

Draft Genome Sequence of the Bacteriocin-Producing *Bradyrhizobium japonicum* Strain FN1

MacLean G. Kohlmeier,^a Harry Yudistira,^a Xiang Li Zhang,^b Brian Fristensky,^b David B. Levin,^c Richard Sparling,^a Ivan J. Oresnik^a

Department of Microbiology, University of Manitoba, Winnipeg, Manitoba, Canada^a; Department of Plant Science, University of Manitoba, Winnipeg, Manitoba, Canada^b; Biosystems Engineering, University of Manitoba, Winnipeg, Manitoba, Canada^c

***Bradyrhizobium japonicum* strain FN1 was found to produce bacteriocin-like zones of clearing when tested against other strains of bradyrhizobia. The genome was sequenced, and several putative bacteriocin-producing genes, in addition to the expected genes involved in nodulation and nitrogen fixation, were identified.**

Received 17 June 2015 Accepted 24 June 2015 Published 30 July 2015

Citation Kohlmeier MG, Yudistira H, Zhang XL, Fristensky B, Levin DB, Sparling R, Oresnik IJ. 2015. Draft genome sequence of the bacteriocin-producing *Bradyrhizobium japonicum* strain FN1. *Genome Announc* 3(4):e00812-15. doi:10.1128/genomeA.00812-15.

Copyright © 2015 Kohlmeier et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 3.0 Unported license](https://creativecommons.org/licenses/by/3.0/).

Address correspondence to Ivan J. Oresnik, ivan.oresnik@umanitoba.ca.

Bradyrhizobium japonicum is a Gram-negative alphaproteobacterium that is capable of existing in either a free-living or a symbiotic state as a bacteroid in plant-derived organs, termed nodules, that develop on the root surface of the host organism *Glycine max*. Within nodules the bacteria reduce atmospheric nitrogen into an organic form that can be used by the plant for growth. For this reason, inocula of *B. japonicum* are commonly used in agriculture to increase crop yield. Rhizobial inoculums are often outcompeted for nodule occupancy by native strains present in the soil (1). Therefore, it is of interest to study factors with the potential to enhance the competitiveness of *B. japonicum* for nodule occupancy. Such factors include bacteriocins, which can be defined as narrow spectrum antibiotics produced by certain bacteria that are only effective against closely related strains (2). Bacteriocins have been shown to play a role in determining nodule occupancy in *Rhizobium leguminosarum* (3, 4).

Bacteriocins have been previously identified in *B. japonicum* (5), and we have isolated a bacteriocin-producing strain of *B. japonicum*, termed strain FN1, from Manitoba soils. In an effort to identify the genes responsible for bacteriocin production, the entire genome of FN1 was sequenced and annotated for further investigation.

The genome of *B. japonicum* strain FN1 was sequenced by the Next Generation Sequencing platform at the Manitoba Institute of Child Health using Illumina MiSeq technologies. Two successful runs both yielded 8,402,786 sequences, all of which were paired 150-bp reads with an average library insert size of 957 bp. Data output was assembled via Optimized-Velvet (6) into two formats; one consisting of 141 contigs and the other further organized into 87 scaffolds. Both data sets were submitted to the Joint Genome Institute's (JGI) Integrated Microbial Genomes-Expert Review (IMG ER) platform (7) for annotation.

The genome consists of 9,138,496 bp, with a GC count of 64%, and has 8,613 coding sequences. It contains a symbiosis island, housing the nodulation and nitrogen fixation genes. Genes encoding the enzymes involved in the Calvin-Benson-Bassham cycle, as well as genes associated with hydrogen uptake, were also detected.

This suggests that FN1 is capable of chemolithoautotrophic growth using H₂ as an electron donor and CO₂ as a source of carbon. In addition, characteristic genes of the Embden-Meyerhoff-Parnas pathway, the Entner-Doudoroff pathway, the pentose phosphate pathway, as well those involved in polyhydroxy alkanolate production, were present. These features are consistent with the published genomes of other *B. japonicum* strains (8–11).

To identify bacteriocin genes the genome was searched with BLAST (12) using the amino acid sequence of RTX-like toxins from *R. leguminosarum* as a query. Subsequent analysis was conducted with two Web-based software tools: antibiotics and Secondary Metabolite Analysis Shell (antiSMASH) (13) and BAGEL (14). These tools identified several putative bacteriocin-producing genes within the genome of FN1. Future work will involve mutating these genes to determine which of them confer the assayed bacteriocin activity.

Nucleotide sequence accession numbers. This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession number [JGCL000000000](https://www.ncbi.nlm.nih.gov/nuccore/JGCL000000000). The version described in this paper is the first version, [JGCL010000000](https://www.ncbi.nlm.nih.gov/nuccore/JGCL010000000).

ACKNOWLEDGMENTS

This work was supported by grants to I.J.O. from the Manitoba Pulse and Soybean Growers Association and the Agri-Food Research and Development Initiative.

REFERENCES

1. Triplett EW, Sadowsky MJ. 1992. Genetics of competition for nodulation of legumes. *Annu Rev Microbiol* 46:399–422. <http://dx.doi.org/10.1146/annurev.mi.46.100192.002151>.
2. Tagg JR, Dajani AS, Wannamaker LW. 1976. Bacteriocins of Gram-positive bacteria. *Bacteriol Rev* 40:722–756.
3. Oresnik IJ, Twelker S, Hynes MF. 1999. Cloning and characterization of a *Rhizobium leguminosarum* gene encoding a bacteriocin with similarities to RTX toxins. *Appl Environ Microbiol* 65:2833–2840.
4. Venter AP, Twelker S, Oresnik IJ, Hynes MF. 2001. Analysis of the genetic region encoding a novel rhizobiocin from *Rhizobium leguminosarum*.

- sarum* bv. *viciae* strain 306. *Can J Microbiol* 47:495–502. <http://dx.doi.org/10.1139/w01-043>.
5. Gross DC, Vidaver AK. 1978. Bacteriocin-like substances produced by *Rhizobium japonicum* and other slow-grow rhizobia. *Appl Environ Microbiol* 36:936–943.
 6. 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>.
 7. Markowitz VM, Mavromatis K, Ivanova NN, Chen IM, Chu K, Kyrpides NC. 2009. IMG ER: a system for microbial genome annotation expert review and curation. *Bioinformatics* 25:2271–2278. <http://dx.doi.org/10.1093/bioinformatics/btp393>.
 8. Sullivan JT, Ronson CW. 1998. Evolution of rhizobia by acquisition of a 500-kb symbiosis island that integrates into a phe-tRNA gene. *Proc Natl Acad Sci U S A* 95:5145–5149. <http://dx.doi.org/10.1073/pnas.95.9.5145>.
 9. Hanus FJ, Maier RJ, Evans HJ. 1979. Autotrophic growth of H₂-uptake-positive strains of *Rhizobium japonicum* in an atmosphere supplied with hydrogen gas. *Proc Natl Acad Sci U S A* 76:1788–1792. <http://dx.doi.org/10.1073/pnas.76.4.1788>.
 10. Kaneko T, Nakamura Y, Sato S, Minamisawa K, Uchiumi T, Sasamoto S, Watanabe A, Idesawa K, Iriguchi M, Kawashima K, Kohara M, Matsumoto M, Shimpo S, Tsuruoka H, Wada T, Yamada M, Tabata S. 2002. Complete genomic sequence of nitrogen-fixing symbiotic bacterium *Bradyrhizobium japonicum* USDA110. *DNA Res* 9:189–197. <http://dx.doi.org/10.1093/dnares/9.6.189>.
 11. Sarma AD, Emerich DW. 2006. A comparative proteomic evaluation of culture grown vs nodule isolated *Bradyrhizobium japonicum*. *Proteomics* 6:3008–3028. <http://dx.doi.org/10.1002/pmic.200500783>.
 12. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. *J Mol Biol* 215:403–410. [http://dx.doi.org/10.1016/S0022-2836\(05\)80360-2](http://dx.doi.org/10.1016/S0022-2836(05)80360-2).
 13. Blin K, Medema MH, Kazempour D, Fischbach MA, Breitling R, Takano E, Weber T. 2013. antiSMASH 2.0—a versatile platform for genome mining of secondary metabolite producers. *Nucleic Acids Res* 41:W204–W212. <http://dx.doi.org/10.1093/nar/gkt449>.
 14. De Jong A, van Heel AJ, Kok J, Kuipers OP. 2010. BAGEL2: mining for bacteriocins in genomic data. *Nucleic Acids Res* 38:W647–W651. <http://dx.doi.org/10.1093/nar/gkq365>.