



## Genome Sequences of Nine Erwinia amylovora Bacteriophages

Ruchira Sharma,<sup>a</sup> Jordan A. Berg,<sup>a</sup> Nolan J. Beatty,<sup>a</sup> Minsey C. Choi,<sup>a</sup> Ashlin E. Cowger,<sup>a</sup> Brooke J. R. Cozzens,<sup>a</sup> Steven G. Duncan,<sup>a</sup> Christopher P. Fajardo,<sup>a</sup> Hannah P. Ferguson,<sup>a</sup> Trevon Galbraith,<sup>a</sup> Jacob A. Herring,<sup>a</sup> Taalin R. Hoj,<sup>a</sup> Jill L. Durrant,<sup>a</sup> Jonathan R. Hyde,<sup>a</sup> Garrett L. Jensen,<sup>a</sup> Si Yang Ke,<sup>a</sup> Shalee Killpack,<sup>a</sup> Jared L. Kruger,<sup>a</sup> Eliza E. K. Lawrence,<sup>a</sup> Ifeanyichukwu O. Nwosu,<sup>a</sup> Tsz Ching Tam,<sup>a</sup> Daniel W. Thompson,<sup>a</sup> Josie A. Tueller,<sup>a</sup> Megan E. H. Ward,<sup>a</sup> Charles J. Webb,<sup>a</sup>† Madison E. Wood,<sup>a</sup> Edward L. Yeates,<sup>a</sup> David A. Baltrus,<sup>b</sup> Donald P. Breakwell,<sup>a</sup> Dsandra Hope,<sup>a</sup> DJulianne H. Grose<sup>a</sup>

<sup>a</sup>Microbiology and Molecular Biology Department, Brigham Young University, Provo, Utah, USA <sup>b</sup>School of Plant Sciences, The University of Arizona, Tempe, Arizona, USA

**ABSTRACT** *Erwinia amylovora* is a plant pathogen belonging to the *Enterobacteriaceae* family, a family containing many plant and animal pathogens. Herein, we announce nine genome sequences of *E. amylovora* bacteriophages isolated from infected apple trees along the Wasatch Front in Utah.

A t an estimated total number of 10<sup>31</sup>, phages are by far the most abundant A biological entity on the planet (1–7). They dramatically influence the evolution of bacteria by their ability to infect and kill their hosts and to transfer genetic material. *Erwinia amylovora* is a rod-shaped facultative anaerobic member of the *Enterobacteriaceae* bacterial family, which includes many well-characterized Gram-negative plant and animal pathogens, such as *Salmonella* spp., *Escherichia coli*, and *Klebsiella* spp. As the causative agent of fire blight, *Erwinia amylovora* infects members of the Rosaceae plant family, causing diseased areas to appear burnt (8–10). The isolation and characterization of phages that infect *E. amylovora* may aid in our understanding of these bacteria and provide potential treatment for this devastating agricultural disease. Herein, we announce the genome sequences of nine *E. amylovora* bacteriophages, vB\_EamM\_Asesino, vB\_EamM\_Alexandra, vB\_EamM\_Bosolaphorus, vB\_EamM\_Desertfox, vB\_EamM\_MadMel, vB\_EamM\_Mortimer, vB\_EamP\_Pavtok, vB\_EamM\_SunLIRen, and vB\_EamM\_Wellington.

Phages were isolated from apple trees along the Wasatch Front in Utah that appeared to harbor fire blight infection. Phages were plaque purified through a minimum of three passages after amplification via enrichment culture (11). All nine phages reported in this announcement infect the *Erwinia amylovora* ATCC 29780 strain, as indicated by plaque assays, and their characteristics are summarized in Table 1. Genomic DNA was extracted (Phage DNA isolation kit; Norgen Biotek), a library was made using the Illumina TruSeq DNA Nano kit, and sample genomes were sequenced by Illumina HiSeq 2500 sequencing (250-bp paired end) and assembled with Geneious (12) version 8.1 using *de novo* assembly with medium-low sensitivity and various percentages of data. All phages circularized upon assembly and were annotated using DNA Master (http://cobamide2.bio.pitt.edu/computer.htm), giving preference for calls that gave full coding potential coverage.

The nine phages were grouped into five distinct clusters by genomic dot plot and average nucleotide identity analyses, as previously described (11), with the first three groups containing jumbo *Myoviridae*. The first jumbo group included four myoviruses, vB\_EamM\_Bosolaphorus, vB\_EamM\_Desertfox, vB\_EamM\_MadMel, and vB\_EamM\_Mortimer, which are similar to previously published *Erwinia* phage Ea35-70 (13), as well as other phages we have isolated (14). The second group included two jumbo myoviruses, vB\_EamM\_Asesino and vB\_EamM\_Wellington, with similarity to the well-characterized *Salmonella* SPN3US phage (15) and related phages. The third is a single

## Received 18 August 2018 Accepted 12 September 2018 Published 11 October 2018

Citation Sharma R, Berg JA, Beatty NJ, Choi MC, Cowger AE, Cozzens BJR, Duncan SG, Fajardo CP, Ferguson HP, Galbraith T, Herring JA, Hoj TR, Durrant JL, Hyde JR, Jensen GL, Ke SY, Killpack S, Kruger JL, Lawrence EEK, Nwosu IO, Tam TC, Thompson DW, Tueller JA, Ward MEH, Webb CJ, Wood ME, Yeates EL, Baltrus DA, Breakwell DP, Hope S, Grose JH. 2018. Genome sequences of nine *Erwinia amylovora* bacteriophages. Microbiol Resour Announc 7:e00944-18. https://doi.org/10.1128/MRA

Editor J. Cameron Thrash, Louisiana State University

**Copyright** © 2018 Sharma et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International license.

Address correspondence to Julianne H. Grose, grosejulianne@gmail.com.

† Deceased. Charles J. Webb did not see or approve the final version of this paper.

			Total no.	No. of reads	Assembly fold coverage		No. of	No. of	0+0
Name	GenBank accession no.	SRA accession no.	of reads	used	(range [mean])	Length (bp)	ORFs <sup>a</sup>	tRNAs	content (%)
vB_EamP_Pavtok	MH426726	SRX4597602	1,301,332	386,192	492–2,086 (1,069)	61,401	62	0	36.9
vB_EamM_SunLlRen	MH426725	SRX4597606	1,301,332	386,192	8,249–42,422 (13,566)	84,559	141	22	36.3
vB_EamM_Wellington	MH426724	SRX4597603	626,048	372,488	133-514 (329.7)	244,950	295	8	50.3
vB_EamM_Asesino	KX397364	SRX4597609	2,222,038	1,022,382	512-1,378 (1,037.7)	246,290	289	12	51.2
vB_EamM_Alexandra	MH248138	SRX4597608	381,540	200,005	63-516 (166.3)	266,532	349	0	49.8
vB_EamM_Bosolaphorus	MG655267	SRX4597604	778,168	326,344	83-555 (248.4)	272,228	321	1	49.4
vB_EamM_Desertfox	MG655268	SRX4597605	1,930,470	1,138,933	115-612 (352.9)	272,458	320	0	49.6
vB_EamM_Mortimer	MG655270	SRX4616109	2,581,160	287,396	47-207 (129.4)	273,914	325	-	49.5
vB_EamM_MadMel	MG655269	SRX4597607	1,604,720	1,443,568	567-1,577 (1,213.9)	275,000	321	0	49.4

jumbo myovirus, EamM\_Alexandra, which has similarity to previously published *Erwinia* phages EamM\_Yoloswag (14) and EamM\_Y3 (16). Podovirus vB\_EamP\_Pavtok and myovirus vB\_EamM\_SunLIRen are similar to *Erwinia* phages PEp14 and phiEa21-4 (17), respectively. The three jumbo myovirus groups package DNA by headful packaging (14) based on homology to phage phiKZ terminase (18), and their bp 1 was chosen by alignment to their phage family. PhageTerm (19) was used to determine the packaging strategy of SunLIRen and Pavtok. SunLIRen appeared to have headful packaging, and its bp 1 was assigned based on homology alignment to *Erwinia* phage phiEa21-4, while the packaging strategy of Pavtok is unknown, and its bp 1 was assigned due to homology to PEp14.

**Data availability.** The GenBank and SRA accession numbers for the nine *Erwinia* bacteriophages are listed in Table 1.

## ACKNOWLEDGMENTS

We thank the Howard Hughes Medical Institute Science Education Alliance–Phage Hunters Advancing Genomics and Evolutionary Science (SEA-PHAGES) for phage analysis training. In addition, we thank Ed Wilcox (BYU DNA Sequencing Center) and Michael Standing (BYU Microscopy Lab).

This work was graciously funded by a USDA grant (to D.A.B., University of Arizona) and the Department of Microbiology and Molecular Biology and the College of Life Sciences at Brigham Young University, as well as a private donor.

## REFERENCES

- Bergh O, Børsheim KY, Bratbak G, Heldal M. 1989. High abundance of viruses found in aquatic environments. Nature 340:467–468. https://doi .org/10.1038/340467a0.
- Wommack KE, Colwell RR. 2000. Virioplankton: viruses in aquatic ecosystems. Microbiol Mol Biol Rev 64:69–114. https://doi.org/10.1128/ MMBR.64.1.69-114.2000.
- 3. Brüssow H, Hendrix RW. 2002. Phage genomics: small is beautiful. Cell 108:13–16. https://doi.org/10.1016/S0092-8674(01)00637-7.
- Wilhelm SW, Jeffrey WH, Suttle CA, Mitchell DL. 2002. Estimation of biologically damaging UV levels in marine surface waters with DNA and viral dosimeters. Photochem Photobiol 76:268–273.
- 5. Hendrix RW. 2003. Bacteriophage genomics. Curr Opin Microbiol 6:506–511. https://doi.org/10.1016/j.mib.2003.09.004.
- Hambly E, Suttle CA. 2005. The viriosphere, diversity, and genetic exchange within phage communities. Curr Opin Microbiol 8:444–450. https://doi.org/10.1016/j.mib.2005.06.005.
- Suttle CA. 2005. Viruses in the sea. Nature 437:356–361. https://doi.org/ 10.1038/nature04160.
- Khan MA, Zhao Y, Korban SS. 2012. Molecular mechanisms of pathogenesis and resistance to the bacterial pathogen *Erwinia amylovora*, causal agent of fire blight disease in rosaceae. Plant Mol Biol Rep 30:247–260. https://doi.org/10.1007/s11105-011-0334-1.
- Schroth M, Thomson S, Hildebrand D, Moller W. 1974. Epidemiology and control of fire blight. Annu Rev Phytopathol 12:389–412. https://doi.org/ 10.1146/annurev.py.12.090174.002133.
- Thomson SV. 2000. Epidemiology of fire blight 2, p 9–36. In Vanneste J (ed), Fire blight: the disease and its causative agent, *Erwinia amylovora*. CABI Publishing, Wallingford, United Kingdom.
- Arens DK, Brady TS, Carter JL, Pape JA, Robinson DM, Russell KA, Staley LA, Stettler JM, Tateoka OB, Townsend MH, Whitley KV, Wienclaw TM, Williamson TL, Johnson SM, Grose JH. 2018. Characterization of two related *Erwinia* myoviruses that are distant relatives of the PhiKZ-like jumbo phages. PLoS One 13:e0200202. https://doi.org/10.1371/journal .pone.0200202.
- Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A. 2012. Geneious Basic: an integrated and extendable

desktop software platform for the organization and analysis of sequence data. Bioinformatics 28:1647–1649. https://doi.org/10.1093/bioinformatics/ bts199.

- Yagubi AI, Castle AJ, Kropinski AM, Banks TW, Svircev AM. 2014. Complete genome sequence of Erwinia *amylovora bacteriophage* vB\_EamM\_Ea35-70. Genome Announc 2:e00413-14. https://doi.org/ 10.1128/genomeA.00413-14.
- Esplin IND, Berg JA, Sharma R, Allen RC, Arens DK, Ashcroft CR, Bairett SR, Beatty NJ, Bickmore M, Bloomfield TJ, Brady TS, Bybee RN, Carter JL, Choi MC, Duncan S, Fajardo CP, Foy BB, Fuhriman DA, Gibby PD, Grossarth SE, Harbaugh K, Harris N, Hilton JA, Hurst E, Hyde JR, Ingersoll K, Jacobson CM, James BD, Jarvis TM, Jaen-Anieves D, Jensen GL, Knabe BK, Kruger JL, Merrill BD, Pape JA, Payne Anderson AM, Payne DE, Peck MD, Pollock SV, Putnam MJ, Ransom EK, Ririe DB, Robinson DM, Rogers SL, Russell KA, Schoenhals JE, Shurtleff CA, Simister AR, Smith HG, Stephenson MB, et al. 2017. Genome sequences of 19 novel *Erwinia amylovora* bacteriophages. Genome Announc 5:e00931-17. https://doi.org/10.1128/genomeA .00931-17.
- Lee JH, Shin H, Kim H, Ryu S. 2011. Complete genome sequence of Salmonella bacteriophage SPN3US. J Virol 85:13470–13471. https://doi .org/10.1128/JVI.06344-11.
- Buttimer C, Born Y, Lucid A, Loessner MJ, Fieseler L, Coffey A. 2018. Erwinia amylovora phage vB\_EamM\_Y3 represents another lineage of hairy Myoviridae. Res Microbiol 30062–30067. https://doi.org/10.1016/j .resmic.2018.04.006.
- Lehman SM, Kropinski AM, Castle AJ, Svircev AM. 2009. Complete genome of the broad-host-range *Erwinia amylovora* phage phiEa21-4 and its relationship to *Salmonella* phage Felix O1. Appl Environ Microbiol 75:2139–2147. https://doi.org/10.1128/AEM.02352-08.
- Mesyanzhinov VV, Robben J, Grymonprez B, Kostyuchenko VA, Bourkaltseva MV, Sykilinda NN, Krylov VN, Volckaert G. 2002. The genome of bacteriophage phiKZ of *Pseudomonas aeruginosa*. J Mol Biol 317:1–19. https://doi.org/10.1006/jmbi.2001.5396.
- Garneau JR, Depardieu F, Fortier L-C, Bikard D, Monot M. 2017. PhageTerm: a fast and user-friendly software to determine bacteriophage termini and packaging mode using randomly fragmented NGS data. Sci Rep 7:8292. https://doi.org/10.1038/s41598-017-07910-5.