Short communication

Draft genome sequence of Bradyrhizobium sp. strain BR 3267, an elite strain recommended for cowpea inoculation in Brazil

Jean Luiz Simões-Araújo\textsuperscript{a}, Jakson Leite\textsuperscript{b}, Samuel Ribeiro Passos\textsuperscript{c}, Gustavo Ribeiro Xavier\textsuperscript{a}, Norma Gouvêa Rumjanek\textsuperscript{a}, Jerri Édson Zilli\textsuperscript{a,∗}

\textsuperscript{a} Embrapa Agrobiologia, Seropédica, Rio de Janeiro, RJ, Brazil
\textsuperscript{b} Universidade Federal Rural do Rio de Janeiro, Departamento de Solos, Seropédica, Rio de Janeiro, RJ, Brazil
\textsuperscript{c} Universidade Federal Rural do Rio de Janeiro, Departamento de Ciências Ambientais, Seropédica, Rio de Janeiro, RJ, Brazil

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\textbf{A B S T R A C T}

The strain BR 3267 is a nitrogen-fixing symbiotic bacteria isolated from soil of semi-arid area of Brazilian Northeast using cowpea as the trap plant. This strain is used as commercial inoculant for cowpea and presents high efficient in nitrogen fixation as consequence of its adaptation potential to semi-arid conditions. We report here the draft genome sequence of Bradyrhizobium sp. strain BR 3267, an elite bacterium used as inoculant for cowpea. Whole genome sequencing of BR 3267 using Illumina MiSeq sequencing technology has 55 scaffolds with a total genome size of 7,904,309 bp and C+G 63%. Annotation was added by the RAST prokaryotic genome annotation service and has shown 7314 coding sequences and 52 RNA genes.

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Cowpea (\textit{Vigna unguiculata} L. Walp) is an important nutrition resource for low-income people living in rural areas of the Brazilian Northeast, due to its high protein content.\textsuperscript{1} Besides its great importance, the crop is well adapted to edaphoclimatic constraints and it is able to fix nitrogen through the process called biological nitrogen fixation – BNF.\textsuperscript{2} BR 3267 is one of the elite strains recommended for cowpea inoculation\textsuperscript{3} and it was isolated from Semi-arid area of Brazilian Northeast.\textsuperscript{4} The strain is a member of \textit{Bradyrhizobium} genus, presents high efficiency in BNF associated with cowpea and it is able to grow at elevated temperature, up to 37 °C.\textsuperscript{4}

Here, we report the draft genome sequence of strain BR 3267, a nitrogen-fixing elite strain for cowpea inoculation in Brazil. The bacterium was grown in YMA medium and genomic DNA was extracted and purified following a firstly described protocol.\textsuperscript{5} The whole genome was sequenced using the 100 bp paired-end Illumina® MiSeq platform (Macrogen, Korea). The sequence reaction provided a total of 12,908,190 reads and 1,303,727,190 total bases, which corresponds to approximately 164 X coverage sequencing. The reads quality was analyzed using FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/), trimmed using FASTX-Toolkit and only

\textsuperscript{∗} Corresponding author.
E-mail: jerri.zilli@embrapa.br (J.É. Zilli).
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bases with quality above 20 (Q20) were used. De novo assembly was performed using ABySS software version 1.9.0\(^6\) and contigs shorter than 200 bp were eliminated.

Identification of coding sequences and annotation of draft genome were carried out using the RAST version 2.0.\(^7\) Contig annotation was also performed by NCBI Prokaryotic Genome Annotation Pipeline (http://www.ncbi.nlm.nih.gov/genomes/static/Pipeline.html), which was used to refine the annotation and registration at the International Nucleotide Sequence Database Collaboration (GenBank – USA).

The draft genome sequence of strain BR 3267 consists of 55 scaffolds and includes 7,904,309 bp with an overall G+C content of 63%. This genome size and C+G content are compatible with other *Bradyrhizobium* genomes deposited in the GenBank. The RAST annotation identified 7314 protein-coding sequences (CDSs), distributed in 501 subsystems, as well as 52 copies of RNA genes, including 50 tRNAs, 1 rRNA and 1 ncRNA. All genes required for the nodulation and biological nitrogen fixation are encoded in the BR 3267 genome. Furthermore, 17 genes involved with denitrification were also identified. The denitrification process is well known for some species of *Bradyrhizobium* genus.\(^5,8\) However, further functional studies with BR 3267 are required to define the expression pattern and relevance of these genes on the denitrification process and N\(_2\)O emission. A total of 188 genes related to stress response were annotated, including: 96 genes for oxidative stress, 21 genes for osmotic stress, and 20 genes encoding heat shock proteins. The genomic information will be important to clarify the mechanisms underlying the BR 3267 nodulation, biological nitrogen fixation and the adaptation of this strain to limited conditions of the Semi-arid soils.

The assembled contigs were deposited in DDBJ/ENA/GenBank and published in the accession number LJYF0000000.1. The version described in this paper is the first version.

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**References**

1. Freire Filho FR. Feijão-caupi no Brasil: Produção, melhoramento genético, avanços e desafios. Teresina: Embrapa Meio Ambiente; 2011.
2. de Freitas ADS, Fernandes Silva A, Valadares de Sá Barrett Sampaio E. Yield and biological nitrogen fixation of cowpea varieties in the semi-arid region of Brazil. Biomass Bioenergy. 2012;45:109–114.
3. Brasil. Ministério da Agricultura Pecuária e Abastecimento. Instrução normativa nº 13 de 24 de março de 2011. Aprova as normas sobre especificações, garantias, registro, embalagem e rotulagem dos inoculantes destinados à agricultura, bem como as relações dos microorganismos autorizados e recomendados para produção de inoculantes no Brasil, na forma dos Anexos I, II, III, desta Instrução Normativa. Diário Oficial da União da República Federativa do Brasil; 2011:3–7 [Seção 1].
4. Martins LMV, Xavier GR, Rangel FW, et al. Contribution of biological nitrogen fixation to cowpea: a strategy for improving grain yield in the semi-arid region of Brazil. Biol Fertil Soils. 2003;38:333–339.
5. 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.
6. 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.
7. Overbeek R, Olson R, Puush GD, et al. The SEED and the Rapid Annotation of microbial genomes using Subsystems Technology (RAST). Nucleic Acids Res. 2013;42:D206–D214.
8. Sánchez C, Tortosa G, Granados A, Delgado A, Bedmar EJ, Delgado MJ. Involvement of Bradyrhizobium japonicum denitrification in symbiotic nitrogen. Soil Biol Biochem. 2011;43:212–217.
9. Tortosa G, Hidalgo A, Salas A, Bedmar EJ, Mesa S, Delgado MJ. Nitrate and flooding induce N\(_2\)O emissions from soybean nodule. Symbiosis. 2015, http://dx.doi.org/10.1007/s13199-015-0341-9.