



Genome Sequence of the Symbiotic Type Strain *Rhizobium tibeticum* CCBAU85039^T

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ABSTRACT *Rhizobium tibeticum* was originally isolated from root nodules of *Trigonella archiducis-nicolai* grown in Tibet, China. This species is also able to nodulate *Medicago sativa* and *Phaseolus vulgaris*. The whole-genome sequence of the type strain, *R. tibeticum* CCBAU85039^T, is reported in this study.

Availability of nitrogen in soils is one of the main concerns for crop cultivation in agriculture. Nitrogen is essential for the biosynthesis of proteins, amino acids, vitamins, and other compounds. Chemical fertilizers commonly ensure sufficient nitrogen availability for crop production. However, these compounds may also cause environmental problems (1, 2). Alternatively, certain bacteria and archaea are able to fix atmospheric dinitrogen via reduction to ammonia. Rhizobia belonging to the classes *Alphaproteobacteria* or *Betaproteobacteria* inhabit soils and are able to enter into nitrogen-fixing symbiosis with leguminous plants. They induce the development of root nodules, where, after colonization and differentiation, bacteroids are able to fix dinitrogen (3). Rhizobia are highly diverse regarding their genetic, metabolic, and taxonomic characteristics (4). *Mesorhizobium loti* MAFF303099 (5) and *Ensifer melliloti* 1021 (6) were the first rhizobia for which complete genome sequences became available. Recently, the genome sequences of 163 further root-nodule bacteria were published, extending our knowledge regarding this group of bacteria (7). However, particular and important species/groups were not covered by the study cited above. An important clade within the group of rhizobia comprises the strains *Rhizobium meso-americanum* CCG502^T (8), *Rhizobium grahamii* CCG501^T (9), *Rhizobium favelukesii* LPU83^T (10), and *Rhizobium tibeticum* CCBAU85039^T (11). While genome sequence information is available for the first three strains, the genome of *R. tibeticum* CCBAU85039^T had not yet been sequenced. The latter strain is able to nodulate *Phaseolus vulgaris*, *Medicago lupulina*, *Medicago sativa*, *Trigonella archiducis-nicolai*, and *Trigonella foenum-graecum*. With the objective to uncover mechanisms of rhizobial diversification and to complement genome sequence information for rhizobial species, the *R. tibeticum* CCBAU85039^T genome was sequenced.

Genomic DNA of *R. tibeticum* CCBAU85039^T was isolated using the GENTRA PureGene kit (Qiagen). A sequencing library was constructed and sequenced on the MiSeq platform applying the Illumina paired-end protocol (Illumina, Inc.). In total, 4,090,570 sequence reads were obtained, yielding a total of 1,102,161,679 bp of sequence information. The Illumina reads were assembled by the GS *de novo* assembler software

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(gsAssembler, version 2.8; Roche) with a final outcome of 206 large (>500 bp) contigs. Of these, 167 contigs were arranged in 128 scaffolds. The estimated genome size is around 7 Mb, and, accordingly, the coverage obtained was approximately 159-fold. The genome features an average G+C content of 59.72%. The N_{50} value for scaffolds was 177,123 bp with an average scaffold size of 54,157 bp.

The genome was annotated applying the Prokka pipeline and GenDB (12, 13), which predicted 6,977 protein-coding sequences (CDSs) and 45 tRNA genes. The rRNA operon was found on a 4-fold overrepresented contig, suggesting the presence of four *rRNA* copies within the genome. Genome comparisons were done within the EDGAR version 2.0 platform (14). More than 5,200 CDSs of *R. tibeticum* (75% of all CDSs) represent orthologs to corresponding *R. favelukesii* LPU83^T genes (15). Moreover, phylogenetic analysis of the concatenated core genomes confirmed a close relationship between both strains, as previously described (16). Further comparative studies will elucidate the similarities and differences among different groups of sequenced rhizobial strains and refine their taxonomic classification.

Accession number(s). This whole-genome shotgun project has been deposited in the EMBL database under the accession numbers [FNXB01000001](https://www.ebi.ac.uk/ena/record/FNXXB01000001) to [FNXB01000167](https://www.ebi.ac.uk/ena/record/FNXXB01000167). The version described in this paper is the first version, FNXB01000000.

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REFERENCES

- Smith DR, Owens PR, Leytem AB, Warnemuende EA. 2007. Nutrient losses from manure and fertilizer applications as impacted by time to first runoff event. *Environ Pollut* 147:131–137. <https://doi.org/10.1016/j.envpol.2006.08.021>.
- Mosier AR. 2001. Exchange of gaseous nitrogen compounds between agricultural systems and the atmosphere. *Plant Soil* 228:17–27. <https://doi.org/10.1023/A:1004821205442>.
- Jones KM, Kobayashi H, Davies BW, Taga ME, Walker GC. 2007. How rhizobial symbionts invade plants: the *Sinorhizobium-Medicago* model. *Nat Rev Microbiol* 5:619–633. <https://doi.org/10.1038/nrmicro1705>.
- Masson-Boivin C, Giraud E, Perret X, Batut J. 2009. Establishing nitrogen-fixing symbiosis with legumes: how many rhizobium recipes? *Trends Microbiol* 17:458–466. <https://doi.org/10.1016/j.tim.2009.07.004>.
- Kaneko T, Nakamura Y, Sato S, Asamizu E, Kato T, Sasamoto S, Watanabe A, Idesawa K, Ishikawa A, Kawashima K, Kimura T, Kishida Y, Kiyokawa C, Kohara M, Matsumoto M, Matsuno A, Mochizuki Y, Nakayama S, Nakazaki N, Shimpo S, Sugimoto M, Takeuchi C, Yamada M, Tabata S. 2000. Complete genome structure of the nitrogen-fixing symbiotic bacterium *Mesorhizobium loti*. *DNA Res* 7:331–338. <https://doi.org/10.1093/dnares/7.6.331>.
- Galibert F, Finan TM, Long SR, Puhler A, Abola P, Ampe F, Barloy-Hubler F, Barnett MJ, Becker A, Boistard P, Bothe G, Boutry M, Bowser L, Buhmester J, Cadieu E, Capela D, Chain P, Cowie A, Davis RW, Dreano S, Federspiel NA, Fisher RF, Gloux S, Godrie T, Goffeau A, Golding B, Gouzy J, Gurjal M, Hernandez-Lucas I, Hong A, Huizar L, Hyman RW, Jones T, Kahn D, Kahn ML, Kalman S, Keating DH, Kiss E, Komp C, Lelaure V, Masuy D, Palm C, Peck MC, Pohl TM, Portetelle D, Purnelle B, Ramsperger U, Surzycki R, Thebault P, Vandenbol M. 2001. The composite genome of the legume symbiont *Sinorhizobium meliloti*. *Science* 293:668–672. <https://doi.org/10.1126/science.1060966>.
- Seshadri R, Reeve WG, Ardley JK, Tennesen K, Woyke T, Kyrpidis NC, Ivanova NN. 2015. Discovery of novel plant interaction determinants from the genomes of 163 root nodule bacteria. *Sci Rep* 5:16825. <https://doi.org/10.1038/srep16825>.
- López-López A, Rogel-Hernández MA, Barois I, Ortiz Ceballos AI, Martínez J, Ormeño-Orrillo E, Martínez-Romero E. 2012. *Rhizobium grahamii* sp. nov., from nodules of *Dalea leporina*, *Leucaena leucocephala* and *Clitoria ternatea*, and *Rhizobium mesoamericanum* sp. nov., from nodules of *Phaseolus vulgaris*, siratro, cowpea and *Mimosa pudica*. *Int J Syst Evol Microbiol* 62:2264–2271. <https://doi.org/10.1099/ijs.0.033555-0>.
- Althabegoiti MJ, Ormeño-Orrillo E, Lozano L, Torres Tejerizo G, Rogel MA, Mora J, Martínez-Romero E. 2014. Characterization of *Rhizobium grahamii* extrachromosomal replicons and their transfer among rhizobia. *BMC Microbiol* 14:6. <https://doi.org/10.1186/1471-2180-14-6>.
- Tejerizo GT, Del Papa MF, Draghi W, Lozano M, Giusti Mde L, Martini C, Salas ME, Salto I, Wibberg D, Szczepanowski R, Weidner S, Schlüter A, Lagares A, Pistorio M. 2011. First genomic analysis of the broad-host-range *Rhizobium* sp. LPU83 strain, a member of the low-genetic diversity Oregon-like *Rhizobium* sp. group. *J Biotechnol* 155:3–10. <https://doi.org/10.1016/j.jbiotec.2011.01.011>.
- Hou BC, Wang ET, Li Y, Jr, Jia RZ, Chen WF, Gao Y, Dong RJ, Chen WX. 2009. *Rhizobium tibeticum* sp. nov., a symbiotic bacterium isolated from *Trigonella archiducis-nicolai* (Sirj.) Vassilcz. *Int J Syst Evol Microbiol* 59:3051–3057. <https://doi.org/10.1099/ijs.0.009647-0>.
- Seemann T. 2014. Prokka: rapid prokaryotic genome annotation. *Bioinformatics* 30:2068–2069. <https://doi.org/10.1093/bioinformatics/btu153>.
- Meyer F, Goesmann A, McHardy AC, Bartels D, Bekel T, Clausen J, Kalinowski J, Linke B, Rupp O, Giegerich R, Pühler A. 2003. GenDB—an open source genome annotation system for prokaryote genomes. *Nucleic Acids Res* 31:2187–2195. <https://doi.org/10.1093/nar/gkg312>.
- Blom J, Kreis J, Spänig S, Juhre T, Bertelli C, Ernst C, Goesmann A. 2016. EDGAR 2.0: an enhanced software platform for comparative gene con-

- tent analyses. *Nucleic Acids Res* 44:W22–W28. <https://doi.org/10.1093/nar/gkw255>.
15. Wibberg D, Tejerizo GT, Del Papa MF, Martini C, Pühler A, Lagares A, Schlüter A, Pistorio M. 2014. Genome sequence of the acid-tolerant strain *Rhizobium* sp. LPU83. *J Biotechnol* 176:40–41. <https://doi.org/10.1016/j.jbiotec.2014.02.008>.
 16. Torres Tejerizo G, Rogel MA, Ormeño-Orrillo E, Althabegoiti MJ, Nilsson JF, Niehaus K, Schlüter A, Pühler A, Del Papa MF, Lagares A, Martínez-Romero E, Pistorio M. 2016. *Rhizobium favelukesii* sp. nov., isolated from the root nodules of alfalfa (*Medicago sativa* L). *Int J Syst Evol Microbiol* [Epub ahead of print.] <https://doi.org/10.1099/ijsem.0.001373>.