

Genome Sequence of *Pseudomonas brassicacearum* DF41

Peter C. Loewen,^a Jack Switala,^a W. G. Dilantha Fernando,^b Teri de Kievit^a

Department of Microbiology^a and Department of Plant Science,^b University of Manitoba, Winnipeg, Manitoba, Canada

***Pseudomonas brassicacearum* DF41, a Gram-negative soil bacterium, is able to suppress the fungal pathogen *Sclerotinia sclerotiorum* through a process known as biological control. Here, we present a 6.8-Mb assembly of its genome, which is the second fully assembled genome of a *P. brassicacearum* strain.**

Received 8 April 2014 Accepted 14 April 2014 Published 8 May 2014

Citation Loewen PC, Switala J, Fernando WGD, de Kievit T. 2014. Genome sequence of *Pseudomonas brassicacearum* DF41. *Genome Announc.* 2(3):e00390-14. doi:10.1128/genomeA.00390-14.

Copyright © 2014 Loewen et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 3.0 Unported license](http://creativecommons.org/licenses/by/3.0/).

Address correspondence to Teri de Kievit, Teresa.Dekievit@ad.umanitoba.ca.

Pseudomonas brassicacearum strain DF41 is a canola (*Brassica napus* L.) root isolate that exhibits excellent antifungal activity against *Sclerotinia sclerotiorum* (Lib.) de Bary in both greenhouse and field trials (1, 2). The fungal pathogen *S. sclerotiorum* is not only versatile but is economically important due to its ability to infect >400 plant species (3). Canola, an edible oilseed crop with low saturates and high protein in the meal, belongs to the family *Brassicaceae* (*Cruciferae*). In canola, *S. sclerotiorum* causes stem rot, resulting in up to 100% yield loss under conducive conditions. Strain DF41 produces a number of compounds that are believed to contribute to biological control, including protease, hydrogen cyanide, and a novel lipopeptide called sclerosin (2, 4). Sclerosin is essential for antifungal activity, as a sclerosin-deficient mutant, DF41-1278, showed dramatically reduced fungal inhibition (2). The expression of antifungal metabolites in DF41 is governed by a complex regulatory cascade that includes the GacS-GacA two-component regulatory system (2), the PdfRI quorum-sensing (QS) system (5), a QS-associated regulator called RfiA (5), and the stringent response (6).

The genome of *P. brassicacearum* DF41 was sequenced in two stages. The first stage employed data generated using an Illumina MiSeq platform, which were assembled into 298 contigs using a combination of MIRA Assembler version 3.9.3 (7), Velvet version 1.2.08 (8), and the MUMmer version 3.23 (9) package. The second stage to complete the genome utilized a Pacific Biosciences data set generated by Genome Québec, which was assembled using the PacBio SMRT Analysis pipeline version 2.0.1, with 222× coverage to give a single contiguous genome sequence. The contigs from the Illumina data were aligned for confirmation. The sequence was annotated by the National Center for Biotechnology Information (NCBI) Prokaryotic Genomes Annotation Pipeline.

Our previous attempts to assign a species eponym to DF41 had been unsuccessful, but finding the *cpn60* gene in DF41 to be >97% identical to that of *P. brassicacearum* allowed us to designate DF41 as a member of this species. The *P. brassicacearum* DF41 genome consists of 6,652,396 bases, with a G+C content of 60.5%. There are 5,574 putative coding sequences, 65 tRNA genes, and 5 rRNA clusters. In addition, the biosynthetic loci for lipopeptide molecules, hydrogen cyanide, and alkaline protease have been identified, consistent with the exoproducts secreted by this

bacterium. A comparison of the genome with the only other completed *P. brassicacearum* genome, that of strain NFM421 (accession no. CP002585.1) (10), using Mauve 2.3.1 (11), revealed only 72% identity and the presence of a very large inversion of almost 2.5 Mb.

Nucleotide sequence accession number. The genome sequence of *P. brassicacearum* DF41 was deposited with NCBI GenBank under the accession no. [CP007410.1](http://www.ncbi.nlm.nih.gov/GenBank/CP007410.1).

ACKNOWLEDGMENTS

This work was supported by grants (DG9600 to P.C.L. and DG249559 to T.D.K.) from the Natural Sciences and Engineering Research Council (NSERC) and by the Canadian Research Chairs (CRC). We acknowledge the strong support of the Manitoba Institute of Child Health and CancerCare Manitoba Foundation. The Manitoba next-generation sequencing platform was supported by funds from the Canadian Foundation for Innovation, Province of Manitoba, University of Manitoba Faculty of Medicine, Manitoba Health Research Council, CancerCare Manitoba Foundation, Manitoba Institute of Child Health, and Manitoba Institute of Cell Biology.

REFERENCES

1. Savchuk S, Dilantha Fernando WG. 2004. Effect of timing of application and population dynamics on the degree of biological control of *Sclerotinia sclerotiorum* by bacterial antagonists. *FEMS Microbiol. Ecol.* 49:379–388. <http://dx.doi.org/10.1016/j.femsec.2004.04.014>.
2. Berry C, Fernando WGD, Loewen PC, de Kievit TR. 2010. Lipopeptides are essential for *Pseudomonas* sp. DF41 biocontrol of *Sclerotinia sclerotiorum*. *Biol. Control* 55:211–218. <http://dx.doi.org/10.1016/j.biocontrol.2010.09.011>.
3. Purdy LH. 1979. *Sclerotinia sclerotiorum*: history, disease and symptomatology, host range, geographic distribution and impact. *Phytopathology* 69:875–880. <http://dx.doi.org/10.1094/Phyto-69-875>.
4. Berry CL, Brassinga AK, Donald LJ, Fernando WG, Loewen PC, de Kievit TR. 2012. Chemical and biological characterization of sclerosin, an antifungal lipopeptide. *Can. J. Microbiol.* 58:1027–1034. <http://dx.doi.org/10.1139/w2012-079>.
5. Berry C, Nandi M, Manuel J, Brassinga AKC, Fernando WGD, Loewen PC, de Kievit TR. 2014. Characterization of the *Pseudomonas* sp. DF41 quorum sensing locus and its role in fungal antagonism. *Biol. Control* 69:82–89. <http://dx.doi.org/10.1016/j.biocontrol.2013.11.005>.
6. Manuel J, Berry C, Selin C, Fernando WG, de Kievit TR. 2011. Repression of the antifungal activity of *Pseudomonas* sp. strain DF41 by the stringent response. *Appl. Environ. Microbiol.* 77:5635–5642. <http://dx.doi.org/10.1128/AEM.02875-10>.
7. Chevreur B, Pfisterer T, Drescher B, Driesel AJ, Müller WE, Wetter T, Suhai S. 2004. Using the miraEST assembler for reliable and automated

- mRNA transcript assembly and SNP detection in sequenced ESTs. *Genome Res.* 14:1147–1159. <http://dx.doi.org/10.1101/gr.1917404>.
8. Zervino 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>.
 9. Kurtz S, Phillippy A, Delcher AL, Smoot M, Shumway M, Antonescu C, Salzberg SL. 2004. Versatile and open software for comparing large genomes. *Genome Biol.* 5:R12. <http://dx.doi.org/10.1186/gb-2004-5-6-p12>.
 10. Ortet P, Barakat M, Lalaouna D, Fochesato S, Barbe V, Vacherie B, Santaella C, Heulin T, Achouak W. 2011. Complete genome sequence of a beneficial plant root-associated bacterium, *Pseudomonas brassicacearum*. *J. Bacteriol.* 193:3146–3147. <http://dx.doi.org/10.1128/JB.00411-11>.
 11. Darling AE, Mau B, Perna NT. 2010. progressiveMauve: multiple genome alignment with gene gain, loss and rearrangement. *PLoS One* 5:e11147. <http://dx.doi.org/10.1371/journal.pone.0011147>.