

Closed Genome Sequence of *Bacillus paralicheniformis* Strain CBMAI 1303, a Bacterium Applied for Phytopathogen Biocontrol

Microbiology

Resource Announcements

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ABSTRACT We report the complete genome sequence and annotation of *Bacillus paralicheniformis* strain CBMAI 1303, a bacterium applied for phytopathogen biocontrol.

Biological control with 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 agent and a target phytopathogen is crucial for enhancing and extending the use of these organisms in agriculture. To gain insight into the use of *Bacillus paralicheniformis* strain CBMAI 1303, a Gram-positive and rod-shaped soil bacterium used for biological control of phytopathogens, we sequenced the genome using the MiSeq platform (Illumina, USA). Several closed related *Bacillus* spp. have been used for phytopathogen biocontrol, including *Magnaporthe grisea* in rice (2) and *Botrytis cinerea* in tomato (3). Moreover, a preliminary study by our group found that *B. paralicheniformis* strain CBMAI 1303 has the potential to control *Rhizoctonia* sp. and *Colletotrichum* sp. growth. This isolate was obtained from the Coleção Brasileira de Micro-organismos de Ambiente e Indústria (CBMAI; http://cbmai.cpqba.unicamp.br) and cultivated in nutrient agar, and the genomic DNA was extracted using the Wizard genomic DNA purification kit (Promega), following the manufacturer's instructions.

Paired-end sequencing libraries (2 \times 250 bp) were constructed with the Nextera XT kit (Illumina), following the manufacturer's instructions. After quality filtering with Trimmomatic version 0.33 (4) (parameters PE; TRAILING:10 LEADING:10 SLIDINGWIN-DOW:4:10), we obtained 1,537,492 reads consisting of approximately 80 \times genome coverage. All reads were *de novo* assembled with the SPAdes genome assembler version 3.12 (5), following default parameters for Illumina data, which generated 847 contigs. Contigs with a length less than 200 bp were discarded, which resulted in a genome sequence assembled into 67 contigs. Scaffolds were generated with the reference-based scaffolder MeDuSa (6) with *Bacillus paralicheniformis* ATCC 9945a (GenBank accession number NC_021362) as a guide for alignment, which resulted in four scaffolds. A proposed genome consensus was manually closed by comparisons against the closest reference genome (*Bacillus paralicheniformis* ATCC 9945a), paired-end read mapping, and a BLAST analysis. Genome completeness and contamination were estimated using CheckM (7) in the lineage-specific mode. Genome statistics were

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Received 5 November 2018 Accepted 12 December 2018 Published 17 January 2019 estimated with QUAST (8), using *Bacillus paralicheniformis* ATCC 9945a as the reference genome. The average nucleotide identity based on BLAST (ANIb) between our genome and its reference was determined by JSpecies (7); ANI scores greater than 95% indicate that they belong to the same species (9, 10).

The final genome sequence consists of a single contiguous circular chromosome. The estimated genome size is 4,476,034 bp, with a G+C content of 45.9%. The genome completeness estimated by CheckM was 99.41%, and the contamination was 0.05%, which classifies it as nearly complete and with low contamination. The genome annotation was performed with PATRIC version 3.5.23 (11), which identified 4,752 coding sequences (CDS), 93 predicted noncoding RNAs (72 tRNAs and 21 rRNAs, encompassing 7 rRNA operons), and 42 repeated regions. The calculated ANIb was 99.89% between *Bacillus paralicheniformis* CBMAI 1303 and *Bacillus paralicheniformis* ATCC 9945a, which classified them as belonging to the same species.

Data availability. This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession number CP033389. The version described in this paper is the first version, CP033389.1. Raw reads are available under the BioProject accession number PRJNA509356.

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REFERENCES

- Canova SP, Petta T, Reyes LF, Zucchi TD, Moraes LAB, 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.
- Tendulkar SR, Saikumari YK, Patel V, Raghotama S, Munshi TK, Balaram P, Chattoo BB. 2007. Isolation, purification and characterization of an antifungal molecule produced by *Bacillus licheniformis* BC98, and its effect on phytopathogen *Magnaporthe grisea*. J Appl Microbiol 103:2331–2339. https://doi.org/10.1111/j.1365-2672.2007.03501.x.
- Lee JP, Lee S-W, Kim CS, Son JH, Song JH, Lee KY, Kim HJ, Jung SJ, Moon BJ. 2006. Evaluation of formulations of *Bacillus licheniformis* for the biological control of tomato gray mold caused by *Botrytis cinereal*. Biol Control 37:329–337. https://doi.org/10.1016/j.biocontrol.2006.01.001.
- Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30:2114–2120. https://doi.org/10 .1093/bioinformatics/btu170.
- Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 19:455–477. https://doi.org/10.1089/cmb.2012.0021.
- Bosi E, Donati B, Galardini M, Brunetti S, Sagot M-F, Lió P, Crescenzi P, Fani R, Fondi M. 2015. MeDuSa: a multi-draft based scaffolder. Bioinformatics 31:2443–2451. https://doi.org/10.1093/bioinformatics/btv171.

- 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.
- 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.
- 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/ijs.0.64483-0.
- Richter M, Rosselló-Móra R, Glöckner FO, Peplies J. 2016. JSpeciesWS: a Web server for prokaryotic species circumscription based on pairwise genome comparison. Bioinformatics 32:929–931. https://doi.org/10.1093/ bioinformatics/btv681.
- 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.