




Complete Genome Sequences of *Paenibacillus larvae* Phages Halcyone, Heath, Scottie, and Unity from Las Vegas, Nevada

Diane G. Yost,^a Carolyn Chang,^a Lucy LeBlanc,^a Erin Cassin,^a Ceara Peterman,^a Padmani Rai,^a Alicia Salisbury,^a Nicolas Barroga,^a Ramiro Cisneros,^a Joseph Fersini,^a Jonathan Juste,^a Juvie Ines,^a Gabriel Leyva,^a Dyanne Macalinalo,^a Spencer Muscelli,^a Gustavo S. Reyes,^a Heather Rhoden,^a Rodney Tan,^a Erika Torres,^a Krystal Tran,^a Georgette Uriarte-Valle,^a Christopher Wallace,^a Simon Wong,^a Kevin Ayala-Pineda,^a Vanessa Cadiz,^a Tiffany Jeanite,^a Sophia Nhan,^a  Julianne H. Grose,^b Christy Strong,^a Penny S. Amy,^a Philippos K. Tsourkas^a

^aSchool of Life Sciences, University of Nevada, Las Vegas, Nevada, USA

^bDepartment of Microbiology and Molecular Biology, Brigham Young University, Provo, Utah, USA

ABSTRACT We present the complete genome sequences of four phages that infect *Paenibacillus larvae*, the causative agent of American foulbrood disease in honeybees. The phages were isolated from beehives and beeswax products from Las Vegas, Nevada. The genomes are 50 to 55 kbp long and use the “direct terminal repeats” DNA-packaging strategy.

American foulbrood, currently the most destructive bacterial disease affecting the honeybee, *Apis mellifera*, is caused by the Gram-positive bacterium *Paenibacillus larvae* (1). There is growing interest in phages that infect and lyse *P. larvae*, as antibiotic resistance is now widespread (2). There are currently 26 complete *P. larvae* phage genome sequences in the literature and more in the process of being published (3–8). Here, we present the complete genome sequences of phages Halcyone and Heath, isolated from soil underneath healthy hives in Gilcrease Orchards in North Las Vegas, Nevada; phage Unity, isolated from material inside a beehive at the University of Nevada Las Vegas (UNLV); and phage Scottie, isolated from commercial hand cream (Burt’s Bees) purchased in the Las Vegas area.

The phages were amplified using *P. larvae* NRRL 2605, an ERIC I genotype strain, and plated on modified brain heart infusion agar with soft agar. Phage DNA was purified with phenol-chloroform extraction at the University of Nevada Las Vegas (UNLV) and sequenced at Brigham Young University with Illumina HiSeq 2500 sequencing with 250-bp paired-end reads. The genome sequences were assembled with Geneious v. 10.2.2 (Biomatters, Auckland, New Zealand) with medium-low sensitivity/fast and manually annotated using DNA Master (9) by students in the course BIOL 209X Phage Discovery at UNLV.

Each phage’s GenBank accession number, isolation source, and assembly results are shown in Table 1. All 4 phages are in the family *Siphoviridae* with linear double-stranded DNA (dsDNA) genomes and use the “direct terminal repeats” (DTR) DNA-packaging strategy (10, 11). The DTR sequence of each phage was identified using Pile-up Analysis Using Starts & Ends (PAUSE) (<https://cpt.tamu.edu/computer-resources/pause/>) and Geneious, looking for a sharply delimited region with double coverage depth (11). Halcyone, Heath, and Scottie have identical DTR sequences 377 bp long, whereas Unity has a different DTR sequence 378 bp long. The genomes were oriented by setting the first base of the DTR sequence to be the first base of the genome.

The genomes for Halcyone, Heath, and Scottie are 55 kbp long, which is at the maximum of the range of the *P. larvae* phage genome length (3–8), and the genome of Unity is 50 kbp long. A multiple alignment of genome sequences with ClustalW shows that Halcyone and Heath are very closely related to each other, while Scottie is

Received 7 August 2018 Accepted 31 August 2018 Published 27 September 2018

Citation Yost DG, Chang C, LeBlanc L, Cassin E, Peterman C, Rai P, Salisbury A, Barroga N, Cisneros R, Fersini J, Juste J, Ines J, Leyva G, Macalinalo D, Muscelli S, Reyes GS, Rhoden H, Tan R, Torres E, Tran K, Uriarte-Valle G, Wallace C, Wong S, Ayala-Pineda K, Cadiz V, Jeanite T, Nhan S, Grose JH, Strong C, Amy PS, Tsourkas PK. 2018. Complete genome sequences of *Paenibacillus larvae* phages Halcyone, Heath, Scottie, and Unity from Las Vegas, Nevada. *Microbiol Resour Announc* 7:e00977-18. <https://doi.org/10.1128/MRA.00977-18>.

Editor Julie C. Dunning Hotopp, University of Maryland School of Medicine

Copyright © 2018 Yost et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to Philippos K. Tsourkas, Philippos.Tsourkas@unlv.edu.

TABLE 1 GenBank accession numbers and genome assembly results for *Paenibacillus larvae* phages

| Phage name | GenBank accession no. | Isolation source | Genome assembly results | | | |
|------------|--------------------------|------------------|-------------------------|------------------|----------------|--------------------------|
| | | | Genome length (bp) | Avg coverage (×) | GC content (%) | DTR sequence length (bp) |
| Halcyone | MH460827 | Soil | 55,560 | 83 | 48.6 | 377 |
| Heath | MH460826 | Soil | 55,560 | 222 | 48.6 | 377 |
| Scottie | MH460825 | Hand cream | 55,990 | 173 | 48.6 | 377 |
| Unity | MH460824 | Beehive | 50,316 | 320 | 49.1 | 378 |

a little more distant and Unity is more distant still; the difference is largely due to a 5-kbp region missing in Unity but present in the other three phages. All four phages encode a large terminase, a portal protein, a major tail protein, two tail assembly proteins, a tail tape measure protein, and an *N*-acetylmuramoyl-L-alanine amidase. The tail assembly proteins appear to have a predicted translational frameshift similar to those of the G and G-T genes in phage Lambda (12, 13), located in the 3' region of the upstream tail assembly protein (gp14). We tentatively identify the heptanucleotide slippery sequence as "TAAAAAA." Current work is ongoing to identify more *P. larvae* phage protein functions and provide a comparative genomic analysis of *P. larvae* phages.

Data availability. The GenBank accession numbers for the four complete *Paenibacillus larvae* phage genome sequences are listed in Table 1.

ACKNOWLEDGMENT

P.K.T. acknowledges support for BIOL 209X from the School of Life Sciences at the University of Nevada Las Vegas.

REFERENCES

- de Graaf DC, Alippi AM, Antúnez K, Aronstein KA, Budge G, De Koker D, De Smet L, Dingman DW, Evans JD, Foster LJ, Fünfhäus A, Garcia-Gonzalez E, Gregore A, Human H, Murray KD, Nguyen BK, Poppinga L, Spivak M, van Engelsdorp D, Wilkins S, Genersch E. 2013. Standard methods for American foulbrood research. *J Apic Res* 52:1–28. <https://doi.org/10.3896/IBRA.1.52.1.11>.
- Tian B, Fadhil NH, Powell JE, Kwong WK, Moran NA. 2012. Long-term exposure to antibiotics has caused accumulation of resistance determinants in the gut microbiota of honeybees. *mBio* 3:e00377-12. <https://doi.org/10.1128/mBio.00377-12>.
- Oliveira A, Melo LDR, Kropinski AM, Azeredo J. 2013. Complete genome sequence of the broad host range *Paenibacillus larvae* phage phiIBB_PI23. *Genome Announc* 1:e00438-13. <https://doi.org/10.1128/genomeA.00438-13>.
- 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:e00101-15. <https://doi.org/10.1128/genomeA.00101-15>.
- Beims H, Wittman 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. <https://doi.org/10.1128/AEM.00804-15>.
- Abraham J, Bousquet A-C, Bruff E, Carson N, Clark A, Connell A, Davis Z, Dums J, Everington C, Groth A, Hawes N, McArthur N, McKenney C, Oufkir A, Pearce B, Rampal S, Rozier H, Schaff J, Slehria T, Carson S, Miller ES. 2016. *Paenibacillus larvae* phage Tripp genome has 378-base-pair terminal repeats. *Genome Announc* 4:e01498-15. <https://doi.org/10.1128/genomeA.01498-15>.
- Tsourkas P, Yost D, 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 of honeybees. *Genome Announc* 3:e01120-15. <https://doi.org/10.1128/genomeA.01120-15>.
- Walker JK, Merrill BD, Berg JA, Dhalai A, Dingman DW, Fajardo CP, Graves K, Hill HL, Hilton JA, Imahara C, Knabe BK, Mangohig J, Monk J, Mun H, Payne AM, Salisbury A, Stamereilers C, Velez K, Ward AT, Breakwell DP, Grose JH, Hope S, Tsourkas PK. 2018. Complete genome sequences of *Paenibacillus larvae* phages BN12, Dragolir, Kiel007, Leyra, Likha, Paggassa, PBL1c, and Tadhana. *Genome Announc* 6:e01601-17. <https://doi.org/10.1128/genomeA.01602-17>.
- Pope WH, Jacobs-Sera D. 2018. Annotation of bacteriophage genome sequences using DNA Master: an overview. *Methods Mol Biol* 1681: 217–229. https://doi.org/10.1007/978-1-4939-7343-9_16.
- Casjens SR, Gilcrease EB. 2009. Determining DNA packaging strategy by analysis of the termini of the chromosomes in tailed-bacteriophage virions. *Methods Mol Biol* 502:91–111. https://doi.org/10.1007/978-1-60327-565-1_7.
- Merrill BD, Ward AT, Grose JH, Hope S. 2016. Software-based analysis of bacteriophage genomes, physical ends, and packaging strategies. *BMC Genomics* 17:679. <https://doi.org/10.1186/s12864-016-3018-2>.
- Xu J, Hendrix RW, Duda RL. 2004. Conserved translational frameshift in dsDNA bacteriophage tail assembly genes. *Mol Cell* 16:11–21. <https://doi.org/10.1016/j.molcel.2004.09.006>.
- Xu J, Hendrix RW, Duda RL. 2013. A balanced ratio of proteins from gene G and frameshift-extended gene GT is required for phage Lambda tail assembly. *J Mol Biol* 425:3476–3487. <https://doi.org/10.1016/j.jmb.2013.07.002>.