

## GENOME ANNOUNCEMENTS

### Genome Sequence of the Biocontrol Agent *Pantoea vagans* Strain C9-1<sup>∇</sup>

Theo H. M. Smits,<sup>1</sup> Fabio Rezzonico,<sup>1</sup> Tim Kamber,<sup>1</sup> Alexander Goesmann,<sup>2</sup> Carol A. Ishimaru,<sup>3</sup>  
Virginia O. Stockwell,<sup>4</sup> Jürg E. Frey,<sup>1</sup> and Brion Duffy<sup>1\*</sup>

Plant Protection Division, Agroscope Changins-Wädenswil (ACW), CH-8820 Wädenswil, Switzerland<sup>1</sup>; Center for Biotechnology (CeBiTec),  
University of Bielefeld, D-33594 Bielefeld, Germany<sup>2</sup>; Department of Botany and Plant Pathology, Oregon State University,  
Corvallis, Oregon 97331<sup>3</sup>; and Department of Plant Pathology, University of Minnesota, St. Paul, Minnesota<sup>4</sup>

Received 21 September 2010/Accepted 5 October 2010

***Pantoea vagans* is a Gram-negative enterobacterial plant epiphyte of a broad range of plants. Here we report the 4.89-Mb genome sequence of *P. vagans* strain C9-1 (formerly *Pantoea agglomerans*), which is commercially registered for biological control of fire blight, a disease of pear and apple trees caused by *Erwinia amylovora*.**

*Pantoea vagans* (syn. *Pantoea agglomerans*, *Erwinia herbicola*) (10), is a common plant epiphyte. Strain C9-1, isolated from apple (*Malus × domestica* ‘Jonathan’) (Michigan) (5), is registered as BlightBan C9-1 (Nufarms America Inc., Burr Ridge, IL) for biocontrol of fire blight caused by the related enterobacterium *Erwinia amylovora*. Applied during bloom, C9-1 provides effective disease control, similar to oxytetracycline and slightly less disease control than streptomycin treatment provides (6). As for most biocontrol agents, growth and efficacy vary among locations and years (6). We sequenced the complete genome of *P. vagans* C9-1 as a step toward elucidating genetic factors that modulate performance reliability.

Genomic DNA, isolated using the Wizard genomic DNA purification kit (Promega, Madison, WI) was whole-genome shot-gun sequenced by 454 Life Sciences (Branford, CT) with four runs on a GS-20 sequencer. This resulted in 1,224,924 high-quality filtered reads with an average read length of 97 bp and coverage equivalent to 25 times. Quality filtered sequences were assembled *in silico* using the 454 Newbler assembler giving 207 contigs, 43 contigs larger than 500 bp. Gap closure and repetitive element sequencing were achieved by PCR walking using the Sanger method on a 3130XL sequencer (Applied Biosystems, Rotkreuz, Switzerland). Final assembly and sequence manipulations were done using the Lasergene package v8.1 (DNASTAR, Madison, WI).

The *P. vagans* C9-1 genome has a 4,025-Mb circular chromosome and three circular plasmids (pPag1 [167,983 bp], pPag2 [165,692 bp], and pPag3 [529,676 bp]). A total of 4,619 coding sequences were predicted (8) using GLIMMER (11) and CRITICA (1). Putative functions of protein-encoding genes were automatically assigned using the GenDB annota-

tion pipeline (9) and manually optimized. The chromosome harbors 7 rRNA operons and 78 tRNAs over the chromosome.

The *P. vagans* C9-1 genome has four large regions containing phage-related genes. Bordering regions could not be identified explicitly (e.g., one phage region is flanked by a tRNA<sup>Met</sup> [AttL] and an AttR region, while others were on the other side of AttR). Transposon-related genes were largely absent, but small genomic islands were observed. Only plasmid pPag2 contains remnants of transposases and recombinases, including an inactivated ISEhe3 and ISEhe4, related to those on pPATH<sub>pag</sub> in *P. agglomerans* pv. *gypsophilae* 824-1 (4).

The *P. vagans* C9-1 genome contains a repertoire of carbohydrate metabolic pathways identified using the KEGG databases (7). Important biosynthetic genes for antibacterial metabolites (i.e., pantocin A and dapdiamide E) and epiphytic fitness genes (e.g., autoinducer 1 [AI-1] quorum-sensing genes, indoleacetic acid, and carotenoid biosynthesis) were identified. No known virulence determinants of enterobacteria (e.g., type III secretion systems, effectors, toxins, and pectolytic enzymes) were found in the C9-1 genome (2, 3, 12–14). The genome sequence of *P. vagans* C9-1 provides a foundation for biosafety assessment of biological control strains introduced into agricultural environments and identification of genes and regulatory cascades that may impact the efficacy of this and related bacterial antagonists used for plant disease control.

**Nucleotide sequence accession numbers.** The *P. vagans* C9-1 chromosome and plasmid pPag1, pPag2, and pPag3 sequences have been deposited in GenBank under accession numbers CP002206 (chromosome), CP001893 (pPag1), CP001894 (pPag2), and CP001895 (pPag3).

We thank B. Frey and C. Pelludat (ACW) for technical support and B. Linke (CeBiTec) for the submission of the sequence to NCBI.

This work was financed by the Swiss Secretariat for Education and Research (SBF C06.0069), the Swiss Federal Office of the Environment (BAFU), and the Swiss Federal Office of Agriculture (BLW Fire Blight Control Project). This work was conducted within the European Science Foundation-funded research networks COST Action 873 and COST Action 864.

\* Corresponding author. Mailing address: Agroscope Changins-Wädenswil (ACW), Swiss National Competence Center for Fire Blight, Postfach, Schloss, CH-8820 Wädenswil, Switzerland. Phone: 41 44 7836416. Fax: 41 44 7836305. E-mail: duffy@acw.admin.ch.

<sup>∇</sup> Published ahead of print on 15 October 2010.

## REFERENCES

1. **Badger, J. H., and G. J. Olsen.** 1999. CRITICA: coding region identification tool invoking comparative analysis. *Mol. Biol. Evol.* **16**:512–524.
2. **Barash, I., and S. Manulis-Sasson.** 2009. Recent evolution of bacterial pathogens: the gall-forming *Pantoea agglomerans* case. *Annu. Rev. Phytopathol.* **47**:133–152.
3. **De Maayer, P., W. Y. Chan, S. N. Venter, I. K. Toth, P. R. J. Birch, F. Joubert, and T. A. Coutinho.** 2010. Genome sequence of *Pantoea ananatis* LMG20103, the causative agent of *Eucalyptus* blight and dieback. *J. Bacteriol.* **192**:2936–2937.
4. **Guo, M., S. Manulis, H. Mor, and I. Barash.** 2002. The presence of diverse IS elements and an *avrPphD* homologue that acts as a virulence factor on the pathogenicity plasmid of *Erwinia herbicola* pv. *gypsophillae*. *Mol. Plant Microbe Interact.* **15**:709–716.
5. **Ishimaru, C. A., E. J. Klos, and R. R. Brubaker.** 1988. Multiple antibiotic production by *Erwinia herbicola*. *Phytopathology* **78**:746–750.
6. **Johnson, K. B., and V. O. Stockwell.** 1998. Management of fire blight: a case study in microbial ecology. *Annu. Rev. Phytopathol.* **36**:227–248.
7. **Kanehisa, M., S. Goto, S. Kawashima, and A. Nakaya.** 2002. The KEGG databases at GenomeNet. *Nucleic Acids Res.* **30**:42–46.
8. **McHardy, A. C., A. Goesmann, A. Pühler, and F. Meyer.** 2004. Development of joint application strategies for two microbial gene finders. *Bioinformatics* **20**:1622–1631.
9. **Meyer, F., A. Goesmann, A. C. McHardy, D. Bartels, T. Bekel, J. Clausen, J. Kalinowski, B. Linke, O. Rupp, R. Giegerich, and A. Pühler.** 2003. GenDB—an open source genome annotation system for prokaryote genomes. *Nucleic Acids Res.* **31**:2187–2195.
10. **Rezzonico, F., T. H. M. Smits, E. Montesinos, J. E. Frey, and B. Duffy.** 2009. Genotypic comparison of *Pantoea agglomerans* plant and clinical strains to address biosafety and taxonomic issues. *BMC Microbiol.* **9**:204.
11. **Salzberg, S. L., A. L. Delcher, S. Kasif, and O. White.** 1998. Microbial gene identification using interpolated Markov models. *Nucleic Acids Res.* **26**:544–548.
12. **Smits, T. H. M., S. Jaenicke, F. Rezzonico, T. Kamber, A. Goesmann, J. E. Frey, and B. Duffy.** 2010. Complete genome sequence of the fire blight pathogen *Erwinia pyrifoliae* DSM 12163<sup>T</sup> and comparative genomic insights into plant pathogenicity. *BMC Genomics* **11**:2.
13. **Smits, T. H. M., F. Rezzonico, T. Kamber, J. Blom, A. Goesmann, J. E. Frey, and B. Duffy.** 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.
14. **Toth, I. K., L. Pritchard, and P. J. R. Birch.** 2006. Comparative genomics reveals what makes an enterobacterial plant pathogen. *Annu. Rev. Phytopathol.* **44**:305–336.