Complete Genome Sequences of 18 Paenibacillus larvae Phages from the Western United States


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ABSTRACT We present here the complete genomes of 18 phages that infect Paenibacillus larvae, the causative agent of American foulbrood in honeybees. The phages were isolated between 2014 and 2016 as part of an undergraduate phage discovery course at Brigham Young University. The phages were isolated primarily from bee debris and lysogens.

Paenibacillus larvae is a Gram-positive bacterium that is the causative agent of American foulbrood, the most destructive bacterial disease affecting the honeybee, Apis mellifera (1). As antibiotic-resistant strains are now widespread (2), there is growing interest in phages that infect P. larvae. There are currently 26 complete P. larvae phage genomes in the literature (3–8). Here, we present 18 complete P. larvae phage genomes isolated over the period 2014 to 2016 by students in the Phage Hunters course at Brigham Young University (BYU). Each phage’s GenBank accession number, isolation source, geographical provenance, and assembly results are shown in Table 1.

All phages were amplified using P. larvae strain ATCC 9545. Phage DNA was isolated from high-titer lysates using DNA isolation kits (Norgen Biotek, Thorold, ON, Canada). Libraries were prepped with TruSeq Nano DNA HT sample preparation kits (Illumina, Inc., Hayward, CA, USA) and then run on a single lane in parallel and barcoded. Genomes were sequenced in the BYU DNA Sequencing Center using the Illumina HiSeq 2500 platform with 250-bp paired-end reads and assembled using Geneious 8 (Biomatters, Inc., Auckland, New Zealand) with medium-low sensitivity/fast and checking for contig circularization. Only genomes that produced circularized contigs were considered complete and published. Genomes were manually annotated by students at the University of Nevada Las Vegas (UNLV) with DNA Master, as previously described (9).

Scanning electron micrographs show that all 18 phages are members of the family Siphoviridae. All the genomes are linear double-stranded DNA molecules. Phages Ash, C7Cdelta, and Ley use the direct terminal repeat (DTR) DNA-packaging strategy, while the other 15 phages use the “cohesive ends with 3’ overhangs” (cos) DNA-packaging strategy (10, 11). The 3’ overhangs were identified by sequence similarity with previously published phages (3–8). The overhangs are CGACTGCC for Arcticfreeze, Bloom, DevRi, Eltigre, Genki, Gryphonian, Honeybear, Jacopo, Kawika, Lucielle, Saudage, and Toothless and CGACGGCCC for LincolnB and Wanderer. The genome ends of Yerffej are still under investigation. For the DTR phages, the DTR sequence was visually identified using Pile-up Analysis Using Starts & Ends (PAUSE) (http://cpt.tamu.edu/computer-resources/pause) and Geneious by looking for a sharply delimited region with double coverage depth (11).
Genome length is bimodal, with the cohesive end phages having genomes in the 37- to 43-kb range and the DTR phages having genomes in the 55- to 56-kb range, which is consistent with previously published *P. larvae* phages (3–8). All phages encode a large terminase, a portal protein, a major capsid protein, two tail assembly proteins, a tail tape measure protein, several tail proteins, and an N-acetylmuramoyl-L-alanine amidase. The tail assembly proteins appear to have a predicted translational frameshift similar to that of the G and G-T genes in phage lambda (12, 13) located in the 3’ region of the upstream tail assembly protein (gp12 in the phages with cohesive ends, gp14 in the DTR phages). We tentatively identify the heptanucleotide slippery sequence as AAAAAAA in Arcticfreeze, Bloom, DevRi, Genki, Gryphonian, Honeybear, Jacopo, Kawika, Lucielle, Saudage, Toothless, and Yerffej, GGAAAAA in LincolnB.

**Data availability.** GenBank accession numbers are listed in Table 1.

### ACKNOWLEDGMENTS


### REFERENCES


### TABLE 1

*P. larvae* phages, GenBank accession numbers, and genome assembly results

<table>
<thead>
<tr>
<th>Phage name</th>
<th>GenBank accession no.</th>
<th>Isolation source</th>
<th>Location</th>
<th>Genomic length (bp)</th>
<th>DNA-packaging strategy</th>
<th>Avg cover (×)</th>
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<td>Arcticfreeze</td>
<td>MH431932</td>
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<td>Idaho</td>
<td>38,518</td>
<td>3’ cos</td>
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<td>Ash</td>
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<td>LincolnB</td>
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