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Ambrosius and Friends: Identification of novel small-genome and temperate Arthrobacter phages

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We are exploring the diversity of bacteriophage that infect *Arthrobacter* to improve our understanding of their evolution, their biological impact, and potential applications. We isolated 22 bacteriophages that infect *Arthrobacter sp.* One, KBurrousTX, was sequenced at University of Pittsburgh and was fully annotated. Using the Illumina MiSeq platform and Sanger sequencing, we generated and completely assembled the genomic sequences for 14 of the remaining phage. These genomes were partially annotated. We also completed the sequencing and annotation of Ambrosius, which was isolated in 2013. Sequence alignments, gene content and genome organization allow assignment of 5 phages to cluster AK, 4 to AO2, and one each to AL and AR.

Two of the new phages clearly form a novel cluster with Ambrosius: Gates51 and Wrastor are 98% identical to each other and 88% identical to Ambrosius, but all 3 exhibit little sequence similarity to other known phages, including the other small-genome phages of *Arthrobacter, Gordonia* and *Microbacterium*. Their 16 kb genomes have identical gene content and organization, including a putative Tailspike/Lipase protein that is shared with *Arthrobacter* phage Galaxy and is also located between minor tail protein genes and an endolysin. Although the Ambrosius-like phages lack a discernible integrase, they generate stable lysogens that exhibit specific immunity, release infectious particles and contain phage DNA that is detected by PCR. The mechanism of lysogenization is unknown. However their genomes contain a putative immunity repressor at a location similar to that in the small *Gordonia* CW phages.

The new phage Bashari also produced true lysogens as shown by specific immunity, release of infectious particles upon induction with Nalidixic Acid, and PCR detection of the phage DNA in the bacteria. We could not obtain the complete sequence of Bashari, but it appears to be a novel phage. Consistent with its temperate behavior, one contig contained similarity to the integrase gene of *MIcrobacterium* phage Min1 and to *Arthrobacter* tRNA –Arg, which might be the integration target. PCR with primers designed against this contig confirmed its presence in the lysogens and in the released infectious particles, whereas it is absent in non-lysogenized *Arthrobacter*. Integrases and temperate behavior are uncommon among *Arthrobacter* phages. It will be interesting to investigate Bashari and the Ambrosius-like phages in greater detail.

Phage-resistant bacteria were also obtained with 8 other bacteriophage, but none were lysogens. The patterns of resistance to all 22 phages were characterized and used for cluster analysis. Some exhibited specific resistance to a particular phage cluster (e.g. AK), but others exhibited more complex patterns that that did not map to a specific phage cluster. This information could be useful for elucidating the mechanisms of resistance.