

Complete Genomic Sequence of *Erwinia amylovora* Phage PhiEaH2

Dóra Dömötör,^a Péter Becságh,^b Gábor Rákhely,^{c,d} György Schneider,^e and Tamás Kovács^a

Department of Biotechnology, Nanophagetherapy Center, Enviroinvest Corporation, Pécs, Hungary^a; Roche Magyarország Kft, Budaörs, Hungary^b; Department of Biotechnology, University of Szeged, Szeged, Hungary^c; Institute of Biophysics, Biological Research Center, Szeged, Hungary^d; and Institute of Medical Microbiology and Immunology, University of Pécs, Pécs, Hungary^e

***Erwinia amylovora* is the causative agent of fire blight, a serious disease of some *Rosaceae* plants. The newly isolated bacteriophage PhiEaH2 is able to lyse *E. amylovora* in the laboratory and has reduced the occurrence of fire blight cases in field experiments. This study presents the sequenced complete genome and analysis of phage PhiEaH2.**

Erwinia amylovora, a member of *Enterobacteriaceae*, is the causative agent of fire blight, a serious disease of some *Rosaceae* plants (8, 9). One alternative treatment to control fire blight could be the application of bacteriophages (2, 6, 7).

We isolated *E. amylovora* phage PhiEaH2 from a soil sample in Hungary. This phage demonstrated strong lytic effect against *E. amylovora* in the laboratory and reduced the occurrence of fire blight cases in a field experiment when no artificial infection was applied (D. Dömötör, G. Schneider, G. Rákhely, B.G. Polyák, and T. Kovács, submitted for publication). These observations indicate that this phage might be able to be used as a biocontrol agent against this plant-pathogenic bacterium.

The genomic DNA of phage PhiEaH2 was extracted and purified by using a Roche High Pure viral nucleic acid kit (Roche Diagnostics GmbH, Germany) according to the protocol supplied. A shotgun library was created and sequenced using two platforms (Roche GS Junior, ABI 3500XL genetic analyzer) with 20-fold coverage of the phage genome. Open reading frame (ORF) prediction was done by using Genemark, Baysys, and Rast. Sequence annotations were performed by Baysys and Rast. The genomic sequence of PhiEaH2 phage is 243,050 bp in length with a G+C content of 51.28 mol%. No phages against *E. amylovora* with such a large genome have been sequenced before (1, 4, 5). The genome showed 262 ORFs, and 205 ORFs were annotated as encoding hypothetical proteins; most of them had the highest similarity to the *Salmonella* phage SPN3US (accession no. JN641803) (3). Additionally, 57 ORFs were annotated as functional genes. Thirty-five ORFs were predicted to encode proteins involved in the structure and assembly of virions, and 15 ORFs were found to encode proteins related to nucleic acid metabolism and modification and DNA replication (thymidylate synthase, thymidylate kinase, DNA adenine methylase, endodeoxyribonuclease, RNase H, dihydrofolate reductase, a transcriptional regulator, DNA-dependent RNA polymerase beta subunits, helicases, and an SMC domain-containing protein). One ORF encodes a protein containing an HD domain, two ORFs encode endolysins, and another two are for acetyltransferases. The product of one ORF is involved in amylovoran biosynthesis, and two proteins contain radical SAM superfamily domains.

In conclusion, we analyzed the complete genomic sequence of the newly isolated *E. amylovora* phage PhiEaH2. PhiEaH2 is a good candidate for use as biocontrol agent against this plant-pathogenic bacterium. However, sequencing its genome revealed the presence of the *amsF* gene, which codes for a protein that is essential in amylovoran biosynthesis. This complex polysaccha-

ride is necessary for *E. amylovora* to evoke the pathogenic process in the host plant. The presence of this gene in PhiEaH2 must be taken into consideration if practical use of this phage strain is intended.

Nucleotide sequence accession number. The complete genome sequence of *E. amylovora* phage PhiEaH2 has been submitted to GenBank and assigned accession number [JX316028](https://www.ncbi.nlm.nih.gov/nuclot/JX316028).

ACKNOWLEDGMENTS

This work was funded by the European Union and by the Hungarian Government; projects GVOP-3.3.3-05/2.-2006-01-0045/3.0, GOP-1.1.1-07/1-2008-0038, GOP-1.3.2.-09-201-0023. The Hungarian National Technology Program (projects FAGCENTER and MFCDiagn) also supported this work.

REFERENCES

- Born Y, et al. 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.
- Jones JB, et al. 2007. Bacteriophages for plant disease control. *Annu. Rev. Phytopathol.* 45:245–262.
- Lee JH, Shin H, Kim H, Ryu S. 2011. Complete genome sequence of *Salmonella* bacteriophage SPN3US. *J. Virol.* 85:13470–13471.
- 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.
- Müller I, Kube M, Reinhardt R, Jelkmann W, Geider K. 2011. Complete genome sequences of three *Erwinia amylovora* phages isolated in north America and a bacteriophage induced from an *Erwinia tasmaniensis* strain. *J. Bacteriol.* 193:795–796.
- Nagy JK, Király L, Schwarczinger I. 2012. Phage therapy for plant disease control with a focus on fire blight. *Cent. Eur. J. Biol.* 7:1–12.
- Svircev AM, Castle AJ, Lehman SM. 2010. Bacteriophages for control of phytopathogens in food production systems, 79–102. In Sabour PM, Griffiths MW (ed), *Bacteriophages in the control of food- and waterborne pathogens*. ASM Press, Washington, DC.
- Van der Zwet T, Beer SV. 1999. Fire blight—its nature, prevention and control: a practical guide to integrated disease management. *Agriculture Information Bulletin No. 631*. U.S. Department of Agriculture, Washington, DC.
- Vanneste JL. 2000. Fire blight: the disease and its causative agent, *Erwinia amylovora*. CABI Publishing, Wallingford, United Kingdom.

Received 18 July 2012 Accepted 18 July 2012

Address correspondence to Tamás Kovács, kovacs@enviroinvest.hu.

Copyright © 2012, American Society for Microbiology. All Rights Reserved.

[doi:10.1128/JVI.01870-12](https://doi.org/10.1128/JVI.01870-12)