



Genome Sequence of the Soybean Cyst Nematode (*Heterodera glycines*) Endosymbiont “*Candidatus Cardinium hertigii*” Strain cHgTN10

Kurt C. Showmaker,^a Kimberly K. O. Walden,^b Christopher J. Fields,^b Kris N. Lambert,^a Matthew E. Hudson^a

^aDepartment of Crop Science, University of Illinois at Urbana-Champaign, Urbana, Illinois, USA

^bCarl R. Woese Institute for Genomic Biology, University of Illinois at Urbana-Champaign, Urbana, Illinois, USA

ABSTRACT In this study, we present the genome sequence of the “*Candidatus Cardinium hertigii*” strain cHgTN10, an endosymbiotic bacterium of the plant-parasitic nematode *Heterodera glycines*. This is the first genome assembly reported for an endosymbiont directly sequenced from a tylenchid nematode.

The soybean cyst nematode (SCN [*Heterodera glycines*]) causes more economic loss to soybean (*Glycine max*) than any other biotic pathogen of this important crop (1). Although an abundance of molecular studies have highlighted the nematode’s interaction with its primary host, soybean, little is known about the molecular interactions between the SCN and its *Cardinium* endosymbiont. Molecular cloning studies of the 16S RNA and *gyrB* genes within an SCN endosymbiont identified the bacterium as “*Candidatus Paenicardinium endonii*,” thereafter taxonomically reassigned to “*Candidatus Cardinium hertigii*” group B (2, 3).

DNA was extracted from surface-sterilized second-stage juveniles of *H. glycines* population TN10 (Hg type 0) cultured on the susceptible soybean cultivar Essex on soil in the greenhouse. Genomic DNA was extracted using a standard proteinase K protocol (4). PacBio RS II and Illumina TruSeq DNA libraries were prepared from the extracted DNA. The PacBio library was sequenced on 20 single-molecule real-time (SMRT) cells. The Illumina DNA library was sequenced as paired ends (2 × 260) on the Illumina HiSeq 2500 platform. The PacBio reads were assembled with the Canu (version 1.4) long-read assembler (5). The resultant metagenomic assembly was error corrected with Pilon (version 1.22) (6) using the Illumina reads aligned with the BWA-MEM (version 0.7.15) alignment algorithm (7). For the identification of potential endosymbiont contigs within the endosymbiont-nematode metagenome assembly, a BlobTools (version 0.9.19.6) (8) database was built with BWA-MEM alignments to the contigs, and the results from the contigs were aligned to the UniProt uniref100 database with the DIAMOND (version 0.9.10) (9) aligner, using the blastx parameter. Additionally, previously identified 16S RNA (GenBank accession no. DQ314214) and *gyrB* (GenBank accession no. DQ314215) genes for “*Ca. Cardinium hertigii*” were aligned with blastn, and *H. glycines* RNA sequencing (RNA-seq) reads from NCBI BioProject no. PRJNA415980 (10) were aligned with TopHat (version 2.1.1) (11). All contigs identified as bacterial by BlobTools were manually inspected for the presence of RNA splicing. A single 1.2-Mb contig was identified as the “*Ca. Cardinium hertigii*” chromosome sequence based upon the following 4 supporting criteria: (i) had a predicted circular link to itself, (ii) was taxonomically identified as bacterial, (iii) contained the “*Ca. Cardinium hertigii*” *gyrB* and 16S RNA alignments, and (iv) did not show evidence of RNA splicing. This contig was circularized using Circlator (version 1.5.1) (12) and subsequently error corrected using 2 passes through Quiver and 2 additional passes through Pilon. The resultant 1.2-Mb genome assembly is reported here.

Received 30 May 2018 Accepted 2 June 2018 Published 28 June 2018

Citation Showmaker KC, Walden KKO, Fields CJ, Lambert KN, Hudson ME. 2018. Genome sequence of the soybean cyst nematode (*Heterodera glycines*) endosymbiont “*Candidatus Cardinium hertigii*” strain cHgTN10. *Genome Announc* 6:e00624-18. <https://doi.org/10.1128/genomeA.00624-18>.

Copyright © 2018 Showmaker 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 Kurt C. Showmaker, kshowmaker@illinois.edu.

Gene prediction with Prokka (version 1.13) (13) resulted in 973 protein-coding genes and 37 tRNAs. BLASTP alignments of the predicted cHgTN10 proteins to the NCBI NR database (accessed 8 February 2018) resulted in 770 (79%) cHgTN10 predicted proteins with a bit score greater than 60, of which 594 (61%) proteins had a highest scoring blastp alignment to 1 of the 2 previously reported “*Ca. Cardinium hertigii*” strains, cEper1 and cBtQ1 (14, 15). Functional annotation with InterProScan (16) resulted in the identification of several proteins and protein domains similar to those of “*Ca. Cardinium hertigii*,” including the following: Ankyrin repeats, ABC transporters, permeases, a WH2 motif, and DEAD box helicase, as reported for cEper1 (14).

Accession number(s). This whole-genome shotgun project has been deposited in DDBJ/ENA/GenBank under the accession no. [CP029619](https://doi.org/10.1093/nar/gky111). The version described in this paper is the first version, CP029619.1.

ACKNOWLEDGMENT

This work was funded by the North Central Soybean Research Program.

REFERENCES

- Wrather A, Koenning S. 2009. Effects of diseases on soybean yields in the United States 1996 to 2007. *Plant Health Progress*. <https://doi.org/10.1094/php-2009-0401-01-rs>.
- Noel GR, Atibalentja N. 2006. “*Candidatus Paenicardinium endonii*”, an endosymbiont of the plant-parasitic nematode *Heterodera glycines* (Nemata: Tylenchida), affiliated to the phylum *Bacteroidetes*. *Int J Syst Evol Microbiol* 56:1697–1702. <https://doi.org/10.1099/ijs.0.64234-0>.
- Nakamura Y, Kawai S, Yukuhiro F, Ito S, Gotoh T, Kisimoto R, Yanase T, Matsumoto Y, Kageyama D, Noda H. 2009. Prevalence of *Cardinium* bacteria in planthoppers and spider mites and taxonomic revision of “*Candidatus Cardinium hertigii*” based on detection of a new *Cardinium* group from biting midges. *Appl Environ Microbiol* 75:6757–6763. <https://doi.org/10.1128/AEM.01583-09>.
- Bekal S, Domier LL, Gonfa B, McCoppin NK, Lambert KN, Bhalerao K. 2014. A novel flavivirus in the soybean cyst nematode. *J Gen Virol* 95:1272–1280. <https://doi.org/10.1099/vir.0.060889-0>.
- Koren S, Walenz BP, Berlin K, Miller JR, Bergman NH, Phillippy AM. 2017. Canu: scalable and accurate long-read assembly via adaptive *k*-mer weighting and repeat separation. *Genome Res* 27:722–736. <https://doi.org/10.1101/gr.215087.116>.
- Walker BJ, Abeel T, Shea T, Priest M, Abouelliel A, Sakthikumar S, Cuomo CA, Zeng Q, Wortman J, Young SK, Earl AM. 2014. Pilon: an integrated tool for comprehensive microbial variant detection and genome assembly improvement. *PLoS One* 9:e112963. <https://doi.org/10.1371/journal.pone.0112963>.
- Li H. 2013. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. *arXiv arXiv:1303.3997 [q-bio.GN]*. <https://arxiv.org/abs/1303.3997>.
- Laetsch DR, Blaxter ML. 2017. BlobTools: interrogation of genome assemblies. *F1000Res* 6:1287. <https://doi.org/10.12688/f1000research.12232.1>.
- Buchfink B, Xie C, Huson DH. 2015. Fast and sensitive protein alignment using DIAMOND. *Nat Methods* 12:59–60. <https://doi.org/10.1038/nmeth.3176>.
- Gardner M, Dhroso A, Johnson N, Davis EL, Baum TJ, Korkin D, Mitchum MG. 2018. Novel global effector mining from the transcriptome of early life stages of the soybean cyst nematode *Heterodera glycines*. *Sci Rep* 8:2505. <https://doi.org/10.1038/s41598-018-20536-5>.
- Kim D, Pertea G, Trapnell C, Pimentel H, Kelley R, Salzberg SL. 2013. TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. *Genome Biol* 14:R36. <https://doi.org/10.1186/gb-2013-14-4-r36>.
- Hunt M, De Silva N, Otto TD, Parkhill J, Keane JA, Harris SR. 2015. Circlator: automated circularization of genome assemblies using long sequencing reads. *Genome Biol* 16:294. <https://doi.org/10.1186/s13059-015-0849-0>.
- Seemann T. 2014. Prokka: rapid prokaryotic genome annotation. *Bioinformatics* 30:2068–2069. <https://doi.org/10.1093/bioinformatics/btu153>.
- Penz T, Schmitz-Esser S, Kelly SE, Cass BN, Muller A, Woyke T, Malfatti SA, Hunter MS, Horn M. 2012. Comparative genomics suggests an independent origin of cytoplasmic incompatibility in *Cardinium hertigii*. *PLoS Genet* 8:e1003012. <https://doi.org/10.1371/journal.pgen.1003012>.
- Santos-García D, Rollat-Farnier P-A, Beitia F, Zchori-Fein E, Vavre F, Mouton L, Moya A, Latorre A, Silva FJ. 2014. The genome of *Cardinium* cBtQ1 provides insights into genome reduction, symbiont motility, and its settlement in *Bemisia tabaci*. *Genome Biol Evol* 6:1013–1030. <https://doi.org/10.1093/gbe/evu077>.
- Quevillon E, Silventoinen V, Pillai S, Harte N, Mulder N, Apweiler R, Lopez R. 2005. InterProScan: protein domains identifier. *Nucleic Acids Res* 33:W116–W120. <https://doi.org/10.1093/nar/gki442>.