JOURNAL OF BACTERIOLOGY, Feb. 2011, p. 795–796 Vol. 193, No. 3
0021-9193/11/$12.00 doi:10.1128/JB.01293-10

Complete Genome Sequences of Three *Erwinia amylovora* Phages Isolated in North America and a Bacteriophage Induced from an *Erwinia tasmaniensis* Strain▽

I. Müller,[1] M. Kube,[2] R. Reinhardt,[2] W. Jelkmann,[1] and K. Geider[1]*

Julius Kühn-Institut, Institut für Pflanzenschutz in Obst- und Weinbau, Schwabenheimer Str. 101, 69221 Dossenheim, Germany[1], and Max-Planck-Institut für Molekulare Genetik, Ihnestr. 63, 14195 Berlin, Germany[2]

Received 26 October 2010/Accepted 12 November 2010

Fire blight, a plant disease of economic importance caused by *Erwinia amylovora*, may be controlled by the application of bacteriophages. Here, we provide the complete genome sequences and the annotation of three *E. amylovora*-specific phages isolated in North America and genomic information about a bacteriophage induced by mitomycin C treatment of an *Erwinia tasmaniensis* strain that is antagonistic for *E. amylovora*. The American phages resemble two already-described viral genomes, whereas the *E. tasmaniensis* phage displays a singular genomic sequence in BLAST searches.

Several *Erwinia amylovora* phages from North America have been partially characterized before by restriction digests and proposed for control of fire blight (5). We determined the genomes of ϕEa1h, ϕEa100, and ϕEa104 by shotgun sequencing on ABI3730XL capillary sequencers (3) and by primer walking. The shotgun sequences were assembled with PHRAP and edited with Consed, GAP4, and Clonemanager 5. Open reading frames (ORFs) were predicted with GLIMMER3. Partial sequences were aligned with the sequence of phage ϕEra103 (GenBank/EMBL/DDB accession number EF160123) for ϕEa1h and ϕEa100 and the sequence of phage ϕEa21-4 (accession number EU710883) for ϕEa104, and gaps were filled by sequencing PCR fragments.

The genomes of ϕEa1h and ϕEa100, members of the *Podoviridae*, have almost identical sizes of 45,522 bp and 45,554 bp, respectively, and average GC contents of 49.7%. They differed in six base pairs and a 32-bp repeat for ϕEa100.

Annotation revealed 50 major ORFs and a putative function for 27 genes, including genes coding for an extracellular polysaccharide (EPS) depolymerase and HNH endonucleases and genes associated with DNA replication, but no tRNA genes.

The highly related genomes of ϕEa1h/ϕEa100 and ϕEra103 diverged in 10 genes and a degenerated terminal repeat of 277 bp. Another peculiarity is a split gene for DNA polymerase and the associated 5′–3′ exonuclease.

The phages ϕEa1h and ϕEa100 may use direct repeats in their genomes for replication as concatemers, similar to *E. coli* phage T7 (2). A 54-mer is repeated twice and is present in both genomes.

Phage ϕEa104 belongs to the *Myoviridae* and has a genome length of 84,565 bp with 43.9% GC content. One hundred eighteen ORFs were identified with similarity to ORFs in ϕEa21-4. Morphogenesis-related proteins were predicted for 10 genes, and an endolysin and a holin gene were identified. ϕEa104 carries genes involved in nucleotide metabolism but no EPS depolymerase. A cluster of 24 tRNAs was detected for ϕEa104, similar to phage ϕEa21-4 (4), with 23 genes coding for standard tRNAs without pseudogenes. For ϕEa104, a circularly permutated linear genome is assumed.

Comparison of ϕEa104 with ϕEa21-4 revealed 98% identity for their genomes of 84,565 and 84,576 bp, respectively. Regions with high levels of mismatch but >97% protein identity were noted for genes encoding RIIA, a hypothetical protein, and DNA polymerase. BLAST search revealed low similarities to *Salmonella* phage Felix O1 (AF320576) (6), *Escherichia coli* O157:H7 phage wV8 (EU877232), and *Staphylococcus* phage SA1 (GU169904). The genome structure of ϕEa104 is completely different from that of ϕEa1h and ϕEa100.

Erwinia tasmaniensis strain Et1/99, antagonistic to *E. amylovora* (1), carries prophage information (3). From the Australian strain Et88, the novel phage ϕEt88 was induced with mitomycin C. It belongs to the *Myoviridae* and has a genome size of 47.3 kb. After shotgun sequencing and assembly, 68 ORFs were predicted. Eight genes encode morphogenesis-related proteins, seven have functions in nucleotide metabolism, and five are related to lysogenicity. Additionally, a lysis protein gene, an associated holin gene, and a single tRNA gene were found.

BLASTX search revealed only low similarity to other viral proteins, including a large terminase subunit, a hypothetical protein, an integrase and a DNA helicase, indicating a singular genomic sequence for ϕEt88.

Nucleotide sequence accession numbers. The sequences of the *E. amylovora* and *E. tasmaniensis* phages have been submitted to the EMBL database under accession no. FQ482084 (ϕEa1h), FQ482086 (ϕEa100), FQ482083 (ϕEa104), and FQ482085 (ϕEt88).

* Corresponding author. Mailing address: Julius Kühn-Institut (JKI) für Pflanzenschutz, Schwabenheimer Str. 101, D-69221 Dossenheim, Germany. Phone: 49-6221-86805-53. Fax: 49-6221-86805-15. E-mail: Klaus.Geider@jki.bund.de.

▽ Published ahead of print on 19 November 2010.

We thank the Julius Kuehn Institute and the Max Planck Society for financial support of the sequencing projects.

REFERENCES

1. **Jakovljevic, V., S. Jock, Z. Du, and K. Geider.** 2008. Hypersensitive response and acyl-homoserine lactone production of the fire blight antagonists *Erwinia tasmaniensis* and *Erwinia billingiae*. Microb. Biotechnol. **1:**416–424.
2. **Kornberg, A.** 1980. DNA replication. W. H. Freeman and Co, San Francisco, CA.
3. **Kube, M., et al.** 2008. The genome of *Erwinia tasmaniensis* strain Et1/99, a non-pathogenic bacterium in the genus *Erwinia*. Environ. Microbiol. **10:**2211–2222.
4. **Lehman, S. M., A. M. Kropinski, A. J. Castle, and A. M. Svircev.** 2009. Complete genome of the broad-host-range *Erwinia amylovora* phage ϕEa21-4 and its relationship to *Salmonella* phage Felix O1. Appl. Environ. Microbiol. **75:**2139–2147.
5. **Schnabel, E. L., and A. L. Jones.** 2001. Isolation and characterization of five *Erwinia amylovora* bacteriophages and assessment of phage resistance in strains of *Erwinia amylovora*. Appl. Environ. Microbiol. **67:**59–64.
6. **Whichard, J. M., et al.** 2010. Complete genomic sequence of bacteriophage Felix O1. Viruses **2:**710–730.