



Complete Genome Sequence of *Bradyrhizobium japonicum* J5, Isolated from a Soybean Nodule in Hokkaido, Japan

Kazuma Kanehara, Kiwamu Minamisawa

Graduate School of Life Sciences, Tohoku University, Sendai, Japan

ABSTRACT Soybean bradyrhizobia form root nodules on soybean plants and symbiotically fix N_2 . Strain J5 is phylogenetically far from well-known representatives within the *Bradyrhizobium japonicum* lineage. The complete genome showed the largest single chromosomal (10.1 Mb) and symbiosis island (998 kb) among complete genomes of soybean bradyrhizobia.

Soybean bradyrhizobia are symbiotic nitrogen-fixing bacteria with soybean plants (1) and are composed of two major species, *Bradyrhizobium diazoefficiens* and *B. japonicum*, in temperate areas (2). The complete genomes of *B. diazoefficiens* USDA110 (3), *B. diazoefficiens* NK6 (4), *B. japonicum* USDA6 (5), and *B. japonicum* E109 (6) revealed their dynamic structures, including a symbiosis island and insertion sequence-mediated rearrangements (4–7). Strain J5 is phylogenetically far from USDA6 and E109 within the diverse *B. japonicum* lineage. Thus, we determined the complete genome sequence of *B. japonicum* J5.

Strain J5 was isolated by Toshikazu Takahashi (Tokachi Federation of Agricultural Cooperative Associations) from the nodule of a soybean plant grown in Shibetsu City in Hokkaido, Japan, in 1998. The genome was sequenced using the PacBio RSII (Pacific Biosciences, Menlo Park, CA USA) and Illumina MiSeq (Illumina, San Diego, CA, USA) platforms. Genomic DNA libraries were prepared for PacBio sequencing using the SMRTbell template prep kit version 1.0, while Illumina libraries were prepared using the Nextera DNA sample preparation kit (Illumina). The resulting reads, generated from the PacBio RSII sequencing procedure, were assembled *de novo* using the Hierarchical Genome Assembly Process (HGAP3.0) approach (SMRT analysis version 2.3.0) with default parameters. The final draft assembly produced two contigs totaling 10.3 Mb in size and with an input read coverage of 28×. Raw reads generated from the Illumina MiSeq sequencing procedure were trimmed using CLC Genomics Workbench version 8.5.1 (CLC Bio, Aarhus, Denmark) with the following parameters: ambiguous limit, 2; quality limit, 0.05; number of 5' terminal nucleotides, 20; number of 3' terminal nucleotides, 5; and minimum number of nucleotides in reads, 70. Trimmed whole-genome shotgun reads were mapped to contigs to update them, with the following CLC Genomics Workbench parameters: mismatch cost, 2; insertion cost, 3; deletion cost, 3; length fraction, 0.5; similarity fraction, 0.8; and no masking. As a result, a single circular contig was assembled. The sequence was annotated using the NCBI Prokaryotic Genome Automatic Annotation Pipeline (8), and the result was manually inspected with respect to positions of start codons for predicted open reading frames using the Microbial Genome Annotation Pipeline (MiGAP; <http://www.migap.org>) and Genome-Matcher (9).

The J5 genome consisted of a single chromosome (10,138,651bp, 63.3% G+C) and 9,067 coding sequences (CDSs). The genome size and CDS numbers were the highest

Received 1 December 2016 Accepted 6 December 2016 Published 9 February 2017

Citation Kanehara K, Minamisawa K. 2017. Complete genome sequence of *Bradyrhizobium japonicum* J5, isolated from a soybean nodule in Hokkaido, Japan. Genome Announc 5:e01619-16. <https://doi.org/10.1128/genomeA.01619-16>.

Copyright © 2017 Kanehara and Minamisawa. 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 Kiwamu Minamisawa, kiwamu@ige.tohoku.ac.jp.

among complete genomes of soybean bradyrhizobia. The length of symbiosis island A (5) reached 998 kb, which is the largest symbiosis island in soybean bradyrhizobia (643 kb to 694 kb). Genome-to-genome distances (high-scoring segment pair length/total length) were calculated using GGDC version 2.1 (<http://ggdc.dsmz.de/distcalc2.php>) (10). The distance between *B. japonicum* strain J5 and strains USDA6 and E109 (0.0441 to 0.0439) was apparently larger than that between USDA6 and E109 (0.0003), indicating that J5 was far from the well-known representatives within the *B. japonicum* lineage in terms of genomics as well. As for denitrification genes relevant to global warming (11), the J5 genome lacked the *nos* gene cluster encoding N₂O reductase.

Accession number(s). The genome sequence of *B. japonicum* strain J5 has been deposited at the GenBank under the accession number [CP017637](https://www.ncbi.nlm.nih.gov/nuccore/CP017637).

ACKNOWLEDGMENTS

This work was supported by the Tokachi Federation of Agricultural Cooperative Associations. We thank Yoshiyuki Ohtsubo (Tohoku University) for the manual annotation by GenomeMatcher; Masato Mikuchi and Ken Arai (Tokachi Federation of Agricultural Cooperative Associations) for providing strain J5; and Takakazu Kaneko (Kyoto Sangyo University) for critically reading the manuscript.

REFERENCES

1. Shiina Y, Itakura M, Choi H, Saeki Y, Hayatsu M, Minamisawa K. 2014. Relationship between soil type and N₂O reductase genotype (*nosZ*) of indigenous soybean bradyrhizobia: *nosZ*-minus populations are dominant in andosols. *Microbes Environ* 29:420–426. <https://doi.org/10.1264/jsm2.ME14130>.
2. Delamuta JR, Ribeiro RA, Ormeño-Orrillo E, Melo IS, Martínez-Romero E, Hungria M. 2013. Polyphasic evidence supporting the reclassification of *Bradyrhizobium japonicum* group Ia strains as *Bradyrhizobium diazoefficiens* sp. nov. *Int J Syst Evol Microbiol* 63:3342–3351. <https://doi.org/10.1099/ijs.0.049130-0>.
3. Kaneko T, Nakamura Y, Sato S, Minamisawa K, Uchiyumi 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. <https://doi.org/10.1093/dnares/9.6.189>.
4. Iida T, Itakura M, Anda M, Sugawara M, Isawa T, Okubo T, Sato S, Chiba-Kakizaki K, Minamisawa K. 2015. Symbiosis island shuffling with abundant insertion sequences in the genomes of extra-slow-growing strains of soybean bradyrhizobia. *Appl Environ Microbiol* 81:4143–4154. <https://doi.org/10.1128/AEM.00741-15>.
5. Kaneko T, Maita H, Hirakawa H, Uchiike N, Minamisawa K, Watanabe A, Sato S. 2011. Complete genome sequence of the soybean symbiont *Bradyrhizobium japonicum* strain USDA6^T. *Genes* 2:763–787. <https://doi.org/10.3390/genes2040763>.
6. Torres D, Revale S, Obando M, Maroniche G, Paris G, Peticari A, Vazquez M, Wisniewski-Dyé F, Martínez-Abarca F, Cassán F. 2015. Genome sequence of *Bradyrhizobium japonicum* E109, one of the most agronomically used nitrogen-fixing rhizobacteria in Argentina. *Genome Announc* 3(1):e01566-14. <https://doi.org/10.1128/genomeA.01566-14>.
7. Siqueira AF, Ormeño-Orrillo E, Souza RC, Rodrigues EP, Almeida LGP, Barcellos FG, Batista JSS, Nakatani AS, Martínez-Romero E, Vasconcelos ATR, Hungria M. 2014. Comparative genomics of *Bradyrhizobium japonicum* CPAC 15 and *Bradyrhizobium diazoefficiens* CPAC 7: elite model strains for understanding symbiotic performance with soybean. *BMC Genomics* 15:420. <https://doi.org/10.1186/1471-2164-15-420>.
8. 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. *Omics* 12:137–141. <https://doi.org/10.1089/omi.2008.0017>.
9. Ohtsubo Y, Ikeda-Ohtsubo W, Nagata Y, Tsuda M. 2008. GenomeMatcher: a graphical user interface for DNA sequence comparison. *BMC Bioinformatics* 9:376. <https://doi.org/10.1186/1471-2105-9-376>.
10. Auch AF, von Jan M, Klenk HP, Göker M. 2010. Digital DNA-DNA hybridization for microbial species delineation by means of genome-to-genome sequence comparison. *Stand Genomic Sci* 2:117–134. <https://doi.org/10.4056/signs.531120>.
11. Itakura M, Uchida Y, Akiyama H, Takada-Hoshino Y, Shimomura Y, Morimoto S, Tago K, Wang Y, Hayakawa C, Uetake Y, Sánchez C, Eda S, Hayatsu M, Minamisawa K. 2013. Mitigation of nitrous oxide emissions from soils by *Bradyrhizobium japonicum* inoculation. *Nat Clim Change* 3:208–212. <https://doi.org/10.1038/nclimate1734>.