AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Resource Announcements

GENOME SEQUENCES

Complete Genome Sequence of *Mesorhizobium ciceri* Strain R30, a Rhizobium Used as a Commercial Inoculant for Chickpea in Argentina

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ABSTRACT We report the complete genome sequence of *Mesorhizobium ciceri* strain R30, a rhizobium strain recommended and used as a commercial inoculant for chickpea in Argentina. The genome consists of almost 7 Mb, distributed into two circular replicons: a chromosome of 6.49 Mb and a plasmid of 0.46 Mb.

As part of sustainable agriculture, legumes may be inoculated with rhizobia that develop nitrogen-fixing symbiosis with the crop and thus prevent nitrogen deficiency (1, 2). Chickpea (*Cicer arietinum* L.) is one of several legumes that establish symbiosis with rhizobia from the genus *Mesorhizobium* (3), more specifically with *Mesorhizobium ciceri*. In Argentina, the Instituto Nacional de Tecnología Agropecuaria (INTA) recommends inoculating chickpea with *M. ciceri* R30 (originally named *Rhizobium ciceri* USDA 3383, it was purchased in the late 1990s from USDA's Agricultural Research Service in Beltsville, MD, USA) (4).

To date, the complete genome sequences of three *M. ciceri* strains are available at NCBI, out of seven assemblies. Only strain CC1192 is symbiotically active with chickpea (5). Here, we announce the complete annotated genome sequence of *M. ciceri* R30.

A pure culture of the strain, provided by INTA, was aerobically grown in liquid yeast extract-mannitol medium at 30°C with 150 rpm rotation until it reached the late exponential growth phase (6). This was the source for the total DNA, obtained using a DNeasy blood and tissue kit (Qiagen) for Illumina sequencing and a Wizard high-molecular-weight (HMW) DNA extraction kit (Promega) for Oxford Nanopore Technologies sequencing. The genome was assembled through a hybrid approach including short Illumina reads and long Oxford Nanopore reads. Illumina sequencing was performed on the P2M platform at Institut Pasteur. The library was prepared using a Nextera XT DNA library preparation kit and sequenced on an Illumina NextSeq 500 instrument, with a paired-end (PE) 150-bp read configuration. Nanopore sequencing was performed at the Oxford Genomics Centre. The sample was processed using an Oxford Nanopore Technologies rapid barcoding sequencing kit (SQK-RBK004) and a native barcoding genomic DNA sequencing kit (SQK-LSK109 with EXP-NBD104). The products of each were sequenced in two Flongle flow cells. Data were base called using Guppy v4.2.2, with the high-accuracy model and the –trim_barcodes option. We obtained 6,189,520 Illumina PE reads predicting 132-fold coverage and 88,970 Nanopore long reads with an N_{50} value of

Editor Julie C. Dunning Hotopp, University of Maryland School of Medicine

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The authors declare no conflict of interest.

Received 28 July 2022
Accepted 5 October 2022

10,948 bp and an average of 5,324 bp, predicting 68-fold coverage. Hybrid genome assembly was performed on the raw reads using the nf-core/bacass pipeline (commit ceebac0) with default parameters (7). This resulted in two contigs that were closed by manually analyzing the overlapped ends using Geneious v2019 2.1 software (8). The reported genome consists of one chromosome (6,489,234 bp; G+C content, 62.7%) and a secondary replicon from the repABC plasmid family (457,705 bp; G+C content, 59.9%) (9). The first nucleotide was assigned at the beginning of the *dnaA* and *repA* genes for the chromosome and plasmid respectively, using the corresponding tool within Geneious. Average nucleotide identity analysis (10) revealed that the R30 chromosome is >98% identical to sequenced *Mesorhizobium ciceri* genomes available at NCBI, such as those for *M. ciceri* strain CC1192 (GenBank accession number CP015062.1) and *M. ciceri* bv. biserrulae strains WSM1271 and WSM1284 (CP002447.1 and CP015064.1, respectively), which is evidence of their close phylogenetic relationship.

The complete genome sequence, which was annotated using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (11–13), consists of 6,452 protein-coding sequences, 2 complete ribosomal operons, and 52 tRNAs. As in other mesorhizobia (14, 15), the genes for nodulation (*nod*) and nitrogen fixation (*nif* and *fix*) in R30 appear to be located on a chromosomic 401-kb symbiosis island (1,263,739 to 1,664,915 bp), flanked by direct repeat sequences identical to those in the ICE region in *M. ciceri* CC1192 and adjacent to one of four serine tRNA genes. This region also harbors biotin and nicotinate biosynthetic clusters, a conjugative type IV secretion system, and *luxI*- and *luxR*-like quorum-sensing genes probably associated with the island's excision and transfer (16).

This complete sequencing of the R30 genome could be crucial for more in-depth research into its symbiotic performance and other biological features.

Data availability. The complete genome sequence of *Mesorhizobium ciceri* R30 is available at NCBI GenBank under accession numbers CP088147 for the chromosome and CP088148 for the plasmid, with BioProject accession number PRJNA782313 and BioSample accession number SAMN23371988. The raw reads are available at NCBI's Sequence Read Archive under accession numbers SRR16992657, SRR16992658, SRR16992659, SRR16992660, and SRR16992661.

ACKNOWLEDGMENTS

This work was supported by grants from the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT) (PID2020-113207GBI00 funded by MCIN/AEI/10.13039/501100011033); by "ERDF: A Way of Making Europe" (P20_0047), funded by the Junta de Andalucía PAIDI/FEDER/EU; and by the Biotechnology and Biosciences Research Council (BBSRC). We are grateful to Plateforme de Microbiologie Mutualisée (P2M) and the Pasteur International Bioresources network (PIBnet) and to Institut Pasteur Paris for providing the resources for Illumina sequencing.

We thank O.G.C. at the Wellcome Centre for Human Genetics for the sequencing data and B.M.R.C. for processing (supported by Wellcome Trust Core Award grant 203141/Z/16/Z and the NIHR Oxford BRC). We are also grateful to Vincent Enouf from Unité de Génétique Moléculaire des Virus à ARN-UMR3569 CNRS, Université de Paris, Centre National de Référence Virus des Infections Respiratoires (dont la grippe) and to F. Sgarlatta for proofreading the manuscript.

The views expressed are those of the authors and not necessarily those of the NHS, the NIHR, or the Department of Health.

REFERENCES

1. Ferguson BJ, Mens C, Hastwell AH, Zhang M, Su H, Jones CH, Chu X, Gresshoff PM. 2019. Legume nodulation: the host controls the party. Plant Cell Environ 42:41–51. https://doi.org/10.1111/pce.13348.
2. Santos MS, Nogueira MA, Hungria M. 2019. Microbial inoculants: reviewing the past, discussing the present and previewing an outstanding future for the use of beneficial bacteria in agriculture. AMB Express 9:205. https://doi.org/10.1186/s13568-019-0932-0.
3. Laranjo M, Alexandre A, Oliveira S. 2014. Legume growth-promoting rhizobia: an overview on the *Mesorhizobium* genus. Microbiol Res 169:2–17. https://doi.org/10.1016/j.micres.2013.09.012.
4. Foresto E, Nievas F, Revale S, Giordano W, Bogino P. 2021. Deciphering the phylogenetic affiliation of rhizobial strains recommended as chickpea inoculants in Argentina. Appl Soil Ecol 166:104069–104104. https://doi.org/10.1016/j.apsoil.2021.104069.
5. Haskett T, Wang P, Ramsay J, O'Hara G, Reeve W, Howieson J, Terpolilli J. 2016. Complete genome sequence of *Mesorhizobium ciceri* strain CC1192, an efficient nitrogen-fixing microsymbiont of *Cicer arietinum*. Genome Announc 4:e00516-16. https://doi.org/10.1128/genomeA.00516-16.
6. Somasegaran PH, Hoben HJ. 1994. Appendix 3: bacterial growth media and plant nutrient solutions, p 333–341. *In* Handbook for rhizobia: methods in

legume-Rhizobium technology. Springer, New York, NY. https://doi.org/10.1007/978-1-4613-8375-8.
7. Ewels PA, Peltzer A, Fillinger S, Patel H, Alneberg J, Wilm A, Garcia MU, Di Tommaso P, Nahnsen S. 2020. The nf-core framework for community-curated bioinformatics pipelines. Nat Biotechnol 38:276–278. https://doi.org/10.1038/s41587-020-0439-x.
8. Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28:1647–1649. https://doi.org/10.1093/bioinformatics/bts199.
9. MacLellan SR, Zaheer R, Sartor AL, MacLean AM, Finan TM. 2006. Identification of a megaplasmid centromere reveals genetic structural diversity within the repABC family of basic replicons. Mol Microbiol 59:1559–1575. https://doi.org/10.1111/j.1365-2958.2006.05040.x.
10. Ciufo S, Kannan S, Sharma S, Badretdin A, Clark K, Turner S, Brover S, Schoch CL, Kimchi A, DiCuccio M. 2018. Using average nucleotide identity to improve taxonomic assignments in prokaryotic genomes at the NCBI. Int J Syst Evol Microbiol 68:2386–2392. https://doi.org/10.1099/ijsem.0.002809.
11. Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J. 2016. NCBI Prokaryotic Genome Annotation Pipeline. Nucleic Acids Res 44:6614–6624. https://doi.org/10.1093/nar/gkw569.
12. Haft DH, DiCuccio M, Badretdin A, Brover V, Chetvernin V, O'Neill K, Li W, Chitsaz F, Derbyshire MK, Gonzales NR, Gwadz M, Lu F, Marchler GH, Song JS, Thanki N, Yamashita RA, Zheng C, Thibaud-Nissen F, Geer LY, Marchler-Bauer A, Pruitt KD. 2018. RefSeq: an update on prokaryotic genome annotation and curation. Nucleic Acids Res 46:D851–D860. https://doi.org/10.1093/nar/gkx1068.
13. Li W, O'Neill KR, Haft DH, DiCuccio M, Chetvernin V, Badretdin A, Coulouris G, Chitsaz F, Derbyshire MK, Durkin AS, Gonzales NR, Gwadz M, Lanczycki CJ, Song JS, Thanki N, Wang J, Yamashita RA, Yang M, Zheng C, Marchler-Bauer A, Thibaud-Nissen F. 2021. RefSeq: expanding the Prokaryotic Genome Annotation Pipeline reach with protein family model curation. Nucleic Acids Res 49:D1020–D1028. https://doi.org/10.1093/nar/gkaa1105.
14. Sullivan JT, Trzebiatowski JR, Cruickshank RW, Gouzy J, Brown SD, Elliot RM, Fleetwood DJ, McCallum NG, Rossbach U, Stuart GS, Weaver JE, Webby RJ, De Bruijn FJ, Ronson CW. 2002. Comparative sequence analysis of the symbiosis island of *Mesorhizobium loti* strain R7A. J Bacteriol 184:3086–3095. https://doi.org/10.1128/JB.184.11.3086-3095.2002.
15. Haskett TL, Terpolilli JJ, Bekuma A, O'Hara GW, Sullivan JT, Wang P, Ronson CW, Ramsay JP. 2016. Assembly and transfer of tripartite integrative and conjugative genetic elements. Proc Natl Acad Sci U S A 113:12268–12273. https://doi.org/10.1073/pnas.1613358113.
16. Ramsay JP, Major AS, Komarovsky VM, Sullivan JT, Dy RL, Hynes MF, Salmond GP, Ronson CW. 2013. A widely conserved molecular switch controls quorum sensing and symbiosis island transfer in *Mesorhizobium loti* through expression of a novel antiactivator. Mol Microbiol 87:1–13. https://doi.org/10.1111/mmi.12079.