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Comparative genome analysis of cluster EF bacteriophages Ajin and OverHedge.

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To further investigate the genetic diversity and evolution of bacteriophages, two new bacteriophages, Ajin and OverHedge were isolated from moist soil in Middle Tennessee. Bacteriophages were purified, amplified to high titer lysates, and analyzed with transmission electron microscopy. Ajin and OverHedge are from the *Siphoviridae* family with an icosahedral capsid and a flexible tail. Bacteriophage DNAs were isolated at Austin Peay State University and were sequenced at the Pittsburgh Bacteriophage Institute. Both bacteriophages are from the cluster EF. Ajin’s DNA size is 56,993 bp with GC content of 62.6% and OverHedge’s DNA size is 56,559 bp with GC content of 63.8%. The genomes of the two bacteriophages were then manually annotated. The bacteriophage Ajin has 86 predicted protein-coding genes (of which we assigned predicted functions for 31 of those genes). Bacteriophage OverHedge has 81 predicted protein-coding genes (of which we assigned predicted function for 34 genes). Both bacteriophages exhibited programmed translational frameshifts in tail assembly chaperone genes 28 and 29 for Ajin and 27 and 28 for OverHedge.   
Based on the Gene Content Similarity (GCS) tool, bacteriophage Ajin is closely related to the bacteriophage TinyMiny (93.74%), which was found in Baltimore, MD, USA. Bacteriophage OverHedge is more closely related to bacteriophage Gilda (96.34%), which was found in Pittsburgh, PA, USA. Based on the GCS tool, Ajin and OverHedge show only 53.89% gene similarity.  
The following unique genes were found in the bacteriophage Overhedge, but not Ajin: head-to-tail stopper, holin, and DNA primase/helicase. On the other hand, the phage Ajin has the following unique genes that are not shown in the phage OverHedge genome: DNA primase, phosphatase and glycosyltransferase. We also identified one additional minor tail protein gene and three additional membrane protein genes in OverHedge compared to Ajin.  
  
Overall, the two bacteriophages have enough differences in their genomes that we might be able to classify them into different subclusters under the same EF cluster.