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Short communication

Draft genome sequence of *Bradyrhizobium* sp. strain BR 3262, an effective microsymbiont recommended for cowpea inoculation in Brazil

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ABSTRACT

The strain BR 3262 was isolated from nodule of cowpea (*Vigna unguiculata* L. Walp) growing in soil of the Atlantic Forest area in Brazil and it is reported as an efficient nitrogen fixing bacterium associated to cowpea. Firstly, this strain was assigned as *Bradyrhizobium elkanii*, however, recently a more detailed genetic and molecular characterization has indicated it could be a *Bradyrhizobium pachyrhizi* species. We report here the draft genome sequence of *B. pachyrhizi* strain BR 3262, an elite bacterium used as inoculant for cowpea. The whole genome with 116 scaffolds, 8,965,178 bp and 63.8% of C+G content for BR 3262 was obtained using Illumina MiSeq sequencing technology. Annotation was added by the RAST prokaryotic genome annotation service and shown 8369 coding sequences, 52 RNAs genes, classified in 504 subsystems.

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Introduction

Cowpea (*Vigna unguiculata* L. Walp) is a leguminous species able to nodulate and fix nitrogen with various rhizobium species in different continents.[1–4] Besides that, it is an important protein source for peoples' nutrition and its cultivation is increasing in Brazil, being the cerrado biome the new agricultural frontier. BR 3262 is on the list of recommended strains for cowpea inoculation in Brazil and it was assigned as *Bradyrhizobium* member.[5] The strain was isolated from nodules of cowpea in the Atlantic Forest area in Brazil, it has the capacity to compete with indigenous rhizobia for nodule occupancy, and it has been reported as an efficient cowpea-associated nitrogen fixer, especially in the cerrado region.[4,5,6] Here, we report on the draft genome sequence of strain BR 3262, an effective cowpea microsymbiont.

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After growing in YMA culture medium the BR3262 bacterial genomic DNA was extracted and purified according to a previously described protocol.[7] The sequencing reaction using a 2 × 50 bp paired-end library construction on the Illumina® MiSeq platform (Macrogen, Korea) yielded a total of 9,632,860 reads and 972,918,860 bases, which corresponds to approximately 108 × genome coverage. The reads were trimmed using the FASTX-Toolkit and only the bases with quality above 20 (Q20) were used for assembly. The ABySS software version 1.9.0[8] was used for *de novo* assembly and contigs shorter than 200 bp were eliminated.

Annotation and identification of metabolic pathways encoded on the draft genome was carried out using the RAST server version 2.0.[9] In addition, the NCBI Prokaryotic Genome Annotation Pipeline (http://www.ncbi.nlm.nih.gov/genomes/static/Pipeline.html) was used for annotation refinement and registration of the genome at the International Nucleotide Sequence Database Collaboration (GenBank, USA).

The *de novo* genome assembly of strain BR 3262 resulted in 116 scaffolds with a genome size of 8,965,178 bp and G+C content of 63.8%. A total of 8369 protein-coding sequences (CDSs) were identified by using RAST annotation distributed in 504 subsystems, as well as 52 copies of RNA: 1 rRNA, 50 tRNAs and 1 ncRNA. The BR 3262 overall nitrogen metabolism requires 71 genes divided in several subcategories, with 20 genes involved in nitrogen fixation, 20 genes related to nitrate and nitrite ammonification and 20 genes associated with ammonia assimilation. Genes involved in denitrification were not found. A large number of CDSs encoding proteins of membrane transport systems (360 genes) and stress response (209 genes) were observed, including 40 CDSs for the type II secretion system and 177 for ABC transporters, 30 genes for osmotic stress response, 110 genes involved in oxidative stress, and 19 genes encoding heat shock proteins. The great gene diversity can be an evolutionary strategy of strain BR 3262 to interact with legumes and fix nitrogen under a variety of environmental conditions. However, further studies on BR 3262 genome are required for functional characterization of genes and the molecular mechanisms underlying the bacterium ability to nodulate and fix nitrogen in symbiosis.

This whole genome sequence has been deposited in DDBJ/ENA/GenBank under the accession no. LJYE00000000.1, the version described in this paper is the first version.

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REFERENCES

1. Bejarano A, Ramírez-Bahena M-H, Velázquez E, Peix A. *Vigna unguiculata* is nodulated in Spain by endosymbionts of genisteae legumes and by a new symbiovar (vignae) of the genus *Bradyrhizobium*. *Syst Appl Microbiol*. 2014;37:533–540.
2. Silva FV, De Meyer SE, Simões de Araujo JL, et al. *Bradyrhizobium manausense* sp. nov., isolated from effective nodules of *Vigna unguiculata* grown in Brazilian Amazon rainforest soils. *Int J Syst Evol Microbiol*. 2014;64:2358–2363.
3. Guimarães AA, Jaramillo PMD, Nóbrega RSA, Florentino LA, Silva KB, Moreira FMS. Genetic and symbiotic diversity of nitrogen-fixing bacteria isolated from agricultural soils in the Western Amazon by using cowpea as the trap plant. *Appl Environ Microbiol*. 2012;78:6726–6733.
4. Silva Júnior EB, Oliveira SS, Oliveira PJD, Boddey RM, Zilli JE, Xavier GR. Cowpea nodulation and production in response to inoculation with different rhizobia densities. *Pesqui Agropecu Bras*. 2014;49:804–812.
5. Zilli JE, Valicheski RR, Rumjanek NG, Simões-Araújo JL, Freire Filho FR, Neves MCP. Symbiotic efficiency of cowpea Bradyrhizobium strains in Cerrado soils. *Pesqui Agropecu Bras*. 2006;41:811–818.
6. Zilli JE, Ferreira EPB, Neves MCP, Rumjanek NG. Efficiency of fast-growing rhizobia capable of nodulating cowpea. *An Acad Bras Ciênc*. 1999;71:553–560.
7. Chen W-P, Kuo T-T. A simple and rapid method for the preparation of gram-negative bacterial genomic DNA. *Nucleic Acids Res*. 1993;21:2260.
8. Simpson JT, Wong K, Jackman SD, Schein JE, Jones SJ, Birol I. ABySS: a parallel assembler for short read sequence data. *Genome Res*. 2009;19:1117–1123.
9. Overbeek R, Olson R, Pusch GD, et al. The SEED and the Rapid Annotation of microbial genomes using Subsystems Technology (RAST). *Nucleic Acids Res*. 2013;42:D206–D214.