



Complete Genome Sequences of *Erwinia amylovora* Phages vB_EamP-S2 and vB_EamM-Bue1

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ABSTRACT Phages vB_EamP-S2 (S2) and vB_EamM-Bue1 (Bue1) infect the plant pathogen *Erwinia amylovora*. S2 has a genome size of 45,495 bp and belongs to the genus *SP6virus*. The genome size of Bue1, related to *Salmonella* phage Vil, is 164,037 bp. Both phages possess a depolymerase enzyme, a frequent feature of *E. amylovora* phages.

The enterobacterium *Erwinia amylovora* is the causative agent of fire blight, a plant disease affecting pome fruit (1). The antibiotic streptomycin is widely used to control the disease (2). However, potential resistance development and public demand for environment-friendly alternatives promote the development of new control strategies (3). One alternative is bacteriophage treatment. *E. amylovora*-specific phages vB_EamP-S2 (S2) and vB_EamM-Bue1 (Bue1) were isolated from soil samples (Swiss apple orchards). Both phages possess a broad host range, infecting 83% (S2) and 96% (Bue1) of the *E. amylovora* strains tested. Transmission electron microscopy identified S2 as a podovirus (4), with an average capsid size of 64 nm (± 4.6 nm), and Bue1 as a myovirus, with an average capsid size of 79 nm (± 2.4 nm) and a 126-nm-long (± 7.4 nm) contractile tail. Phage DNA was extracted as described previously (4) and sheared into 550-bp fragments on an E220 ultrasonicator (Covaris, Woburn, MA). Libraries were prepared on a NeoPrep system (Illumina, San Diego, CA) using a TruSeq Nano DNA kit (Illumina) with six PCR cycles, according to the manufacturer's instructions. Paired-end sequencing of 300 bp was performed on a MiSeq instrument (Illumina) using a 600-cycle MiSeq reagent kit version 3 (Illumina), according to the manufacturer's instructions. This generated 4,387,300 (S2) and 4,642,900 (Bue1) raw reads. *De novo* assemblies were created using SeqMan NGen (Lasergene Genomics package version 12.1.0; DNASTar, Madison, WI). The average coverages were 5,463 \times (S2) and 7,668 \times (Bue1).

Coding sequences (CDS) were annotated using RAST 2.0 (5) and BLAST (6) comparisons with the nonredundant GenBank database. ARAGORN (7) and tRNAscan-SE 2.0 (8) identified tRNA sequences. Overall nucleotide sequence identities were analyzed using EMBOSS stretcher (9).

The S2 genome is 45,495 bp long. Primer walking toward the expected ends determined direct terminal repeats (297 bp). The G+C content is 49.8%. Of the 49 CDS annotated, 26 were assigned a putative function. No tRNA was found. S2 shares a nucleotide identity of 76.7% with *E. amylovora* phage Era103 (GenBank accession number [NC_009014](https://ncbi.nlm.nih.gov/nuccore/NC_009014); SP6-like) and 54.1% with *Salmonella* phage SP6 (GenBank accession number [NC_004831](https://ncbi.nlm.nih.gov/nuccore/NC_004831)), the type species of the genus *SP6virus* (10), placing S2 into the subfamily *Autographivirinae*, genus *SP6virus*.

The double-stranded linear DNA of Bue1 is 164,037 bp long, with a G+C content of

Received 25 June 2018 Accepted 27 June 2018 Published 26 July 2018

Citation Knecht LE, Born Y, Pothier JF, Loessner MJ, Fieseler L. 2018. Complete genome sequences of *Erwinia amylovora* phages vB_EamP-S2 and vB_EamM-Bue1. *Microbiol Resour Announc* 7:e00891-18. <https://doi.org/10.1128/MRA.00891-18>.

Editor Jason Stajich, University of California, Riverside

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50.2% containing 175 annotated CDS, with 64 with assigned putative functions and one tRNA^{Lys} sequence. The circularly permuted/terminally redundant genome was opened upstream of the rIIA lysis gene for annotation. Due to the nucleotide identity of 92.1% with *E. amylovora* phage phiEa2809 (GenBank accession number [NC_027340](#)) and 52.9% with the *Salmonella* phage Vi01 (GenBank accession number [NC_015296](#)), Bue1 can be assigned to the family *Ackermannviridae* (11).

Both S2 and Bue1 encode putative exopolysaccharide (EPS) depolymerases, which degrade the amylovan component of the host's capsule (12). Similar genes are present in *E. amylovora* phages vB_EamP-L1 (GenBank accession number [NC_019510](#); T7virus) (4), Ea9-2 (GenBank accession number [NC_023579](#); Ea92virus) (13), and phiEa2809 (14). This widespread prevalence of EPS depolymerases among *E. amylovora* podoviruses and myoviruses indicates an importance in host infection and specificity.

Data availability. The annotated sequences of the two *Erwinia amylovora* phage genomes were deposited at GenBank under the accession numbers [MG736918](#) (vB_EamP-S2) and [MG973030](#) (vB_EamM-Bue1).

ACKNOWLEDGMENTS

L.E.K., Y.B., and J.F.P. performed the experiments and wrote the paper, and M.J.L. and L.F. wrote the paper.

L.E.K. and Y.B. were funded by Swiss National Science Foundation (SNF) grant 310030_156947.

We declare no conflicts of interest.

REFERENCES

- Bonn WG, van der Zwet T. 2000. Distribution and economic importance of fire blight, p 37–53. In Vanneste JL (ed), *Fire blight: the disease and its causative agent, Erwinia amylovora*. CAB International, Wallingford, United Kingdom.
- Stockwell VO, Duffy B. 2012. Use of antibiotics in plant agriculture. *Rev Sci Tech* 31:199–210. <https://doi.org/10.20506/rst.31.1.2104>.
- Buttimer C, McAuliffe O, Ross RP, Hill C, O'Mahony J, Coffey A. 2017. Bacteriophages and bacterial plant diseases. *Front Microbiol* 8:34. <https://doi.org/10.3389/fmicb.2017.00034>.
- Born Y, Fieseler L, Marazzi J, Lurz R, Duffy B, Loessner MJ. 2011. Novel virulent and broad-host-range *Erwinia amylovora* bacteriophages reveal a high degree of mosaicism and a relationship to *Enterobacteriaceae* phages. *Appl Environ Microbiol* 77:5945–5954. <https://doi.org/10.1128/AEM.03022-10>.
- Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST server: Rapid Annotations using Subsystems Technology. *BMC Genomics* 9:75. <https://doi.org/10.1186/1471-2164-9-75>.
- Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. *J Mol Biol* 215:403–410. [https://doi.org/10.1016/S0022-2836\(05\)80360-2](https://doi.org/10.1016/S0022-2836(05)80360-2).
- Laslett D, Canback B. 2004. ARAGORN, a program to detect tRNA genes and tmRNA genes in nucleotide sequences. *Nucleic Acids Res* 32:11–16. <https://doi.org/10.1093/nar/gkh152>.
- Lowe TM, Chan PP. 2016. tRNAscan-SE on-line: integrating search and context for analysis of transfer RNA genes. *Nucleic Acids Res* 44:W54–W57. <https://doi.org/10.1093/nar/gkw413>.
- Rice P, Longden I, Bleasby A. 2000. EMBOSS: the European Molecular Biology Open Software Suite. *Trends Genet* 16:276–277. [https://doi.org/10.1016/S0168-9525\(00\)02024-2](https://doi.org/10.1016/S0168-9525(00)02024-2).
- King AMQ, Adams MJ, Carstens EB, Lefkowitz E. 2012. Virus taxonomy: classification and nomenclature of viruses: ninth report of the International Committee on Taxonomy of Viruses. Academic Press, London, United Kingdom.
- Adriaenssens EM, Wittmann J, Kuhn JH, Turner D, Sullivan MB, Dutilh BE, Jang HB, van Zyl LJ, Klumpp J, Lobočka M, Moreno Switt AI, Rumnieks J, Edwards RA, Uchiyama J, Alfenas-Zerbini P, Petty NK, Kropinski AM, Barylski J, Gillis A, Clokie MRC, Prangishvili D, Lavigne R, Aziz RK, Duffy S, Krupovic M, Poranen MM, Knezevic P, Enault F, Tong Y, Oksanen HM, Brister JR. 2018. Taxonomy of prokaryotic viruses: 2017 update from the ICTV Bacterial and Archaeal Viruses Subcommittee. *Arch Virol* 163: 1125–1129. <https://doi.org/10.1007/s00705-018-3723-z>.
- Born Y, Fieseler L, Klumpp J, Eugster MR, Zurfluh K, Duffy B, Loessner MJ. 2014. The tail-associated depolymerase of *Erwinia amylovora* phage L1 mediates host cell adsorption and enzymatic capsule removal, which can enhance infection by other phage. *Environ Microbiol* 16:2168–2180. <https://doi.org/10.1111/1462-2920.12212>.
- 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, Fuhrman 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>.
- Lagonenko AL, Sadovskaya O, Valentovich LN, Evtushenkov AN. 2015. Characterization of a new VII-like *Erwinia amylovora* bacteriophage phiEa2809. *FEMS Microbiol Lett* 362:fnv031. <https://doi.org/10.1093/femsle/fnv031>.