

Genome Sequence of an *Erwinia amylovora* Strain with Pathogenicity Restricted to *Rubus* Plants[∇]

Rachel Powney,^{1,2,3,4,†} Theo H. M. Smits,^{5,†} Tim Sawbridge,⁶ Beatrice Frey,⁵ Jochen Blom,⁷
Jürg E. Frey,⁵ Kim M. Plummer,^{1,3} Steven V. Beer,² Joanne Luck,^{1,4}
Brion Duffy,^{5,*} and Brendan Rodoni^{1,4}

Cooperative Research Centre for National Plant Biosecurity, LPO Box 5012, Bruce, ACT 2617, Australia¹; Department of Plant Pathology and Plant-Microbe Biology, Cornell University, Ithaca, New York 14853²; Department of Botany, La Trobe University, Bundoora, VIC 3086, Australia³; Department of Primary Industries, Knoxfield, VIC 3180, Australia⁴; Agroscope Changins-Wädenswil ACW, Plant Protection Division, CH-8820 Wädenswil, Switzerland⁵; Department of Primary Industries, Victorian AgriBiosciences Centre, Bundoora, VIC 3086, Australia⁶; and CeBiTec, University of Bielefeld, Bielefeld, Germany⁷

Received 10 November 2010/Accepted 19 November 2010

Here, we present the genome of a strain of *Erwinia amylovora*, the fire blight pathogen, with pathogenicity restricted to *Rubus* spp. Comparative genomics of ATCC BAA-2158 with *E. amylovora* strains from non-*Rubus* hosts identified significant genetic differences but support the inclusion of this strain within the species *E. amylovora*.

Erwinia amylovora is a Gram-negative enterobacterial phytopathogen that was first reported from the Northeastern United States in the late 1790s (2). Most strains of *E. amylovora* have broad-spectrum pathogenicity to plants of the Spiraeoideae subfamily (revised Maloideae), particularly commercial apple and pear and ornamental and amenity species that include members of the genera *Sorbus*, *Crataegus*, and *Cotoneaster* (2). However, a less common, genetically distinct group of *E. amylovora* strains with a host range restricted to *Rubus* species has also been described from North America (9, 13). Here, we report the first genome sequence of a *Rubus*-pathogenic *E. amylovora* strain, ATCC BAA-2158 (syn. Ea246, Bb-1, IL-5), isolated from thornless blackberry in Illinois (9).

This sequence augments genomic data from recently sequenced Spiraeoideae-pathogenic strains of *E. amylovora* ATCC 49946 (10) and CFBP 1430 (12) and genomes of the closely related *Erwinia* species *E. pyrifoliae* DSM 12163^T (pathogen of Asian pear) (11), *E. tasmaniensis* Et1/99 (epiphyte of uncertain pathogenicity) (5), and the nonpathogenic *E. billingiae* Eb661 (4). Our objective was to facilitate comparative studies that may elucidate the host-range determinants and evolutionary origins of this important phytopathogenic bacterial species.

Whole-genome pyrosequencing (454 Life Sciences) from two independent runs (3/8 of a 454 Titanium run and one 454 GS Junior run) yielded 344,879 high-quality filtered reads with an average read length of 375 bp and 31-times genome coverage. A consensus assembly of 32 contigs was obtained by assembly with Newbler (454 Life Sciences), and *in silico* gap closure performed with Lasergene (DNASStar, Madison, WI).

Assembly was confirmed by realigning reads against the consensus using NGen 2.0 (DNASStar).

The *E. amylovora* ATCC BAA-2158 genome contains a chromosome (3.81 Mb in 29 contigs with 53.6% G+C content) and three circular plasmids, pEA29 (28,138 bp with 50% G+C), pEAR5.2 (5,251 bp with 52.2% G+C), and pEAR4.3 (4,369 bp with 51.5% G+C). A total of 3,869 coding sequences (CDS) and putative functions of the encoding genes were automatically assigned to the genome using GenDB (8) with manual optimization (11, 12). Plasmid pEA29 shares 99% sequence identity (100% coverage) with previously described pEA29 plasmids in genotypically diverse strains of *E. amylovora* (6, 10, 12). Plasmids pEAR5.2 (6 CDS) and pEAR4.3 (4 CDS) are unique to strain ATCC BAA-2158 and share 88% and 89% sequence identity (57% and 53% coverage) with pEP5 of *E. pyrifoliae* DSM 12163^T (11).

Genomic sequence comparison using EDGAR (1) revealed approximately 373 singletons from ATCC BAA-2158 that were absent or highly divergent in the genomes of Spiraeoideae-infecting strains CFBP 1430 and ATCC 49946 of *E. amylovora* (10, 12). However, genomic sequence comparison of ATCC BAA-2158 with these strains of *E. amylovora* and the closely related species *E. pyrifoliae* DSM 12163^T (11), *E. tasmaniensis* Et1/99 (5), and *E. billingiae* Eb661 (4) corroborates other analyses (e.g., DNA-DNA hybridization and sequencing of housekeeping genes) that retain ATCC BAA-2158 within the species *E. amylovora* (3, 7).

Nucleotide sequence accession numbers. The 29 contigs of the draft chromosome of *E. amylovora* strain ATCC BAA-2158 were deposited at EMBL under accession numbers FR719181 to FR719209, and the plasmids under accession numbers FR719212 (pEA29), FR719210 (pEAR4.3), and FR719211 (pEAR5.2).

We acknowledge the support of the Australian Government's Cooperative Research Centres Program, Horticulture Australia, a Special

* Corresponding author. Mailing address: Agroscope Changins-Wädenswil ACW, Postfach, Schloss, CH-8820 Wädenswil, Switzerland. Phone: 41 44 7836416. Fax: 41 44 7836305. E-mail: duffy@acw.admin.ch.

† R. Powney and T. H. M. Smits are joint first authors.

∇ Published ahead of print on 3 December 2010.

Grant provided by the USDA CSREES for research on fire blight in New York, the European Union ESF-FP7-KBBE Project Q-Detect (grant no. 245047), the Swiss Secretariat for Education and Research (SER no. C09.0029), and the Swiss Federal Office of Agriculture (BLW no. 08.02).

We thank Jean M. Bonasera (Cornell University) and F. Rezzonico (Agroscope Changins-Wädenswil ACW) for technical support.

REFERENCES

1. **Blom, J., et al.** 2009. EDGAR: a software framework for the comparative analysis of prokaryotic genomes. *BMC Bioinform.* **10**:154.
2. **Bonn, W. G., and T. van der Zwet.** 2000. Distribution and economic importance of fire blight, p. 37–54. In J. L. Vanneste (ed.), *Fire blight: the disease and its causative agent, Erwinia amylovora*. CAB International, Wallingford, United Kingdom.
3. **Geider, K., et al.** 2006. *Erwinia tasmaniensis* sp. nov., a non-phytopathogenic bacterium from apple and pear trees. *Int. J. Syst. Evol. Microbiol.* **56**:2937–2943.
4. **Kube, M., et al.** 2010. Genome comparison of the epiphytic bacteria *Erwinia billingiae* and *E. tasmaniensis* with the pear pathogen *E. pyrifoliae*. *BMC Genomics* **11**:393.
5. **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.
6. **McGhee, G. C., and A. L. Jones.** 2000. Complete nucleotide sequence of ubiquitous plasmid pEA29 from *Erwinia amylovora* strain Ea88: gene organization and intraspecies variation. *Appl. Environ. Microbiol.* **66**:4897–4907.
7. **McGhee, G. C., et al.** 2002. Relatedness of chromosomal and plasmid DNAs of *Erwinia pyrifoliae* and *Erwinia amylovora*. *Appl. Environ. Microbiol.* **68**:6182–6192.
8. **Meyer, F., et al.** 2003. GenDB: an open source genome annotation system for prokaryote genomes. *Nucleic Acids Res.* **31**:2187–2195.
9. **Ries, S. M., and A. G. Otterbacher.** 1977. Occurrence of fire blight on thornless blackberry in Illinois. *Plant Dis. Rep.* **61**:232–235.
10. **Sebaihia, M., et al.** 2010. Complete genome sequence of the plant pathogen *Erwinia amylovora* strain ATCC 49946. *J. Bacteriol.* **192**:2020–2021.
11. **Smits, T. H. M., et al.** 2010. Complete genome sequence of the fire blight pathogen *Erwinia pyrifoliae* DSM 12163^T and comparative genomic insights into plant pathogenicity. *BMC Genomics* **11**:2.
12. **Smits, T. H. M., et al.** 2010. Complete genome sequence of the fire blight pathogen *Erwinia amylovora* CFBP 1430 and comparison to other *Erwinia* spp. *Mol. Plant-Microbe Interact.* **23**:384–393.
13. **Starr, M. P., C. Cardona, and D. Folsom.** 1951. Bacterial fire blight of raspberry. *Phytopathology* **41**:914–919.