

UC Davis

UC Davis Previously Published Works

Title

Finished Genome Sequences of *Xanthomonas fragariae*, the Cause of Bacterial Angular Leaf Spot of Strawberry

Permalink

<https://escholarship.org/uc/item/9g79m8cw>

Journal

Microbiology Resource Announcements, 4(6)

ISSN

2576-098X

Authors

Henry, Peter M
Leveau, Johan HJ

Publication Date

2016-12-29

DOI

10.1128/genomea.01271-16

Peer reviewed

Finished Genome Sequences of *Xanthomonas fragariae*, the Cause of Bacterial Angular Leaf Spot of Strawberry

Peter M. Henry, Johan H. J. Leveau

Department of Plant Pathology, University of California Davis, Davis, California, USA

***Xanthomonas fragariae* is a foliar pathogen of strawberry that is of significant concern to nursery production of strawberry transplants and field production of strawberry fruit. Long-read sequencing was employed to generate finished genomes for two isolates (each with one chromosome and two plasmids) from symptomatic plants in northern California.**

Received 19 September 2016 Accepted 22 September 2016 Published 10 November 2016

Citation Henry PM, Leveau JHJ. 2016. Finished genome sequences of *Xanthomonas fragariae*, the cause of bacterial angular leaf spot of strawberry. *Genome Announc* 4(6): e01271-16. doi:10.1128/genomeA.01271-16.

Copyright © 2016 Henry and Leveau. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to Johan H. J. Leveau, jleveau@ucdavis.edu.

Xanthomonas fragariae causes angular leaf spot (ALS) of strawberry (*Fragaria* × *ananassa*) and is an international quarantine pathogen (1, 2). This species of Gram-negative bacteria colonizes strawberry foliage and enters the leaves through wounds or stomata (3), where it may remain quiescent before initiating growth and the profuse production of exopolysaccharides which is associated with typical water-soaked leaf lesions (2, 3). ALS symptoms may be mitigated by the use of certified disease-free planting stock or the foliar application of copper (1). A draft genome of a Belgian *X. fragariae* strain (LMG 25863) was published previously (4). The abundance of insertion sequences on this genome greatly complicated the assembly of Illumina reads (draft status at 96 contigs). To obtain a complete reference genome for future resequencing projects, long-read sequencing technology (PacBio) was used on two strains of *X. fragariae* (*FaP21* and *FaP29*) isolated in 2011 from symptomatic strawberry leaf tissue in Siskiyou County, California.

Genomic DNA was extracted from cells growing exponentially in liquid Wilbrinks-N (5) using a DNeasy blood and tissue kit (Qiagen, Valencia, CA). PacBio SMRTbell libraries were prepared at the UC Davis DNA Technologies Core (Davis, CA), size-selected to >20-kb fragments with BluePippin (Sage Science, Beverly, MA), and sequenced on the PacBio RS II platform (Pacific Biosciences, Menlo Park, CA). Reads were assembled by the hierarchical genome assembly process (HGAP3) protocol in smrt-analysis v2.3.0 (6) to yield for each isolate a single chromosome-length contig and two plasmid contigs. Ends of each contig were checked in Gepard (7) for overlapping regions that were trimmed and joined to yield complete circular chromosome and plasmid sequences (with the exception of one of the plasmids in *FaP29*, which could not be circularized). The beginning of each circularized chromosome was set to the start codon of the *dnaA* gene. Assemblies were quality-checked with high-fidelity 150-bp, paired-end Illumina MiSeq reads (UC Davis DNA Technologies Core). Bowtie2 mapped 99.25% of reads to assemblies with mean coverages of >400× (8). Using Pilon (9), we corrected 33 and 39 indel errors in the *FaP21* and *FaP29* genomes, respectively. Gene

prediction was done using the Rapid Annotation using Subsystem Technology (RAST) server (10).

The genomes of *X. fragariae* *FaP21* and *FaP29* were highly similar in size (4.2827 and 4.2826 Mbp, respectively), G+C content (62.27% for both), and number of RAST-predicted genes (4,149 and 4,141, respectively). The genomes harbored multiple gene clusters for copper resistance and an arsenal of type II, IV, VI, and VII secretion systems. Similar to LMG 25863 (4), genes for type III secretion and TAL effectors were absent in the *FaP* genomes. The largest of the two plasmids (29.1 kb) in both strains showed homology to a 27.2-kb plasmid from the xylem-limited sugarcane pathogen *Xanthomonas albilineans*. Mapping of the 96 contigs of LMG 25863 onto the *FaP* genomes revealed that the ends of these contigs consistently represented highly repeated regions on the genome, showing very clearly the benefit of using long-read technology to close bacterial genomes.

Accession number(s). The complete genome sequences for *FaP21* and *FaP29* have been deposited at DDBJ/EMBL/GenBank under the accession numbers [CP016830](https://accession.gtrdb.org/CP016830) (*FaP21* chromosome), [CP016831](https://accession.gtrdb.org/CP016831) (plasmid *pFaP21-1*), [CP016832](https://accession.gtrdb.org/CP016832) (plasmid *pFaP21-2*), [CP016833](https://accession.gtrdb.org/CP016833) (*FaP29* chromosome), [CP016834](https://accession.gtrdb.org/CP016834) (plasmid *pFaP29-1*), and [CP016835](https://accession.gtrdb.org/CP016835) (plasmid *pFaP29-2*).

ACKNOWLEDGMENTS

This project was made possible by a gift from Lassen Canyon Nursery (Redding, CA). We thank Joseph Fass from the University of California at Davis Bioinformatics Core for technical assistance.

REFERENCES

- Roberts PD, Berger RD, Jones JB, Chandler CK, Stall RE. 1997. Disease progress, yield loss, and control of *Xanthomonas fragariae* on strawberry plants. *Plant Dis* 81:917–921. <http://dx.doi.org/10.1094/PDIS.1997.81.8.917>.
- Turechek WW, Hartung JS, McCallister J. 2008. Development and optimization of a real-time detection assay for *Xanthomonas fragariae* in strawberry crown tissue with receiver operating characteristic curve analysis. *Phytopathology* 98:359–368. <http://dx.doi.org/10.1094/PHTO-98-3-0359>.
- Kastelein P, Krijger M, Czajkowski R, van der Zouwen PS, van der

- Schoor R, Jalink H, van der Wolf JM. 2014. Development of *Xanthomonas fragariae* populations and disease progression in strawberry plants after spray inoculation of leaves. *Plant Pathol* 63:255–263. <http://dx.doi.org/10.1111/ppa.12090>.
4. Vandroemme J, Cottyn B, Baeyen S, De Vos P, Maes M. 2013. Draft genome sequence of *Xanthomonas fragariae* reveals reductive evolution and distinct virulence-related gene content. *BMC Genomics* 14:829. <http://dx.doi.org/10.1186/1471-2164-14-829>.
 5. Koike H. 1965. The aluminium-cap method for testing sugarcane varieties against leaf scald disease. *Phytopathology* 55:317–319.
 6. Chin CS, Alexander DH, Marks P, Klammer AA, Drake J, Heiner C, Clum A, Copeland A, Huddleston J, Eichler EE, Turner SW, Korlach J. 2013. Nonhybrid, finished microbial genome assemblies from long-read SMRT sequencing data. *Nat Methods* 10:563–569. <http://dx.doi.org/10.1038/nmeth.2474>.
 7. Krumsiek J, Arnold R, Rattei T. 2007. Gepard: A rapid and sensitive tool for creating dotplots on genome scale. *Bioinformatics* 23:1026–1028. <http://dx.doi.org/10.1093/bioinformatics/btm039>.
 8. Langmead B, Salzberg SL. 2012. Fast gapped-read alignment with bowtie 2. *Nat Methods* 9:357–359. <http://dx.doi.org/10.1038/nmeth.1923>.
 9. Walker BJ, Abeel T, Shea T, Priest M, Abouelliel A, Sakthikumar S, Cuomo CA, Zeng Q, Wortman J, Young SK, Earl AM. 2014. Pilon: an integrated tool for comprehensive microbial variant detection and genome assembly improvement. *PLoS One* 9:e112963. <http://dx.doi.org/10.1371/journal.pone.0112963>.
 10. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST server: Rapid Annotations using Subsystems Technology. *BMC Genomics* 9:75. <http://dx.doi.org/10.1186/1471-2164-9-75>.