



# Closed Genome Sequence of Phytopathogen Biocontrol Agent *Bacillus velezensis* Strain AGVL-005, Isolated from Soybean

Victor Satler Pylro,<sup>a</sup> Armando Cavalcante Franco Dias,<sup>b</sup> Fernando Dini Andreote,<sup>a</sup> Daniel Kumazawa Morais,<sup>c</sup> Alessandro de Mello Varani,<sup>d</sup> Cristiane Cipolla Fasanella Andreote,<sup>b</sup> Eduardo Roberto de Almeida Bernardo,<sup>e</sup> Tiago Zucchi<sup>e</sup>

<sup>a</sup>Soil Microbiology Laboratory, Luiz de Queiroz College of Agriculture, University of São Paulo (ESALQ/USP), Piracicaba, São Paulo, Brazil

<sup>b</sup>Andrios Assessoria, Piracicaba, São Paulo, Brazil

<sup>c</sup>Institute of Microbiology, Czech Academy of Sciences (CAS), Prague, Czech Republic

<sup>d</sup>Departamento de Tecnologia, Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista (Unesp), Jaboticabal, São Paulo, Brazil

<sup>e</sup>Agrivalle Brasil Indústria e Comércio de Produtos Agrícolas Ltda., Salto, São Paulo, Brazil

**ABSTRACT** We report here the closed and near-complete genome sequence and annotation of *Bacillus velezensis* strain AGVL-005, a bacterium isolated from soybean seeds in Brazil and used for phytopathogen biocontrol.

**B**iological control using bacteria shows great potential as an eco-friendly and feasible alternative to replace, or at least diminish, the application of chemicals for pathogen control (1). Understanding the mechanisms involved in the interaction between any biological control agents and target phytopathogen is crucial to enhance and extend the use of these organisms in agriculture. To gain insight into the use of *Bacillus velezensis* strain AGVL-005 (a Gram-positive and rod-shaped soil bacterium) for the biological control of phytopathogens, we sequenced the genome using the MinION Mk1B device (MIN-101B; Oxford Nanopore Technologies, UK).

Briefly, ~1 µg of unsheared genomic DNA was submitted to end repair and dA-tailing steps using the NEBNext Ultra end repair/dA-tailing module (New England BioLabs, USA) and then treated with the 1D Genomic DNA sequencing kit for the MinION device (catalog number SQK-LSK-108; Oxford Nanopore Technologies, UK). The resulting library was sequenced using a flow cell Spot-ON Mk1 (FLO-MIN 106 R9 version; Oxford Nanopore Technologies), with the R9 version library loading bead kit (EXP-LLB001; Oxford Nanopore Technologies). The raw reads were acquired using the MinKNOW software version 1.7.14 in a 48-h run experiment and base called using the Albacore software version 2.0.2.

A total of 810,820 reads were obtained, with sizes ranging from 31 to 69,980 bp in length. All reads were *de novo* assembled using Canu version 1.5 (2), using default parameters for Nanopore data. The genome was assembled into 14 contigs. Analysis based on the SIS software (3) and BLAST indicated that most of the contig ends were related to rRNA operon genes. A proposed genome consensus was manually closed by comparisons against the closest reference genome (*Bacillus amyloliquefaciens* subsp. *plantarum* strain FZB42 [GenBank accession number NC\_009725]) and BLAST analysis. An improved consensus sequence for the draft assembly was obtained with the Nanopolish software version 0.7.2 (<https://github.com/jts/nanopolish>), using default parameters. Genome completeness and contamination were estimated using CheckM (4) in the lineage-specific mode. Genome statistics were estimated with QUAST (5) using *B. amyloliquefaciens* subsp. *plantarum* strain FZB42 as a reference genome. The average nucleotide identity based on BLAST (ANIB) between our genome and its

Received 15 January 2018 Accepted 17 January 2018 Published 15 February 2018

**Citation** Pylro VS, Dias ACF, Andreote FD, Morais DK, Varani ADM, Andreote CCF, Bernardo ERDA, Zucchi T. 2018. Closed genome sequence of phytopathogen biocontrol agent *Bacillus velezensis* strain AGVL-005, isolated from soybean. *Genome Announc* 6:e00057-18. <https://doi.org/10.1128/genomeA.00057-18>.

**Copyright** © 2018 Pylro 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 Victor Satler Pylro, victor.pylro@brmicrobiome.org, or Tiago Zucchi, tiago.zucchi@agrivalle.com.br.

reference was determined by JSpecies (6); ANI scores of >95% indicate that they belong to the same species (7, 8).

The final genome consists of a single contiguous circular chromosome. The estimated genome size is 4,146,154 bp, with a G+C content of 45.98%. The genome completeness estimated by CheckM was 90.66%, and the contamination was 0.64%, being classified as a near-complete and low-contaminated genome. Genome annotation was performed with PATRIC version 3.2.45beta (9). We identified 6,261 coding sequences (CDSs) and 92 predicted noncoding RNAs (71 tRNA and 21 rRNA). The calculated ANIb was 98.3% between *B. velezensis* AGVL-005 and *B. amyloliquefaciens* subsp. *plantarum* (reference strain), which classified them as belonging to the same species. It is important to highlight that *B. amyloliquefaciens* subsp. *plantarum* was recently classified as a later heterotypic synonym of *Bacillus velezensis* (10); therefore, our isolate was identified according to this reclassification.

**Accession number(s).** This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession number [CP024922](https://doi.org/10.11274/010-0412-9). The version described in this paper is the first version, CP024922.

## ACKNOWLEDGMENTS

We thank Lucia Žiřčáková (Institute of Microbiology of the CAS/Czech Republic) for her help during the MinION sequencing procedures and Marc Redmile-Gordon for critical comments and review of the written English in the manuscript.

This work was supported by the National Council for Scientific and Technological Development (Conselho Nacional de Desenvolvimento Científico e Tecnológico/CNPq) under project grant number 443815/2014-3. V.S.P. received a fellowship from FAPESP (processes 14/50320-4 and 16/02219-8). This work was also supported by the Brazilian Microbiome Project (<http://www.brnmicrobiome.org>) and the National Institute of Science and Technology (Microbiome [<http://www.inct-microbiome.org>]).

## REFERENCES

1. Canova SP, Petta T, Reyes LF, Zucchi TD, Moraes LA, Melo IS. 2010. Characterization of lipopeptides from *Paenibacillus* sp. (IIRAC30) suppressing *Rhizoctonia solani*. *World J Microbiol Biotechnol* 26:2241–2247. <https://doi.org/10.1007/s11274-010-0412-9>.
2. Koren S, Walenz BP, Berlin K, Miller JR, Bergman NH, Phillippy AM. 2017. Canu: scalable and accurate long-read assembly via adaptive k-mer weighting and repeat separation. *Genome Res* 27:722–736. <https://doi.org/10.1101/gr.215087.116>.
3. Dias Z, Dias U, Setubal JC. 2012. SIS: a program to generate draft genome sequence scaffolds for prokaryotes. *BMC Bioinformatics* 13:96. <https://doi.org/10.1186/1471-2105-13-96>.
4. Parks DH, Imelfort M, Skennerton CT, Hugenholtz P, Tyson GW. 2015. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. *Genome Res* 25:1043–1055. <https://doi.org/10.1101/gr.186072.114>.
5. Gurevich A, Saveliev V, Vyahhi N, Tesler G. 2013. QUAST: quality assessment tool for genome assemblies. *Bioinformatics* 29:1072–1075. <https://doi.org/10.1093/bioinformatics/btt086>.
6. Richter M, Rosselló-Móra R. 2009. Shifting the genomic gold standard for the prokaryotic species definition. *Proc Natl Acad Sci U S A* 106: 19126–19131. <https://doi.org/10.1073/pnas.0906412106>.
7. Goris J, Konstantinidis KT, Klappenbach JA, Coenye T, Vandamme P, Tiedje JM. 2007. DNA-DNA hybridization values and their relationship to whole-genome sequence similarities. *Int J Syst Evol Microbiol* 57:81–91. <https://doi.org/10.1099/ijts.0.64483-0>.
8. Richter M, Rosselló-Móra R, Glöckner FO, Peplies J. 2015. JSpeciesWS: a web server for prokaryotic species circumscription based on pairwise genome comparison. *Bioinformatics* 32:929–931. <https://doi.org/10.1093/bioinformatics/btv681>.
9. Wattam AR, Abraham D, Dalay O, Disz TL, Driscoll T, Gabbard JL, Gillespie JJ, Gough R, Hix D, Kenyon R, Machi D, Mao C, Nordberg EK, Olson R, Overbeek R, Pusch GD, Shukla M, Schulman J, Stevens RL, Sullivan DE, Vonstein V, Warren A, Will R, Wilson MJC, Yoo HS, Zhang C, Zhang Y, Sobral BW. 2014. PATRIC, the bacterial bioinformatics database and analysis resource. *Nucleic Acids Res* 42:D581–D591. <https://doi.org/10.1093/nar/gkt1099>.
10. Dunlap CA, Kim SJ, Kwon SW, Rooney AP. 2016. *Bacillus velezensis* is not a later heterotypic synonym of *Bacillus amyloliquefaciens*; *Bacillus methylotrophicus*, *Bacillus amyloliquefaciens* subsp. *plantarum* and “*Bacillus oryzicola*” are later heterotypic synonyms of *Bacillus velezensis* based on phylogenomics. *Int J Syst Evol Microbiol* 66:1212–1217. <https://doi.org/10.1099/ijsem.0.000858>.