

Complete Genome Sequence of the Model Rhizosphere Strain *Azospirillum brasilense* Az39, Successfully Applied in Agriculture

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We present the complete genome sequence of *Azospirillum brasilense* Az39, isolated from wheat roots in the central region of Argentina and used as inoculant in extensive and intensive agriculture during the last four decades. The genome consists of 7.39 Mb, distributed in six replicons: one chromosome, three chromids, and two plasmids.

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Azospirillum sp. is one of the best-studied plant-growth-promoting rhizobacteria at present. Members of this genus colonize more than 100 plant species and significantly improve their growth and productivity under field conditions (1). One of the main characteristics of *Azospirillum* sp. proposed to explain plant growth promotion has been related to its ability to produce plant growth regulators as auxins, cytokinins, gibberellins, ethylene, abscisic acid, nitric oxide, and polyamines (2–8). *Azospirillum brasilense* Az39 was isolated in 1982 from surface-sterilized wheat seedlings in Marcos Juarez, Argentina, and selected for inoculant formulation based on its ability to increase crop yields of maize and wheat under agronomic conditions (9). The potential mechanisms responsible for growth promotion in strain Az39 have been partially unraveled (10–13).

The genome sequence was obtained using a combined whole-genome shotgun and 8-kb paired-end strategy with a 454 GS FLX Titanium pyrosequencer at INDEAR (Argentina), resulting in a 21-fold genome coverage. Sequencing reads were *de novo* assembled (Newbler v 2.8), resulting in 6 scaffolds (>160 kbp each; N_{50} , 1,908,534 bp). The closure of the gap intra- and interscaffolds was achieved by detailed observation of relevant sequencing reads using the Geneious R7 software platform (14). Optical mapping analysis was performed with an OpGen Argus optical mapper at TGAC (United Kingdom) to validate the final assembly. In agreement with the bioinformatic data, pulsed-field gel electrophoresis (PFGE) analysis of total DNA revealed the presence of six replicons in *A. brasilense* Az39, defined as one chromosome, three chromids, and two plasmids. The presence of six to seven replicons is a common feature of *Azospirillum* genomes (15–17). Replicon sizes and their G + C contents were 3,064,393 bp (68.4%) for the chromosome; 1,901,707 bp (68.4%), 933,960 bp (68.6%), and 641,573 bp (69.2%) for the chromids (AbAZ39_p1, AbAZ39_p2,

and AbAZ39_p4); and 686,487 bp (69.5%) and 163,159 bp (65.6%) for the plasmids (AbAZ39_p3 and AbAZ39_p5).

Genome annotation was done using the NCBI Prokaryotic Genomes Automatic Annotation Pipeline (PGAAP) (18). The complete genome consists of 6,311 protein-coding sequences (2,763 on the chromosome, 1,605 on AbAZ39_p1, 744 on AbAZ39_p2, 534 on AbAZ39_p3, 557 on AbAZ39_p4, and 108 on AbAZ39_p5). Similarly to other species of the *Azospirillum* genera, Az39 contains multiple ribosomal operons at different replicons (15–17). Eight rRNA operons are complete and one lacks the 5S rRNA subunit. Complete operons are distributed with two in the chromosome, four in AbAZ39_p1, and two in AbAZ39_p4, while the incomplete one is located on the chromid AbAZ39_p2. Eighty-seven tRNA loci (distributed 44 on the chromosome, 42 on the chromids, and 1 on the plasmids) were identified. The putative genes involved in plant growth promotion mechanisms of Az39 were determined by the use of the RAST annotation server (19) and KAAS (20).

The *A. brasilense* Az39 genome contains genes related to nitrogen fixation; phytohormones and plant growth regulators biosynthesis; biofilms formation and type I, II, and VI secretion systems. The genome sequence of Az39 provides a genomic basis for in-depth comparative genome analyses, to elucidate the specific mechanisms of *Azospirillum*-plant interactions.

Nucleotide sequence accession numbers. The complete genome sequence of *Azospirillum brasilense* Az39 is available at NCBI GenBank under the accession numbers CP007793 for the chromosome and CP007794 to CP007798 for the other replicons.

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