



Genome Sequence of *Azospirillum brasilense* REC3, Isolated from Strawberry Plants

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ABSTRACT The genome sequence of a plant growth-promoting bacterium and biocontrol agent, *Azospirillum brasilense* REC3, isolated from strawberry roots, is reported here. The *A. brasilense* REC3 total genome contains 7,229,924 bp and has a G+C content of 68.7 mol%.

Azospirillum brasilense REC3 was isolated from inner-root tissue samples of strawberry plants cultivated in Tucumán, Argentina (1). This strain exhibits the main characteristics that define plant growth-promoting bacteria, namely, nitrogen fixation and the production of siderophores and indoles, and contributes to the mineral nutrition of strawberry plants, reinforcing their physiological and cellular characteristics. Previous studies reported that, among other characteristics, *A. brasilense* REC3 enhanced levels of soluble phenolic compounds, reduced lipid peroxidation, and promoted the upregulation of strawberry genes involved in bacterial recognition and defense and H₂O₂ depuration (2–4). By using a green fluorescent protein-tagged gene, it was demonstrated that REC3 is capable of colonizing strawberry roots, promoting their growth under controlled environmental and field conditions, and colonizing newborn plants via stolons (5–7). *A. brasilense* REC3 is also an active participant in the systemic protection of strawberry plants against anthracnose disease caused by *Colletotrichum acutatum* (8). Moreover, besides its association with strawberry plants, *A. brasilense* REC3 has been shown to improve grain yields of rain-fed rice crops (9).

In this study, the whole genome of *A. brasilense* REC3 was sequenced using the Illumina HiSeq platform. The total size was 7,229,924 bp, representing a genome coverage of approximately 99.9×. Quality-filtered reads were assembled using Velvet version 1.1.04 (10), which generated 137 contigs. The contigs were ordered against *A. brasilense* Az39 (11) using progressive MAUVE multiple-genome alignment software (12), which identified 103 contigs coming from the plasmid DNA. The maximum contig size for the plasmid DNA was 333,173 bp, confirming the presence of large plasmids in *A. brasilense* REC3. Annotation with the Rapid Annotations using Subsystems Technology (RAST) server (13) revealed that the total genome contains approximately 6,526 coding genes, 84 tRNAs, and 513 subsystems. The genome of this strain has a high G+C content (68.7%). Putative functions of the coding genes related to plant growth promotion, including nitrogen fixation, phytohormone and plant growth-regulator biosynthesis, biofilm formation, and type I, II, and VI secretion systems, were automatically identified using the RAST server.

Further comparative genome analyses with other strains from different sources are in progress, with the goal of investigating the specific mechanisms of *Azospirillum* plant-pathogen interactions to elucidate the potential of this microorganism to be used as a biocontrol agent.

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REFERENCES

- Pedraza RO, Motok J, Tortora ML, Salazar SM, Díaz Ricci JC. 2007. Natural occurrence of *Azospirillum brasilense* in strawberry plants. *Plant Soil* 295:169–178. <https://doi.org/10.1007/s11104-007-9273-x>.
- Tortora ML, Díaz Ricci JC, Pedraza RO. 2012. Protection of strawberry plants (*Fragaria ananassa* Duch.) against anthracnose disease induced by *Azospirillum brasilense*. *Plant Soil* 356:279–290. <https://doi.org/10.1007/s11104-011-0916-6>.
- Guerrero-Molina MF, Lovaisa NC, Salazar SM, Díaz-Ricci JC, Pedraza RO. 2013. Elemental composition of strawberry plants inoculated with the plant growth-promoting bacterium *Azospirillum brasilense* REC3 assessed by scanning electron microscopy and energy dispersive X-ray analysis. *Plant Biol* 16:726–731.
- Guerrero-Molina MF, Lovaisa NC, Salazar SM, Martínez-Zamora MG, Díaz-Ricci JC, Pedraza RO. 2015. Physiological, structural and molecular traits activated in strawberry plants after inoculation with the plant growth-promoting bacterium *Azospirillum brasilense* REC3. *Plant Biol* 17: 766–773. <https://doi.org/10.1111/plb.12270>.
- Pedraza RO, Motok J, Salazar SM, Ragout A, Mentel MI, Tortora ML, Guerrero Molina MF, Winik BC, Díaz Ricci JC. 2010. Growth-promotion of strawberry plants inoculated with *Azospirillum brasilense*. *World J Microbiol Biotechnol* 26:265–272. <https://doi.org/10.1007/s11274-009-0169-1>.
- Guerrero-Molina MF, Winik BC, Pedraza RO. 2012. More than rhizosphere colonization of strawberry plants by *Azospirillum brasilense*. *Appl Soil Ecol* 61:205–212. <https://doi.org/10.1016/j.apsoil.2011.10.011>.
- Salazar SM, Lovaisa NC, Guerrero-Molina MF, Ragout AL, Kirschbaum DS, Díaz-Ricci JC, Pedraza RO. 2012. Fruit yield of strawberry plants inoculated with *Azospirillum brasilense* RLC1 and REC3 under field conditions. *Rev Agronómica Noroeste Argent* 32:63–66.
- Tortora L, Díaz Ricci JC, Pedraza RO. 2011. *Azospirillum brasilense* siderophores with antifungal activity against *Colletotrichum acutatum*. *Arch Microbiol* 193:275–286. <https://doi.org/10.1007/s00203-010-0672-7>.
- Pedraza RO, Bellone CH, Bellone SC, Boa Sorte PMF, Teixeira KRS. 2009. *Azospirillum* inoculation and nitrogen fertilization effect on grain yield and on the diversity of endophytic bacteria in the phyllosphere of rice rainfed crop. *Eur J Soil Biol* 45:36–43. <https://doi.org/10.1016/j.ejsobi.2008.09.007>.
- Zerbino DR, Birney E. 2008. Velvet: algorithms for de novo short read assembly using de Bruijn graphs. *Genome Res* 18:821–829. <https://doi.org/10.1101/gr.074492.107>.
- Rivera D, Revale S, Molina R, Gualpa J, Puente M, Maroniche G, Paris G, Baker D, Clavijo B, McLay K, Spaepen S, Peticari A, Vazquez M, Wisniewski-Dye F, Watkins C, Martinez-Abarca F, Vanderleyden J, Cassan F. 2014. Complete genome sequence of the model rhizosphere strain *Azospirillum brasilense* Az39, successfully applied in agriculture. *Genome Announc* 2(4):e00683-14. <https://doi.org/10.1128/genomeA.00683-14>.
- Rissman AI, Mau B, Biehl BS, Darling AE, Glasner JD, Perna NT. 2009. Reordering contigs of draft genomes using the Mauve aligner. *Bioinformatics* 25:2071–2073. <https://doi.org/10.1093/bioinformatics/btp356>.
- Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST server: Rapid Annotations using Subsystems Technology. *BMC Genomics* 9:75. <https://doi.org/10.1186/1471-2164-9-75>.