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GENOME SEQUENCES

Draft Genome Sequences of Two *Xanthomonas fragariae* Strains

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ABSTRACT *Xanthomonas fragariae* is the causal agent of angular leaf spot of strawberry. Short-read sequences were generated for two *X. fragariae* strains with different virulence phenotypes on the Illumina HiSeq 2000 platform. These genome sequences will contribute to a better understanding of pathogen evolution and the genes contributing to virulence in *X. fragariae*.

Xanthomonas fragariae causes angular leaf spot of strawberry, a problematic disease in strawberry nursery production (1–4). Genome sequences of *X. fragariae* strains with similar virulences have been published (1–3). Here, we present the draft genome sequence assemblies of two *X. fragariae* strains reported as having different virulence phenotypes. Xf100 and Xf1431 were isolated in the 1990s from symptomatic plants in Florida; Xf1431 was reported as nonpathogenic on cultivar Dover (5, 6). The strains were received from John Hartung (USDA-ARS) and stored at −80°C.

A single colony per strain was transferred from a 3- to 4-day-old culture on sucrose peptone agar into sucrose peptone broth for 1 to 2 days at 25°C; genomic DNA was extracted from the colonies using a DNeasy blood and tissue kit (Qiagen, Germantown, MD) and sent to BGI (Shenzhen, Guangdong, China) for sequencing on the Illumina HiSeq 2000 system. For each strain, two libraries (~470-bp and ~6,300-bp inserts) were prepared (7) and sequenced with 90- and 50-bp reads, respectively. The sequences were processed with bioinformatics tools using default parameters unless stated otherwise. The raw reads were quality controlled with Trimmomatic v0.39 (8). After eliminating the low-quality reads, the clean reads (>160× coverage per strain) were used for *de novo* genome assembly with SOAPdenovo r242 (9, 10). Gene prediction and annotation were performed with the prokaryotic genome annotation program PROKKA v1.14.5 (11). The similarity of amino acid sequences to the LMG-25863 reference genome (GenBank accession no. GCA_000376745.1) (3) was measured using AAI-Profiler (12). The trimmed and paired reads were aligned to LMG-25863 with HISAT2 v2.2.1 (13); genomic variants were identified with the GATK haplotype caller v4.1.9.0 (14). Variants in the coding regions were functionally annotated with SnpEff v5.0 (15). The LMG-25863 protein sequences from the types II, III, IV, and VI secretion systems and the toxin-antitoxin (TA) system were compared against those of Xf100 and Xf1431 using BLASTP.

Xf100 and Xf1431 are highly similar to LMG-25863 (Table 1); all strains lacked several critical genes for pathogen-host interactions found in other xanthomonads, including the *xcs* genes in the type II secretion system, phenolics degradation I and II, glyoxylate shunt, and xylan degradation clusters I to III. All three strains share the same type II-*xps*, III, IV, and VI secretion systems, except for the ClpB T6SS protein present in Xf100 and Xf1431 but highly fragmented in LMG-25863. For genes in the TA system, Xf1431 harbors two copies of RelB and RelE, while Xf100 and LMG-25863 each have one; DinJ and YafO in Xf100 shared 44% and 88% identity to those in Xf1431 and LMG-25863. Only 148 variant sites (142 single nucleotide polymorphisms [SNPs], 3 insertions, and

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TABLE 1 Sequencing statistics of Xf100 and Xf1431 in comparison to the reference *X. fragariae* strain LMG-25863

Strain	No. of reads	No. of bases (Mbp)	Assembly size (bp)	No. of contigs	N_{50} (kbp)	GC content (%)	No. of protein-coding genes[a]
Xf100	5,365,874	755	4,155,724	136	77.3	62.30	3,841
Xf1431	4,678,916	688	4,203,715	121	91.5	62.27	3,896
LMG-25863	8,185,858	1,022	4,182,545	96	131.4	62.20	3,919

[a] As predicted with PROKKA.

3 deletions) were found between Xf100 and Xf1431, which may explain their different virulence phenotypes, but they shared increased variation (685 SNPs, 30 insertions, and 34 deletions) from LMG-25863 (isolated in Belgium), which could be attributed to their geographical distance. The genome sequences of Xf100 and Xf1431 will be important additional resources for understanding virulence in *X. fragariae*.

Data availability. The assembled sequences are available under GenBank assembly accession no. GCA_016792245.1 and GCA_016792185.1; the raw reads are available under SRA accession no. SRR13617564 and SRR13617565 for Xf100 and SRR13618019 and SRR13618020 for Xf1431.

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REFERENCES

1. Gétaz M, van der Wolf JM, Blom J, Pothier JF. 2017. Complete genome sequences of three isolates of Xanthomonas fragariae, the bacterium responsible for angular leaf spots on strawberry plants. Genome Announc 5:e00642-17. https://doi.org/10.1128/genomeA.00642-17.
2. Henry PM, Leveau JHJ. 2016. Finished genome sequences of *Xanthomonas fragariae*, the cause of bacterial angular leaf spot of strawberry. Genome Announc 4:e01271-16. https://doi.org/10.1128/genomeA.01271-16.
3. 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. https://doi.org/10.1186/1471-2164-14-829.
4. Wang H, McTavish C, Turechek WW. 2018. Colonization and movement of *Xanthomonas fragariae* in strawberry tissues. Phytopathology 108:681–690. https://doi.org/10.1094/PHYTO-10-17-0356-R.
5. Roberts PD, Hodge NC, Bouzar H, Jones JB, Stall RE, Berger RD, Chase AR. 1998. Relatedness of strains of Xanthomonas fragariae by restriction fragment length polymorphism, DNA-DNA reassociation, and fatty acid analyses. Appl Environ Microbiol 64:3961–3965. https://doi.org/10.1128/AEM.64.10.3961-3965.1998.
6. Pooler MR, Ritchie DF, Hartung JS. 1996. Genetic relationships among strains of Xanthomonas fragariae based on random amplified polymorphic DNA PCR, repetitive extragenic palindromic PCR, and enterobacterial repetitive intergenic consensus PCR data and generation of multiplexed PCR primers useful for the identification of this phytopathogen. Appl Environ Microbiol 62:3121–3127. https://doi.org/10.1128/AEM.62.9.3121-3127.1996.
7. Sinha A, Phillips-Salemka S, Niraula T-A, Short KA, Niraula NP. 2019. The complete genomic sequence of Streptomyces spectabilis NRRL-2792 and identification of secondary metabolite biosynthetic gene clusters. J Ind Microbiol Biotechnol 46:1217–1223. https://doi.org/10.1007/s10295-019-02172-8.
8. Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30:2114–2120. https://doi.org/10.1093/bioinformatics/btu170.
9. Li R, Li Y, Kristiansen K, Wang J. 2008. SOAP: short oligonucleotide alignment program. Bioinformatics 24:713–714. https://doi.org/10.1093/bioinformatics/btn025.
10. Li R, Zhu H, Ruan J, Qian W, Fang X, Shi Z, Li Y, Li S, Shan G, Kristiansen K, Li S, Yang H, Wang J, Wang J. 2010. De novo assembly of human genomes with massively parallel short read sequencing. Genome Res 20:265–272. https://doi.org/10.1101/gr.097261.109.
11. Seemann T. 2014. Prokka: rapid prokaryotic genome annotation. Bioinformatics 30:2068–2069. https://doi.org/10.1093/bioinformatics/btu153.
12. Medlar AJ, Törönen P, Holm L. 2018. AAI-profiler: fast proteome-wide exploratory analysis reveals taxonomic identity, misclassification and contamination. Nucleic Acids Res 46:W479–W485. https://doi.org/10.1093/nar/gky359.
13. Kim D, Paggi JM, Park C, Bennett C, Salzberg SL. 2019. Graph-based genome alignment and genotyping with HISAT2 and HISAT-genotype. Nat Biotechnol 37:907–915. https://doi.org/10.1038/s41587-019-0201-4.
14. McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, Garimella K, Altshuler D, Gabriel S, Daly M, DePristo MA. 2010. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res 20:1297–1303. https://doi.org/10.1101/gr.107524.110.
15. Cingolani P, Platts A, Wang LL, Coon M, Nguyen T, Wang L, Land SJ, Lu X, Ruden DM. 2012. A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of Drosophila melanogaster strain w^{1118}; iso-2; iso-3. Fly (Austin) 6:80–92. https://doi.org/10.4161/fly.19695.