

Draft Genome Sequence of *Pseudomonas syringae* pv. *syringae* ALF3 Isolated from Alfalfa

James Harrison,^a Melinda R. Dornbusch,^b Deborah Samac,^{b,c}  David J. Studholme^a

Biosciences, University of Exeter, Devon, United Kingdom^a; USDA-ARS, Plant Science Research Unit, St. Paul, Minnesota, USA^b; Department of Plant Pathology, University of Minnesota, St. Paul, Minnesota, USA^c

We report here the annotated draft genome sequence of *Pseudomonas syringae* pv. *syringae* strain ALF3, isolated in Wyoming. A comparison of this genome sequence with those of closely related strains of *P. syringae* adapted to other hosts will facilitate research into interactions between this pathogen and alfalfa.

Received 16 December 2015 Accepted 21 December 2015 Published 11 February 2016

Citation Harrison J, Dornbusch MR, Samac D, Studholme DJ. 2016. Draft genome sequence of *Pseudomonas syringae* pv. *syringae* ALF3 isolated from alfalfa. *Genome Announc* 4(1):e01722-15. doi:10.1128/genomeA.01722-15.

Copyright © 2016 Harrison et al. 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 David J. Studholme, dj.studholme@exeter.ac.uk.

Pseudomonas syringae pv. *syringae* causes bacterial stem blight of alfalfa (*Medicago sativa*, also known as lucerne), the most widely cultivated perennial forage crop worldwide (1). The disease is widespread in the central and western United States and occasionally occurs in eastern states. It has also been reported in Australia and Europe, and it was recently reported in western Iran (2); however, little is known about the pathogen. The bacterium penetrates host stems primarily at frost injury sites and forms water-soaked lesions that extend down the stem, becoming amber with dried bacterial exudate that blackens with age. Leaves become water soaked and then chlorotic and necrotic. Plants with the disease are stunted, with spindly stems that are easily broken. Strain ALF3 was isolated from alfalfa plants with symptoms of bacterial stem blight from an area near Cheyenne, WY, and shown to be highly pathogenic on alfalfa, *Medicago truncatula*, and snap bean seed pods (3).

We used the Illumina HiSeq 2500 to generate 13 million pairs of 100-bp reads from genomic DNA; the raw data are available in the Sequence Read Archive (4). *De novo* assembly with Velvet 1.2.03 (5) resulted in 29 scaffolds comprising a total of 130 contigs. The contig N_{50} and scaffold N_{50} were 127,021 bp and 500,801 bp, respectively. The genome sequence was annotated using the NCBI Prokaryotic Genome Annotation Pipeline (6) version 2.6 (rev. 440435). This predicted 4,842 protein-coding genes and 38 pseudogenes, as well as 46 tRNA genes and three rRNA genes (5S, 16S, and 23S). Intriguingly, the genome sequence of ALF3 is almost identical to that of *P. syringae* B576 (GenBank accession no. JRUO00000000.1), with which it shares 99.9% average nucleotide identity (ANI) over 99.9% of the genome. Little information is available for strain B756 other than that it was isolated from a nonspecified plant in western China. Comparisons of whole-genome sequences revealed that apart from strain B756, strain ALF3 is most similar to *P. syringae* pv. *japonica* M301072 from barley and *P. syringae* pv. *aptata* DSM 50252 (7) from beet, sharing 98.7% ANI with each. However, approximately 6% of the ALF3 genome shares no detectable nucleotide sequence similarity with genome sequences of M301072 and DSM 50252. This portion

includes putative phage-associated genes and a gene encoding an HrpZ harpin (GenBank accession no. KFF85522.1).

The availability of this genome sequence will enable studies on the molecular basis for adaptation to alfalfa, facilitated by the availability of sequences from other *P. syringae* pv. *syringae* strains not known to infect this host (7–12) and by the availability of genome sequences from the pathogen responsible for the other major bacterial disease of alfalfa, namely, *Xanthomonas alfalfae* subsp. *alfalfae*, which causes bacterial leaf spot (13).

Nucleotide sequence accession numbers. This whole-genome shotgun project has been deposited in DDBJ/ENA/GenBank under the accession no. [JPNN00000000](https://www.ncbi.nlm.nih.gov/nuclink/JPNN00000000). The version described in this paper is the first version, JPNN01000000.

ACKNOWLEDGMENTS

Genome sequencing was performed by the Exeter Sequencing Service and Computational Core Facilities at the University of Exeter. These facilities are supported by a Medical Research Council Clinical Infrastructure award (grant MR/M008924/1), the Wellcome Trust Institutional Strategic Support Fund (grant WT097835MF), a Wellcome Trust Multi-User Equipment award (grant WT101650MA), and a BBSRC LOLA award (grant BB/K003240/1). James Harrison was supported by a Ph.D. studentship from the Biotechnology and Biological Sciences Research Council (BBSRC). The research was supported by USDA-ARS CRIS project 5062-12210-002-00D. The funders had no role in the study design, data collection and interpretation, or the decision to submit the work for publication.

FUNDING INFORMATION

Biotechnology and Biological Sciences Research Council (BBSRC) provided funding to James Harrison.

Funding was also provided by USDA-ARS CRIS project 5062-12210-002-00D.

REFERENCES

1. Gray FA, Hollingsworth CR. 2015. Bacterial stem blight, p. 60–62. In Samac DA, Rhodes LH, Lamp WO (ed), *Compendium of alfalfa diseases and pests*, 3rd ed. APS Press, St. Paul, MN.

2. Harighi B. 2007. Occurrence of alfalfa bacterial stem blight disease in Kurdistan Province, Iran. *J Phytopathol* 155:593–595. <http://dx.doi.org/10.1111/j.1439-0434.2007.01284.x>.
3. Samac D, Studholme DJ, Ao S. 2014. Characterization of the bacterial stem blight pathogen of alfalfa, *Pseudomonas syringae* pv. *syringae* ALF3. *Phytopathology* 104:102.
4. Leinonen R, Sugawara H, Shumway M, International Nucleotide Sequence Database Collaboration. 2011. The Sequence Read Archive. *Nucleic Acids Res* 39:D19–D21. <http://dx.doi.org/10.1093/nar/gkq1019>.
5. Zerbino DR, Birney E. 2008. Velvet: algorithms for *de novo* short read assembly using de Bruijn graphs. *Genome Res* 18:821–829. <http://dx.doi.org/10.1101/gr.074492.107>.
6. Angiuoli SV, Gussman A, Klimke W, Cochrane G, Field D, Garrity G, Kodira CD, Kyrpides N, Madupu R, Markowitz V, Tatusova T, Thomson N, White O. 2008. Toward an online repository of Standard Operating Procedures (SOPs) for (meta)genomic annotation. *Omi AJ Integr. J Biol* 12:137–141.
7. Baltrus DA, Nishimura MT, Romanchuk A, Chang JH, Mukhtar MS, Cherkis K, Roach J, Grant SR, Jones CD, Dangl JL. 2011. Dynamic evolution of pathogenicity revealed by sequencing and comparative genomics of 19 *Pseudomonas syringae* isolates. *PLoS Pathog* 7:e1002132. <http://dx.doi.org/10.1371/journal.ppat.1002132>.
8. Sohn KH, Jones JDG, Studholme DJ. 2012. Draft genome sequence of *Pseudomonas syringae* pathovar *syringae* strain FF5, causal agent of stem tip dieback disease on ornamental pear. *J Bacteriol* 194:3733–3734. <http://dx.doi.org/10.1128/JB.00567-12>.
9. Ravindran A, Jalan N, Yuan JS, Wang N, Gross DC. 2015. Comparative genomics of *Pseudomonas syringae* pv. *syringae* strains B301D and HS191 and insights into intrapathovar traits associated with plant pathogenesis. *Microbiologyopen* 4:553–573. <http://dx.doi.org/10.1002/mbo3.261>.
10. Dudnik A, Dudler R. 2013. High-quality draft genome sequence of *Pseudomonas syringae* pv. *syringae* strain SM, isolated from wheat. *Genome Announc* 1(4):e00610-13. <http://dx.doi.org/10.1128/genomeA.00610-13>.
11. Feil H, Feil WS, Chain P, Larimer F, DiBartolo G, Copeland A, Lykidis A, Trong S, Nolan M, Goltsman E, Thiel J, Malfatti S, Loper JE, Lapidus A, Detter JC, Land M, Richardson PM, Kyrpides NC, Ivanova N, Lindow SE. 2005. Comparison of the complete genome sequences of *Pseudomonas syringae* pv. *syringae* B728a and pv. *tomato* DC3000. *Proc Natl Acad Sci USA* 102:11064–11069. <http://dx.doi.org/10.1073/pnas.0504930102>.
12. Clarke CR, Cai R, Studholme DJ, Guttman DS, Vinatzer BA. 2010. *Pseudomonas syringae* strains naturally lacking the classical *P. syringae* *hrp/hrc* locus are common leaf colonizers equipped with an atypical type III secretion system. *Mol Plant Microbe Interact* 23:198–210. <http://dx.doi.org/10.1094/MPMI-23-2-0198>.
13. Jacques MA, Bolot S, Charbit E, Darrasse A, Briand M, Arlat M, Gagnevin L, Koebnik R, Noël LD, Portier P, Carrère S, Boureau T. 2013. High-quality draft genome sequence of *Xanthomonas alfalfae* subsp. *alfalfae* strain CFBP 3836. *Genome Announc* 1(6):e01035-13. <http://dx.doi.org/10.1128/genomeA.01035-13>.