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Paenibacillus larvae Phage Tripp Genome Has 378-Base-Pair Terminal Repeats

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Paenibacillus larvae **bacteriophage Tripp was isolated from an American foulbrood diseased honey bee hive in North Carolina, USA. The 54,439-bp genome is 48.3% G+C, encodes 92 proteins, no tRNAs, and has 378-bp direct terminal repeats. It is currently unique in Genbank.**

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Bacteriophage Tripp was isolated on *Paenibacillus larvae* strain ATCC 9545, a bacterium that causes American foulbrood disease (AFB) of honey bees (1). The 9545 host used is designated the type strain with the alternate designations of 846 [NRRL B-2605, http://www.atcc.org/]. Comb swab samples from the frame of an AFB diseased hive in Lincoln County, North Carolina, USA were incubated in brain heart infusion broth plus thiamine and glucose with *P. larvae* 9545. Clarified and filtered (0.22 μm) enrichment broth (30°C, 48 h) was plated in soft agar overlays with fresh *P. larvae* cells and plaques were identified. A phage Tripp plaque was purified three times and then amplified using the whole plate lysis method. DNA was extracted from the high titer lysate, and a sequencing library was prepared and analyzed using Illumina MiSeq procedures as described previously (2). FastQ files were assembled using CLC genomics workbench software (release 2014) with 10,071-fold coverage, and annotation was performed using DNA Master (http://cobamide2.bio.pitt.edu), GeneMark (3), NCBI BLASTp (4), ShineFind in the Galaxy bioinformatics suite (https://galaxyproject.org/), and HHPred (5). The 54,439-bp genome is 48.3% G+C, and encodes 92 proteins and no tRNAs.

The genome of *P. larvae* phage Tripp differs considerably from the genomes of other *Siphoviridae P. larvae*-infecting bacteriophages isolated in North Carolina (2) and from elsewhere in the world (6–8). While all other *P. larvae* phages to date are *cos* type with 5′ or 3′ overhanging ends, Tripp has terminal repeats of 378 bp. Tripp does show nucleotide and coding sequence (CDS) similarities to prophage sequences in the annotated *P. larvae* genome DSM 25430 (9) (GenBank accession number CP003355). Phage Tripp, although isolated as forming clear plaques, is likely a temperate phage with the capacity to form lysogens on certain *P. larvae* strains. Several biologically interesting properties of the Tripp genome were identified, including an encoded protein resembling the anti-toxin HicB, an anti-repressor, a transposase gene with an apparent −2 frameshift, and the terminally redundant direct repeats not present in the other deposited *P. larvae* bacteriophage genomes. One copy related to the repeat sequence (357/378 bp; 94%), occurs in the *P. larvae* DSM 25430 genome at base position 2,669,085 near other Tripp-like phage sequences. This suggests that strain DSM 25430 harbors a defective prophage lacking one of the terminal repeats.

Compared to the several Diva-like phages infecting *P. larvae* that have relatively short 5′- or 3′-overhanging DNA termini, the direct terminal repeats of the Tripp genome suggests it replicates and/or packages by a different mechanism. The growing number of sequenced phages that infect *P. larvae* provides increased opportunities for comparative phage genomics, new resources for genetic manipulation, and potential biotechnology applications involving AFB and other systems using *Paenibacillus* species.

Nucleotide sequence accession number. The complete genome of *Paenibacillus larvae* bacteriophage Tripp is available at Genbank under accession number KT755656.

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REFERENCES

1. **Genersch E.** 2010. American foulbrood in honeybees and its causative agent, *Paenibacillus larvae*. J Invertebr Pathol **103**:S10–S19. http://dx.doi.org/10.1016/j.jip.2009.06.015.

2. **Carson S, Bruff E, DeFoor W, Dums J, Groth A, Hatfield T, Iyer A, Joshi K, McAdams S, Miles D, Miller D, Oufkir A, Raynor B, Riley S, Roland S, Rozier H, Talley S, Miller ES**. 2015. Genome sequences of six *Paenibacillus larvae Siphoviridae* phages. Genome Announc **3**(3):e00101-15. http://dx.doi.org/10.1128/genomeA.00101-15.
3. **Besemer J, Lomsadze A, Borodovsky M**. 2001. GeneMarkS: a self-training method for prediction of gene starts in microbial genomes. Implications for finding sequence motifs in regulatory regions. Nucleic Acids Res **29:** 2607–2618. http://dx.doi.org/10.1093/nar/29.12.2607.
4. **Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ**. 1990. Basic local alignment search tool. J Mol Biol **215:**403–410. http://dx.doi.org/10.1016/S0022-2836(05)80360-2.
5. **Söding J, Biegert A, Lupas AN**. 2005. The HHpred interactive server for protein homology detection and structure prediction. Nucleic Acids Res **33:**W244–W248.
6. **Oliveira A, Melo LDR, Kropinski AM, Azeredo J**. 2013. Complete genome sequence of the broad-host-range *Paenibacillus larvae* phage phiIBB_Pl23. Genome Announc **1**(5):e00438-13. http://dx.doi.org/10.1128/genomeA.00438-13.
7. **Beims H, Wittmann J, Bunk B, Spröer C, Rohde C, Günther G, Rohde M, von der Ohe W, Steinert M**. 2015. *Paenibacillus larvae*-directed bacteriophage HB10c2 and its application in American foulbrood-affected honey bee larvae. Appl Environ Microbiol **81:**5411–5419. http://dx.doi.org/10.1128/AEM.00804-15.
8. **Tsourkas PK, Yost DG, Krohn A, LeBlanc L, Zhang A, Stamereilers C, Amy PS**. 2015. Complete genome sequences of nine phages capable of infecting *Paenibacillus larvae*, the causative agent of American foulbrood disease in honeybees. Genome Announc **3**(5):e01120-15. http://dx.doi.org/10.1128/genomeA.01120-15.
9. **Djukic M, Brzuszkiewicz E, Fünfhaus A, Voss J, Gollnow K, Poppinga L, Liesegang H, Garcia-Gonzalez E, Genersch E, Daniel R**. 2014. How to kill the honey bee larva: genomic potential and virulence mechanisms of *Paenibacillus larvae*. PLoS One **9:**e90914. http://dx.doi.org/10.1371/journal.pone.0090914.