



# Draft Genome Sequences of *Azospirillum brasilense* Strains Ab-V5 and Ab-V6, Commercially Used in Inoculants for Grasses and Legumes in Brazil

Mariangela Hungria,<sup>a</sup> Renan Augusto Ribeiro,<sup>b</sup> Marco Antonio Nogueira<sup>a</sup>

<sup>a</sup>Embrapa Soja, Soil Biotechnology Laboratory, Londrina, Paraná, Brazil

<sup>b</sup>CNPq, SHIS QI 1 Conjunto B, Brasília, Federal District, Brazil

**ABSTRACT** *Azospirillum brasilense* strains Ab-V5 and Ab-V6 are largely used in commercial inoculants for grasses and legumes in Brazil. Their genomes were estimated at 6,934,595 and 7,197,196 bp, respectively, and encompass genes related to nitrogen fixation, synthesis of phytohormones, and environmental adaptation. Although the strains differ in phenotypic properties, their genomes are highly similar.

The use of inoculants carrying plant growth-promoting bacteria (PGPB) is increasing, stimulated by a global search for a more productive but sustainable agriculture, and the genus *Azospirillum* comprises one of the most studied and used PGPB. Brazil has a long tradition of studying *Azospirillum* spp. (1), but it was only in 2009 that the first strains started to be used in commercial inoculants (2). Since then, two strains—Ab-V5 (CNPSO 2083) and Ab-V6 (CNPSO 2084)—identified by our research group (2) have been used in inoculants for grasses (maize [*Zea mays* L.], wheat [*Triticum aestivum* L.] [2, 3], and pastures with brachiaria [*Urochloa* spp.] [4]), and for coinoculation of legumes (soybean [*Glycine max* L. Merr.] and common bean [*Phaseolus vulgaris* L.] (5, 6). In the 2017 to 2018 crop season, about 5 million doses of inoculants carrying strains Ab-V5 and Ab-V6 were commercialized in Brazil, and their use is expanding in neighboring countries. Here, we report the draft genomes of these two strains.

DNA extraction and paired-end sequencing on the MiSeq platform (Illumina) were performed as described before (7), and sequences were assembled with the A5-miseq pipeline (*de novo*). Shotgun sequences allowed genome coverages of 245- and 60-fold for Ab-V5 and Ab-V6, respectively. The genome of strain Ab-V5 was estimated at 6,934,595 bp, with a G+C content of 68.4 mol%, and the genome of Ab-V6 was estimated at 7,197,196 bp, with a G+C content of 68.3 mol%; both genomes were assembled with 63 contigs. The sizes are within the range of other sequenced *A. brasilense* strains, including CBG497 (6,473,208 bp) (8), Az39 (7,391,279 bp) (9), and Sp245 (7,530,241 bp) (8). *A. brasilense* strains usually carry plasmids (8, 9), and from Eckhart gel electrophoresis, we observed that Ab-V5 and Ab-V6 have at least two common plasmids (of about 285,000 and 100,000 bp) and that Ab-V6 carries an extra plasmid of about 150,000 bp. Besides that, the Ab-V5 and Ab-V6 genomes are highly similar, with an average nucleotide identity estimated at 100%.

Sequences were submitted to the Rapid Annotations using Subsystems Technology (RAST) server (10), and 6,349 DNA coding sequences (CDSs) were identified in Ab-V5 and 6,625 CDSs in Ab-V6, classified into 510 and 519 subsystems, respectively; 53 and 54% of the CDSs were not classified in any subsystem, respectively. Both strains carry similar *nif* and *fix* genes that confer their ability to fix atmospheric nitrogen. Although the strains differ in their capacity to synthesize phytohormones (11, 12), both share the same genes related to the synthesis of auxins. One important feature of Ab-V5 and Ab-V6 is their capacity to induce genes related to tolerance of biotic and abiotic stresses

Received 31 March 2018 Accepted 2 April 2018 Published 17 May 2018

**Citation** Hungria M, Ribeiro RA, Nogueira MA. 2018. Draft genome sequences of *Azospirillum brasilense* strains Ab-V5 and Ab-V6, commercially used in inoculants for grasses and legumes in Brazil. *Genome Announc* 6: e00393-18. <https://doi.org/10.1128/genomeA.00393-18>.

**Copyright** © 2018 Hungria 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 Mariangela Hungria, [mariangela.hungria@embrapa.br](mailto:mariangela.hungria@embrapa.br).

in plants (11, 13), and the strains also carry several stress response genes, the majority of which are related to oxidative stresses. The reported niche adaptation of *A. brasilense* (8) might be attributed to a whole range of genes related to motility and chemotaxis, phosphorus metabolism, and resistance to antibiotics, among others. As reported before (12), we found several copies of genes encoding quorum-sensing LuxR but not LuxI proteins; in addition, there were no genes related to type III secretion systems.

**Accession number(s).** The whole-genome shotgun project for strain Ab-V5 has been deposited at DDBJ/EMBL/GenBank under the accession number [POQV00000000](https://www.ncbi.nlm.nih.gov/nuccore/POQV00000000) (accession number SUBID SUB3474034, BioProject PRJNA429443, and BioSample SAMN08346097); the version described in this paper is the first version, POQV01000000. The whole-genome shotgun project for strain Ab-V6 has been deposited at DDBJ/EMBL/GenBank under the accession number [POTD00000000](https://www.ncbi.nlm.nih.gov/nuccore/POTD00000000) (accession number SUBID SUB3488520, BioProject PRJNA429631, and BioSample SAMN08354664); the version described in this paper is the first version, POTD01000000.

## ACKNOWLEDGMENTS

This work was funded by CNPq-Universal (400468/2016-6), Embrapa (02.13.08.001.00.00), and INCT-Plant-Growth Promoting Microorganisms for Agricultural Sustainability and Environmental Responsibility (CNPq 465133/2014-2, Fundação Araucária-STI, CAPES).

## REFERENCES

- Dobereiner J, Marriel IE, Nery M. 1976. Ecological distribution of *Spirillum lipoferum* Beijerinck. *Can J Microbiol* 22:1464–1473. <https://doi.org/10.1139/m76-217>.
- Hungria M, Campo RJ, Souza EM, Pedrosa FO. 2010. Inoculation with selected strains of *Azospirillum brasilense* and *A. lipoferum* improves yields of maize and wheat in Brazil. *Plant Soil* 331:413–425. <https://doi.org/10.1007/s11104-009-0262-0>.
- Fukami J, Nogueira MA, Araujo RS, Hungria M. 2016. Accessing inoculation methods of maize and wheat with *Azospirillum brasilense*. *AMB Express* 6:1–13. <https://doi.org/10.1186/s13568-015-0171-y>.
- Hungria M, Nogueira MA, Araujo RS. 2016. Inoculation of *Brachiaria* spp. with the plant growth-promoting bacterium *Azospirillum brasilense*: an environment-friendly component in the reclamation of degraded pastures in the tropics. *Agric Ecosyst Environ* 221:125–131. <https://doi.org/10.1016/j.agee.2016.01.024>.
- Hungria M, Nogueira MA, Araujo RS. 2013. Co-inoculation of soybeans and common beans with rhizobia and azospirilla: strategies to improve sustainability. *Biol Fertil Soils* 49:791–801. <https://doi.org/10.1007/s00374-012-0771-5>.
- Hungria M, Nogueira MA, Araujo RS. 2015. Soybean seed co-inoculation with *Bradyrhizobium* spp. and *Azospirillum brasilense*: a new biotechnological tool to improve yield and sustainability. *Am J Plant Sci* 6:811–817. <https://doi.org/10.4236/ajps.2015.66087>.
- Megías E, Reis Junior FB, Ribeiro RA, Ollero FJ, Megías M, Hungria M. 2017. Genome sequence of *Pantoea ananatis* strain AMG 501, a plant-growth promoting bacterium isolated from rice leaves grown in paddies of Southern Spain. *Genome Announc* 5(34):e00848-17. <https://doi.org/10.1128/genomeA.00848-17>.
- Wisniewski-Dyé F, Lozano L, Acosta-Cruz E, Borland S, Drogue B, Prigent-Combaret C, Rouy Z, Barbe V, Herrera AM, González V, Mavingui P. 2012. Genome sequence of *Azospirillum brasilense* CBG497 and comparative analyses of *Azospirillum* core and accessory genomes provide insight into niche adaptation. *Genes* 3:576–602. <https://doi.org/10.3390/genes3040576>.
- 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>.
- 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>.
- Fukami J, Ollero FJ, Megías M, Hungria M. 2017. Phytohormones and induction of plant-stress tolerance and defense genes by seed and foliar inoculation with *Azospirillum brasilense* cells and metabolites promote maize growth. *AMB Express* 7:153. <https://doi.org/10.1186/s13568-017-0453-7>.
- Fukami J, Abrantes JLF, del Cerro P, Nogueira MA, Ollero FJ, Megías M, Hungria M. 2018. Revealing different strategies of quorum sensing in *Azospirillum brasilense* strains Ab-V5 and Ab-V6. *Arch Microbiol* 200:47–56. <https://doi.org/10.1007/s00203-017-1422-x>.
- Fukami J, De La Osa C, Ollero FJ, Megías M, Hungria M. 2018. Co-inoculation of maize with *Azospirillum brasilense* and *Rhizobium tropici* as a strategy to mitigate salinity stress. *Functional Plant Biol* 45:328–339. <https://doi.org/10.1071/FP17167>.