

Complete Genome Sequence of *Erwinia amylovora* Bacteriophage vB_EamM_Ea35-70

Abdelbaset I. Yagubi,^{a,d} Alan J. Castle,^a Andrew M. Kropinski,^{b,c} Travis W. Banks,^e Antonet M. Svircev^f

Department of Biological Sciences, Brock University, St. Catharines, Ontario, Canada^a; Laboratory for Foodborne Zoonoses, Public Health Agency of Canada, Guelph, Ontario, Canada^b; Department of Molecular and Cellular Biology, University of Guelph, Guelph, Ontario, Canada^c; Al-Jabal Al-Gharbi University, Gherian, Libya^d; Vineland Research and Innovation Centre, Vineland Station, Ontario, Canada^e; Agriculture and Agri-Food Canada, Vineland Station, Ontario, Canada^f

The complete genome of an *Erwinia amylovora* bacteriophage, vB_EamM_Ea35-70 (Ea35-70), is 271,084 bp, encodes 318 putative proteins, and contains one tRNA. Comparative analysis with other *Myoviridae* genomes suggests that Ea35-70 is related to the *Phikzlikevirus* genus within the family *Myoviridae*, since 26% of Ea35-70 proteins share homology to proteins in *Pseudomonas* phage ϕ KZ.

Received 14 April 2014 Accepted 16 July 2014 Published 21 August 2014

Citation 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(4):e00413-14. doi:10.1128/genomeA.00413-14.

Copyright © 2014 Yagubi et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 3.0 Unported license](http://creativecommons.org/licenses/by/3.0/).

Address correspondence to Abdelbaset I. Yagubi, abdelbaset.yagubi@agr.gc.ca, or Antonet M. Svircev, Antonet.Svircev@agr.gc.ca.

Bacteriophages and their carrier bacterium, *Pantoea agglomerans*, have been developed as a combined biological control agent for the fire blight pathogen, *Erwinia amylovora* (1–4). *E. amylovora* phage vB_EamM_Ea35-70 will be incorporated in the phage mixture–carrier system that is applied onto open blossoms during the spring. Multiyear field-based trials have demonstrated that phages and the phage carrier bacterium *P. agglomerans* can control *E. amylovora* at efficacies comparable to those of antibiotics (1). Knowledge of phage genome structure is critical to our understanding of the biological relationship between phages and their bacterial hosts and of phage detection in the field.

The complete genome sequence of Ea35-70, a member of the family *Myoviridae*, is presented here. Ea35-70 was isolated from soil samples collected under infected pear trees in southern Ontario, Canada (4). The *Myoviridae* isolates in our collection produce clear plaques and have high titers on bacterial host cells with low exopolysaccharide production (5), while in contrast, this phage produces clear plaques on *E. amylovora* cells that produce high amounts of exopolysaccharides.

The genomic DNA of Ea35-70 was extracted and purified with the Norgen phage DNA isolation kit (Norgen Biotek Corp., Ontario, Canada).

The genome sequence was obtained from McGill University and the Génome Québec Innovation Centre (Montreal, Quebec, Canada). The sequencing library was constructed using an Illumina Nextera XL kit. The average insert size was 800 bp. The library was sequenced on an Illumina MiSeq generating 2 × 150 paired-end reads. The ends were determined by the assembly algorithm as the initiation and termination points of the De Bruijn graph. The genome was assembled using SeqMan NGen 3 (DNASTar, Madison, WI, USA). Contigs >1 kb were exported, reassembled, and proofread using SeqMan Pro (DNASTar). The final contig was oriented in the same manner as for *Pseudomonas* phage ϕ KZ since it is the nearest genetically related phage in the database. The genomes were autoannotated using MyRAST (6),

with the massaged GenBank flatfile (*.gbk) imported into Kodon (Applied Maths, Austin, TX, USA), and the annotation quality was proofread. The presence of one tRNA was identified using tRNAscan-SE 1.21 (7). The annotated sequence was searched for missed coding sequences (CDSs) and incorrect initiation codons. To determine the level of relatedness to other phages, tBLASTx, with default settings, was used to compare the predicted protein sequences to the genome sequences of all other phages in NCBI.

The genome of Ea35-70 is 271,084 bp long, with a G+C content of 49.9%. The genome contains 318 CDSs, of which 284 CDSs were annotated as encoding hypothetical proteins. Only 34 open reading frames (ORFs) were annotated as functional genes encoding structural proteins, proteins involved in nucleic acid modification, and those involved in replication.

Nucleotide sequence accession number. The complete genome sequence of *E. amylovora* phage Ea35-70 has been submitted to GenBank and assigned the accession no. [KF806589](https://www.ncbi.nlm.nih.gov/nuccore/KF806589).

ACKNOWLEDGMENTS

This work was supported by a Growing Forward II grant from Agriculture and Agri-Food Canada and Brock University. Financial support for the graduate student was obtained from the Ministry of Higher Education and Scientific Research of Libya.

REFERENCES

- Lehman SM. 2007. Development of a bacteriophage-based biopesticide for fire blight. Ph.D. thesis. Brock University, St. Catharines, Ontario, Canada.
- Svircev AM, Castle AJ, Lehman SM. 2010. Bacteriophages for control of phytopathogens in food production systems, p 79–102. In Sabour PM, Griffiths MW (ed), *Bacteriophages in the control of food- and waterborne pathogens*. ASM Press, Washington, DC.
- Svircev AM, Lehman SM, Sholberg PL, Roach DR, Castle AJ. 2011. Phage biopesticides and soil bacteria: multilayered and complex interactions, p 215–235. In Witzany G (ed), *Soil biology*, vol 23: biocommunication in soil microorganisms. Springer Verlag, New York, NY.
- Gill JJ, Svircev AM, Smith R, Castle AJ. 2003. Bacteriophages of *Erwinia amylovora*. *Appl. Environ. Microbiol.* 69:2133–2138. <http://dx.doi.org/10.1128/AEM.69.4.2133-2138.2003>.

5. Roach DR, Sjaarda DR, Castle AJ, Svircev AM. 2013. Host exopolysaccharide quantity and composition impact *Erwinia amylovora* bacteriophage pathogenesis. *Appl. Environ. Microbiol.* 79:3249–3256. <http://dx.doi.org/10.1128/AEM.00067-13>.
6. Overbeek R, Olson R, Pusch GD, Olsen GJ, Davis JJ, Disz T, Edwards RA, Gerdes S, Parrello B, Shukla M, Vonstein V, Wattam AR, Xia F, Stevens R. 2013. The SEED and the Rapid Annotation of microbial genomes using Subsystems Technology (RAST). *Nucleic Acids Res.* 42: D206–D214. <http://dx.doi.org/10.1093/nar/gkt1226>.
7. Schattner P, Brooks AN, Lowe TM. 2005. The tRNAscan-SE, snoscan and snoGPS web servers for the detection of tRNAs and snoRNAs. *Nucleic Acids Res.* 33:W686–W689. <http://dx.doi.org/10.1093/nar/gki366>.