**A 2024 Report on the Characteristics of Phages Assigned to Cluster AZ4.**

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**Isolation and Morphological Characteristics**

As of June 2024, 5 phages have been assigned to Actinobacteriophage cluster AZ4. Cluster AZ4 consists of putative lytic phages that were isolated from environmental samples collected in one state, using *Arthrobacter sulfureus* as the host. A sampling of transmission electron micrographs reveals these phages to have siphoviral morphologies, while photographs of plaque assays reveal small to medium plaques. Examples of phages displaying these morphologies include phage Emotion and VroomVroom.

**Genometrics**
The genomes of phages in this cluster have an average length of 44,161bp (range: 43,320 bp – 45,092 bp) with an average GC content of 67.04% (range: 66.8% – 67.9%). The genomes have 3PRI ends.

**Gene Content**

1. A survey of all manually annotated genomes in this cluster (i.e. excluding draft genomes) reveal the number of genes called per genome to range between 66 - 73 genes, which represent a total of 85 gene phamilies (or phams).
2. Of these 85 phams, 52 are fully conserved across the cluster. These phams are assigned putative functions that are involved in structure and assembly (terminase, portal protein, major and minor capsid proteins, tape measure, tail assembly chaperones, and minor tail proteins), replication and recombination (DNA primase/polymerase, DNA polymerase alpha subunit, DNA helicase, and resolvase), and lysis (endolysin). Some other notable putative functions include ThyX-like thymidylate synthase, several HNH endonucleases, NrdH-like glutaredoxin, and SprT-like protease
3. Of the 137 genes in subcluster AZ4, 104 genes are fully conserved across the subcluster. The other 33 genes have varying levels of conservation.
4. There are 5 tRNA genes identified in this cluster. There are also no identifiable integrase or repressor functions, suggesting that phages of this cluster do not form lysogens and are thus may be lytic phages.
5. The genomes are organized with genes involved in structure and assembly located within the first third of the genome while those involved in replication and recombination are scattered across the remaining two-thirds of the genome. Genes involved in lysis are located at the boundary of these two regions (e.g. Emotion gp21). All genes are transcribed in the forward direction, with the exception of 1 highly conserved gene (Emotion gp41) that is transcribed in the reserve direction.
6. Notably, AZ4 phages do not have an identifiable integrase, unlike other AZ subclusters. Although this may suggest these phages are fully lytic, some of these phages form turbid plaques.

**References:** Below are prior publications that include a description of this cluster (consider excluding genome announcements)

Klyczek KK, et al. Tales of diversity: Genomic and morphological characteristics of forty-six Arthrobacter phages. PLoS One. 2017 Jul 17;12(7).

Kapinos A, et al. Novel Cluster AZ Arthrobacter phages Powerpuff, Lego, and YesChef exhibit close functional relationships with Microbacterium phages. PLoS One. 2022 Jan 13;17(1)

AZ team, Comparative genomic analysis of cluster AZ Arthroabacter phages reveal complex evolutionary histories, in preparation.

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