



Draft Genome Sequence of *Bacillus subtilis* 2C-9B, a Strain with Biocontrol Potential against Chili Pepper Root Pathogens and Tolerance to Pb and Zn

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ABSTRACT *Bacillus subtilis* 2C-9B, obtained from the rhizosphere of wild grass, exhibits inhibition against root rot causal pathogens in *Capsicum annuum*, Pb and Zn tolerance, and plant growth promotion in medium supplemented with Pb. The genome of *B. subtilis* 2C-9B was sequenced and the draft genome assembled, with a length of 4,215,855 bp and 4,723 coding genes.

iocontrol of phytopathogens in horticultural crops can greatly benefit from the identification of new strains of bacterial species as alternatives to counteract specific crop diseases. In addition, tolerance to heavy metals and plant growthpromoting activity are other characteristics that, if present in the isolated bacterial strain, could be of great biotechnological relevance for the bioremediation of heavy metal soil-polluted regions. Bacillus subtilis is well known to possess properties of phytopathogen inhibition, plant growth promotion, and heavy metal absorption (1–3); moreover, in other Bacillus and Halobacillus species, the improvement of resistance in plants to heavy metals has been reported (4, 5). In this study, we report the draft genome sequence of Bacillus subtilis 2C-9B, which has inhibitory activity against Phytophthora capsici, Fusarium solani, and Rhizoctonia solani, pathogens that cause root rot in chili pepper. Moreover, this strain shows tolerance to Pb (2,500 ppm) and Zn (400 ppm), promotes plant growth in Arabidopsis thaliana in medium supplemented with Pb, and synthesizes indoleacetic acid. The genome was sequenced using the MiSeq platform (Illumina, San Diego, CA, USA) in a 2×75 paired-end run. The genome library was prepared according to Nextera kit instructions, and the library quality was analyzed in a Bioanalyzer 2010 (Agilent Technologies). Genome assembly was performed using the SPAdes genome assembler (6), and the quality was analyzed using QUAST 4.1 (7). For genome annotation, the NCBI Prokaryotic Genome Annotation Pipeline was used (8). In the draft genome of B. subtilis strain 2C-9B, a total of 4,823 genes are reported, of which, 4,000 are coding genes, 100 are RNA genes (22 rRNAs, 73 tRNAs, and 5 noncoding RNAs [ncRNAs]), and 723 are pseudogenes.

Two nonribosomal peptide synthetases and a beta-glucanase gene were found in the genome of this bacterium, suggesting a role of these genes in the observed antifungal activity (9). Also, a butanediol dehydrogenase gene and a spermidine synthase gene were found, with butanediol being a potential inducer of systemic resistance in plants (10) and the spermidine gene associated with plant growth promotion (11). Although no *pbr* genes were found, other genes related to heavy metal resistance were identified by sequence homology. Among these were *zntR*, Received 4 December 2017 Accepted 5 December 2017 Published 18 January 2018

Citation Muñoz-Moreno CY, De La Cruz-Rodríguez Y, Vega-Arreguín J, Alvarado-Rodríguez M, Gómez-Soto JM, Alvarado-Gutiérrez A, Fraire-Velázquez S. 2018. Draft genome sequence of *Bacillus subtilis* 2C-9B, a strain with biocontrol potential against chili pepper root pathogens and tolerance to Pb and Zn. Genome Announc 6:e01502-17. https://doi.org/10.1128/genomeA.01502-17.

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Address correspondence to Saúl Fraire-Velázquez, sfraire@uaz.edu.mx. which mediates the expression of the zinc export protein *zntA*; *zntB*, which is involved in the transport of zinc (12); and a *merR* family transcriptional regulator which activates transcription in response to metal ions (13). Also, a cadmium-translocating P-type ATPase, a copper-translocating P-type ATPase, a copper-binding protein, and the copper transporter CopZ were found. Furthermore, CheA and CheC are present in the genome of this bacterium; these proteins are usually involved in chemotaxis and adaptation (14, 15). An endoglucanase and an *N*-acetylglucosamine-6-phosphate deacetylase, which are enzymes involved mainly in the consumption of carbon sources, were also found.

Considering the gene profile of this bacterial strain, *B. subtilis* 2C-9B can be seen from the perspective of a biotechnological tool with multipurpose applications, including biocontrol of phytopathogens, bioremediation of Pb- and/or Zn-contaminated areas, and increase in crop yields.

Accession number(s). This whole-genome shotgun project has been deposited at DDBJ/ENA/GenBank under the accession number MOXE00000000. The version described in this paper is version MOXE01000000.

ACKNOWLEDGMENTS

This research was supported by CONACYT under grant INFR-2014-01-226183 to S.F.-V. and partially supported by CONACYT under grant FORDECYT-Doctores 174509 to S.F.-V. We thank CONACYT for the scholarship to C.Y.M.-M. (CVU: 636975).

REFERENCES

- 1. Beveridge TJ, Murray RG. 1980. Sites of metal deposition in the cell wall of *Bacillus subtilis*. J Bacteriol 141:876–887.
- Rekha K, Baskar B, Srinath S, Usha B. 2017. Plant-growth-promoting rhizobacteria *Bacillus subtilis* RR4 isolated from rice rhizosphere induces malic acid biosynthesis in rice roots. Can J Microbiol 18:1–8. https://doi .org/10.1139/cjm-2017-0409.
- Falcäo LL, Silva-Werneck JO, Vilarinho BR, da Silva JP, Pomella AW, Marcellino LH. 2014. Antimicrobial and plant growth-promoting properties of the cacao endophyte *Bacillus subtilis* ALB629. J Appl Microbiol 116:1584–1592. https://doi.org/10.1111/jam.12485.
- Chen W, Ouyang LM, Kong PJ, Yang ZY, Wu W, Zhu DL, Zhang LL. 2015. Rhizospheria bacteria of *Poplus euphratica* improve resistance of wood plants to heavy metals. Ying Yong Sheng Tai Xue Bao 26:2811–2816.
- Desale P, Patel B, Singh S, Malhotra A, Nawani N. 2014. Plant growth promoting properties of *Halobacillus* sp. and *Halomonas* sp. in presence of salinity and heavy metals. J Basic Microbiol 54:781–791. https://doi .org/10.1002/jobm.201200778.
- 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.
- 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.
- Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J. 2016. NCBI prokaryotic

genome annotation pipeline. Nucleic Acids Res 44:6614-6624. https://doi .org/10.1093/nar/gkw569.

- Tapi A, Chollet-Imbert M, Scherens B, Jacques P. 2010. New approach for the detection of non-ribosomal peptide synthetase genes in *Bacillus strains* by polymerase chain reaction. Appl Microbiol Biotechnol 85: 1521–1531. https://doi.org/10.1007/s00253-009-2176-4.
- Yi HS, Ahn YR, Song GC, Ghim SY, Lee S, Lee G, Ryu CM. 2016. Impact of a bacterial volatile 2,3-butanediol on *Bacillus subtilis* rhizosphere robustness. Front Microbiol 7:993. https://doi.org/10.3389/fmicb.2016.00993.
- Xie SS, Wu HJ, Zang HY, Wu LM, Zhu QQ, Gao XW. 2014. Plant growth promotion by spermidine-producing *Bacillus subtilis* OKB105. Mol Plant Microbe Interact 27:655–663. https://doi.org/10.1094/MPMI-01-14-0010-R.
- Chaoprasid P, Nookabkaew S, Sukchawalit R, Mongkolsuk S. 2015. Roles of *Agrobacterium tumefaciens* C58 ZntA and ZntB and the transcriptional regulator ZntR in controlling CD²⁺/Zn²⁺/Co²⁺ resistance and the peroxide stress response. Microbiology 161:1730–1740. https://doi.org/10 .1099/mic.0.000135.
- Brown NL, Stoyanov JV, Kidd SP, Hobman JL. 2003. The MerR family of transcriptional regulators. FEMS Microbiol Rev 27:145–163. https://doi .org/10.1016/S0168-6445(03)00051-2.
- Yuan W, Glekas GD, Allen GM, Walukiewicz HE, Rao CV, Ordal GW. 2012. The importance of the interaction of CheD with CheC and the chemoreceptors compared to its enzymatic activity during chemotaxis in *Bacillus subtilis*. PLoS One 7:e50689. https://doi.org/10.1371/journal.pone.0050689.
- Gullett JM, Bible A, Alexandre G. 2017. Distinct domains of CheA confer unique functions in chemotaxis and cell length in *Azospirillum brasilense* Sp7. J Bacteriol 199:e00189-17. https://doi.org/10.1128/JB.00189-17.