Draft Genome Sequence of *Bacillus velezensis* BZR 277, a Prospective Biocontrol Agent against Phytoparasitic Nematodes

Anzhela M. Asaturova,a Anna I. Homyak,a Alexander E. Kozitsyn,a Margarita V. Shternshis,a Andrey L. Rakitin,b Alexey V. Beletsky,b Andrey V. Mardanov,b Nikolai V. Ravinb

aFederal Research Center of Biological Plant Protection, Krasnodar, Russia
bInstitute of Bioengineering, Research Center of Biotechnology, Russian Academy of Sciences, Moscow, Russia

**ABSTRACT** *Bacillus velezensis* strain BZR 277 is a rhizobacterium isolated from the rhizoplane of a winter oilseed rape plant from the Krasnodar region in Russia. This study presents the genome sequence of the rhizobacterium *Bacillus velezensis* BZR 277, which exhibited high antagonistic activity against the root-knot nematode *Meloidogyne incognita* Kof. The data on the genome sequence can help to identify pathways for the biosynthesis of metabolites responsible for nematicidal activity in order to improve crop protection against plant-parasitic nematodes.

*Meloidogyne incognita* is a serious pest of vegetable crops. To control *M. incognita*, several species of *Bacillus* bacteria have been registered, including *B. amyloliquefaciens* (syn. *B. velezensis*), *B. cereus*, *B. circulans*, *B. coagulans*, *B. firmus*, *B. licheniformis*, *B. megaterium*, *B. polymyxa*, and *B. subtilis* (1). It is known that the synthesis of extracellular lytic enzymes and antibiotic compounds by *Bacillus* species can destroy the physiological integrity of nematode eggs and adults (2, 3).

The *B. velezensis* BZR 277 strain was isolated from the rhizoplane of winter oilseed rape plants at the experimental plots of the Bioresource Collection of the Federal State Budgetary Scientific Institution Federal Research Center for Biological Plant Protection (FSBSI FSCBPP) in Krasnodar, Russia. The strain was previously described as *B. amyloliquefaciens* BZR 277 based on its morphological features and analysis of the 16S rRNA gene sequence and was included in the departmental collection of useful microorganisms for agricultural purposes of the Russian Collection of Agricultural Microorganisms (accession number RCAM05296) as a producer of hydrolytic enzymes of the protease and lipase group and as an antagonist of the root-knot nematode *Meloidogyne incognita* Kof. (4, 5). Strain *B. velezensis* BZR 277 was registered as a promising biopreparation producer strain for the protection of vegetable, ornamental, and flower crops against root-knot nematode (6).

A liquid culture of the *B. velezensis* BZR 277 strain was obtained by cultivation in the original nutrient medium. The total DNA was isolated using the DNeasy PowerSoil kit (Qiagen). Genomic DNA was sequenced using Illumina and Oxford Nanopore platforms. The shotgun genome library was prepared using the NEBNext Ultra II DNA library prep kit (New England BioLabs, USA). The sequencing of this library on a MiSeq instrument (Illumina, San Diego, CA) generated 2,613,855 read pairs (2 × 300-nucleotide [nt] mode). In addition, genomic DNA was sequenced on a MinION device (Oxford Nanopore, UK) using the ligation sequencing kit 1D and FLO-MIN110 cells. Nanopore sequencing generated 79,498 reads with an average length of 5,881 nt. Paired overlapping MiSeq reads were merged using FLASH (7), and low-quality sequences were trimmed using Sickle v.1.33 (q = 30) (https://github.com/najoshi/sickle). Nanopore reads were de novo assembled using Flye v.2.7 (8). The consensus sequence was corrected with Pilon v.1.22 (9) using MiSeq reads.
As a result, the complete circular 3,913,640-bp-long genome with a G+C content of 46.6% was obtained; no plasmids were detected. Gene search and annotation were performed using the National Center for Biotechnology Information (NCBI) Prokaryotic Genome Annotation Pipeline (10). The default settings were used for all software. In the assembled genome, 9 16S-23S-5S rRNA gene clusters were found. Comparison of the 16S rRNA gene sequence of \textit{B. velezensis} BZR 277 with the GenBank database showed that the strain belongs to the genus \textit{Bacillus}. The strain was classified as \textit{Bacillus velezensis} based on 97.6% average nucleotide sequence identity to \textit{Bacillus velezensis} strain NRRL B-41580.

**Data availability.** The complete genome sequence of \textit{B. velezensis} BZR 277 has been deposited in GenBank under the accession number CP064845. The version described in this paper is the first version (CP064845.1). The raw sequences have been deposited in the Sequence Read Archive under the accession numbers SRX9502288 and SRX9502287.

**ACKNOWLEDGMENTS**

The work was carried out on the material and technical base of the Unique Scientific Facility (program 671367) using the new strains from the Bioresource Collection of the Federal State Budgetary Scientific Institution Federal Research Center for Biological Plant Protection (State Collection of Entomocariphages and Microorganisms). Genome sequencing was performed using the scientific equipment of the Core Research Facility “Bioengineering” (Research Center of Biotechnology RAS).

The studies were supported by the Kuban Science Foundation (grant MFI-20.1/68).

**REFERENCES**

1. Xiang N, Lawrence KS, Kloepper JW, Donald PA, McInroy JA, Lawrence GW. 2017. Biological control of \textit{Meloidogyne incognita} by spore-forming plant growth-promoting rhizobacteria on cotton. Plant Dis 101:774–784. https://doi.org/10.1094/PDIS-09-16-1369-RE.

2. Mota MS, Gomes CB, Souza Júnior IT, Moura AB. 2017. Bacterial selection for biological control of plant disease: criterion determination and validation. Braz J Microbiol 48:62–70. https://doi.org/10.1016/j.bjm.2016.09.003.

3. Choi TG, Maung CEH, Lee DR, Henry AB, Lee YS, Kim KY. 2020. Role of bacterial antagonists of fungal pathogens, \textit{Bacillus thuringiensis} KYC and \textit{Bacillus velezensis} CE 100 in control of root-knot nematode, \textit{Meloidogyne incognita} and subsequent growth promotion of tomato. Biocont Sci Technol 30:685–700. https://doi.org/10.1080/09598315.2020.1765980.

4. Asaturova AM, Dubyaga VM, Tomashevich NS, Zhamnikova MD. 2012. Selection of perspective biological control agents for fall wheat protection against fusarium diseases. (In Russian.) Russian Academy of Agricultural Sciences, Krasnodar, Russia. http://ej.kubagro.ru/2012/01/pdf/37.pdf.

5. Lychagina SV, Migunova VD, Asaturova AM. 2017. The influence of \textit{Bacillus} bacteria on the development of cucumber plants affected by the gall nematode. Theory Practice Parasitic Dis Control 18:227–229.

6. Sidorov NM, Asaturova AM, Dubyaga VM, Homyak AI. May 2021. \textit{Bacillus amyloliquefaciens} bacterial strain, possessing nematicidal activity against root-knot nematodes. Russian patent 2745156.

7. Magoc T, Salzberg S. 2011. FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics 27:2957–2963. https://doi.org/10.1093/bioinformatics/btr507.

8. Kolmogorov M, Yuan J, Lin Y, Pevzner PA. 2019. Assembly of long, error-prone reads using repeat graphs. Nat Biotechnol 37:540–546. https://doi.org/10.1038/s41587-019-0072-8.

9. Walker BJ, Abeel T, Shea T, Priest M, Abouelliel A, Sakhikumar S, Cuomo CA, Zeng Q, Wortman J, Young SK, Earl AM. 2014. Pilon: an integrated tool for comprehensive microbial variant detection and genome assembly improvement. PLoS One 9:e112963. https://doi.org/10.1371/journal.pone.0112963.

10. 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.