similar to each other than with other AZ phages.
- Maureen and Liebe shared 100% of their gene content and are less similar to other AZ phages.

Clusters AZ and EH Exhibit High Intercluster Gene Content Similarity

- Little similarity (< 3% GCS) shown between temperate *Arthrobacter* clusters and Cluster AZ.
- The most similar cluster to Cluster AZ is Cluster EH, which infects *Microbacterium*. This suggests that host has no effect on gene content similarity.

Conserved Genomic Region Between Clusters AZ and EH May Contain Immunity Genes

- There is a conserved genomic region between Cluster AZ and EH that contains a DNA binding protein (pham 63728), SprT-like protease, and serine integrase.
- These are genetic indicators of possible immunity genes within this region.
- Pham 63728 has multiple functions listed that are dubious. It could be an immunity repressor or related gene due to its proximity to the integrase.

Future Directions

- Gene knockout experiments and characterization on the gene of interest
- Extending research to other Actinobacteriophages in PhagesDB [5] that contain genes labeled 'RNA sigma factor' or other mismatched gene labels.
- Performing a host range assay on Cluster AZ phages to explore their potential in infecting *Microbacterium* hosts and vice versa with Cluster EH and *Arthrobacter*.
- Performing co-infection assays with Cluster AZ and EH phages to see if superinfection immunity is granted by the high gene content similarity.

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