

Draft genome sequence of *Bacillus velezensis* strains AOA1 and AKS2, the potential plant growth-promoting rhizobacteria

Olubukola Oluranti Babalola,¹ Akinlolu Olalekan Akanmu,¹ Ayansina Segun Ayangbenro¹

AUTHOR AFFILIATION See affiliation list on p. 2.

ABSTRACT This report describes the draft genome sequence of *Bacillus velezensis* strains AOA1 and AKS2 isolated from maize rhizosphere soil in South Africa. *Bacillus velezensis* plays important biological roles as plant growth promoting rhizobacterium (PGPR). *Bacillus velezensis* strains also exhibit numerous biotechnological application potentials in agriculture and diverse industrial settings.

KEYWORDS bacteria, PGPR, Illumina Nextera, genome assembly, DNA extraction, KBase, soil microbe extraction

Bacillus species are endospore-forming and rod-shaped bacteria, which are either aerobic or facultative anaerobe. Many species exhibit a wide range of physiological traits, which enable their survival in a diverse natural environment (1). *Bacillus velezensis* was formerly classified as strains of *Bacillus amyloliquefaciens* but reclassified based on genomic analysis (2, 3). Comparative genomics and DNA-DNA relatedness calculations had, however, showed that *B. velezensis* was taxonomically distinct from *B. amyloliquefaciens* (4). Many of the *B. velezensis* strains are gaining relevance for their applications in medicine, pharmaceuticals, food industry, and environment (5). Moreover, this species is harmless to both humans and animals, and environmentally safe (4).

Experimental samples were collected at the North-West University Research Farm, Molelwane, South Africa (25°47'26.4" S 25°37'01.6" E) on March 2022. Maize plants were randomly selected, uprooted, and 10 g of soil tightly adhering to maize roots was scraped. Isolation of bacteria from the soil samples was carried out by serial dilution, plated on Luria-Bertani (LB) agar plates and incubated at 28°C ± 2°C for 24 h. Individual bacterial colonies were picked and streaked on LB plates for further purification. The growth from a single colony was selected and confirmed through morphological and biochemical tests in-line with the protocol described by Majeed et al. (6).

A ZF Fungal/Bacterial DNA MiniPrep Extraction Kit by Zymo Research, USA was used for genomic DNA extraction from pure culture grown on solid agar. The genome of each strain was subsequently sequenced at Novogene Co. Ltd., Singapore. Library was prepared from the DNA sample using Illumina Nextera DNA Flex Library Preparation Kit. The prepared library was sequenced using NovaSeq 6000 (2 × 150 bp).

The whole-genome sequencing analysis was conducted with KBase (7). FastQC v43.1.0.4 (8) was employed in examining the read quality, while adapter regions and low-quality reads were filtered using Trimmomatic v1.2.14 (9). The total read pairs of 4,394,405 and 3,455,403 were obtained after filtering and trimming for *Bacillus velezensis* strains AOA1 and AKS2, respectively. The genome assembly was performed using SPAdes v1.2.4 (10). The assembled genomes were subjected to BLAST assessment using GTDB-Tk v1.7.0 (11), which showed FastANI taxonomy of 98.13 and 98.08 with *Bacillus velezensis*, respectively. The presence of secondary metabolites, such as iturins, fengycins, and surfactins, was revealed in both strains with antiSMASH (version 7.0.1) (12). The

Editor Elinne Becket, California State University San Marcos, USA

Address correspondence to Olubukola Oluranti Babalola, olubukola.babalola@nwu.ac.za.

The authors declare no conflict of interest.

See the funding table on p. 2.

Received 16 September 2023

Accepted 6 February 2024

Published 27 February 2024

Copyright © 2024 Babalola 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/).

TABLE 1 Whole-genome sequencing characteristics of *Bacillus velezensis* strains AOA1 and AKS2

Parameter	<i>Bacillus velezensis</i>	
	Strain AOA1	Strain AKS2
Genome size	3,919,636	3,935,018
G + C contents (%) (bp)	46.43	46.38
Number of contigs	12	21
Total read pairs	4,394,405	3,455,403
N50 (bp)	2,072,719	1,238,655
L50	1	2
FastANI taxonomy	98.13	98.08
Number of genes (total)	3,873	3,888
DNA sequences (CDSs)	3,820	3,734
Number of proteins	3,746	3,734
Number of rRNAs	1, 2, 1 (5S, 16S, 23S)	2, 3, 2 (5S, 16S, 23S)
Number of tRNAs	44	56
Number of pseudogenes	74	86
Noncoding sequences	53	68
Noncoding RNAs	5	5

annotation for prediction of gene functions was conducted using the NCBI Prokaryotic Genome Annotation Pipeline (13) (Table 1).

ACKNOWLEDGMENTS

O.O.B. acknowledges the National Research Foundation of South Africa for the funds (grant numbers UID123634 and UID 132595) that support this research.

O.O.B. conceptualized this study. A.O.A. and A.S.A. designed and carried out the experiments. O.O.B., A.O.A. and A.S.A. conducted the bioinformatic analyses. O.O.B. thoroughly critiqued the article. All authors approved the manuscript for publication.

AUTHOR AFFILIATION

¹Food Security and Safety Focus Area, Faculty of Natural and Agricultural Sciences, North-West University, Mafikeng, South Africa

AUTHOR ORCIDs

Olubukola Oluranti Babalola  <http://orcid.org/0000-0003-4344-1909>

Akinlolu Olalekan Akanmu  <http://orcid.org/0000-0003-2816-9820>

Ayansina Segun Ayangbenro  <http://orcid.org/0000-0002-3220-1873>

FUNDING

Funder	Grant(s)	Author(s)
National Research Foundation (NRF)	UID123634, UID132595	Olubukola Oluranti Babalola

AUTHOR CONTRIBUTIONS

Olubukola Oluranti Babalola, Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing – review and editing | Akinlolu Olalekan Akanmu, Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Writing – review and editing, Writing – original draft | Ayansina Segun Ayangbenro, Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – review and editing

DATA AVAILABILITY

The whole-genome sequences of *Bacillus velezensis* strains AOA1 and AKS2 have been deposited at DDBJ/ENA/GenBank under the accession numbers [JAVHYM000000000](#) and [JAVKYN000000000](#). The versions described in this paper are versions [JAV-HYM000000000.1](#) and [JAVKYN000000000.1](#), respectively. Also, the raw reads are available under BioProject accession numbers [PRJNA1010530](#) and [PRJNA1014219](#), with the BioSample accession numbers [SAMN37190764](#) and [SAMN37321166](#). The sequence data obtained in this work have been deposited in the NCBI. Sequence Read Archive under the accession numbers [SRR25784552](#) and [SRR25949163](#) for strains AOA1 and AKS2, respectively.

REFERENCES

1. To HTA, Chhetri V, Settachaimongkon S, Prakitchaiwattana C. 2022. Stress tolerance-*Bacillus* with a wide spectrum bacteriocin as an alternative approach for food bio-protective culture production. *Food Control* 133:108598. <https://doi.org/10.1016/j.foodcont.2021.108598>
2. Adeniji AA, Loots DT, Babalola OO. 2019. *Bacillus velezensis*: phylogeny, useful applications, and avenues for exploitation. *Appl Microbiol Biotechnol* 103:3669–3682. <https://doi.org/10.1007/s00253-019-09710-5>
3. Li L, Wang R, Liang X, Gai Y, Jiao C, Wang M. 2023. Characterization of a *Bacillus velezensis* with antibacterial activity and its inhibitory effect on gray mold germ. *Agronomy* 13:1553. <https://doi.org/10.3390/agronomy13061553>
4. Ye M, Tang X, Yang R, Zhang H, Li F, Tao F, Li F, Wang Z. 2018. Characteristics and application of a novel species of *Bacillus*: *Bacillus velezensis*. *ACS Chem Biol* 13:500–505. <https://doi.org/10.1021/acscchembio.7b00874>
5. Quach NT, Vu THN, Nguyen TTA, Ha H, Ho P-H, Chu-Ky S, Nguyen L-H, Van Nguyen H, Thanh TTT, Nguyen NA, Chu HH, Phi Q-T. 2022. Structural and genetic insights into a poly-γ-glutamic acid with *in vitro* antioxidant activity of *Bacillus velezensis* VCN56. *World J Microbiol Biotechnol* 38:173. <https://doi.org/10.1007/s11274-022-03364-8>
6. Majeed A, Abbasi MK, Hameed S, Imran A, Rahim N. 2015. Isolation and characterization of plant growth-promoting rhizobacteria from wheat rhizosphere and their effect on plant growth promotion. *Front Microbiol* 6:198. <https://doi.org/10.3389/fmicb.2015.00198>
7. Arkin AP, Cottingham RW, Henry CS, Harris NL, Stevens RL, Maslov S, Dehal P, Ware D, Perez F, Canon S, et al. 2018. KBase: the United States department of energy systems biology knowledgebase. *Nat Biotechnol* 36:566–569. <https://doi.org/10.1038/nbt.4163>
8. Andrews S. 2010. FastQC: a quality control tool for high throughput sequence data. Babraham Bioinformatics, Babraham Institute, Cambridge, United Kingdom.
9. 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>
10. Nurk S, Bankevich A, Antipov D, Gurevich AA, Korobeynikov A, Lapidus A, Prjibelski AD, Pyshkin A, Sirotkin A, Sirotkin Y, Stepanauskas R, Clingenpeel SR, Woyke T, McLean JS, Lasken R, Tesler G, Alekseyev MA, Pevzner PA. 2013. Assembling single-cell genomes and mini-metagenomes from chimeric MDA products. *J Comput Biol* 20:714–737. <https://doi.org/10.1089/cmb.2013.0084>
11. Chaumeil P-A, Mussig AJ, Hugenholtz P, Parks DH. 2019. GTDB-Tk: a toolkit to classify genomes with the genome taxonomy database. *Bioinformatics* 36:1925–1927. <https://doi.org/10.1093/bioinformatics/btz848>
12. Blin K, Shaw S, Augustijn HE, Reitz ZL, Biermann F, Alanjary M, Fetter A, Terlouw BR, Metcalf WW, Helfrich EJN, van Wezel GP, Medema MH, Weber T. 2023. antiSMASH 7.0: new and improved predictions for detection, regulation, chemical structures and visualisation. *Nucleic Acids Res* 51:W46–W50. <https://doi.org/10.1093/nar/gkad344>
13. Haft DH, DiCuccio M, Badretdin A, Brover V, Chetverin V, O'Neill K, Li W, Chitsaz F, Derbyshire MK, Gonzales NR, Gwadz M, Lu F, Marchler GH, Song JS, Thanki N, Yamashita RA, Zheng C, Thibaud-Nissen F, Geer LY, Marchler-Bauer A, Pruitt KD. 2018. RefSeq: an update on prokaryotic genome annotation and curation. *Nucleic Acids Res* 46:D851–D860. <https://doi.org/10.1093/nar/gkx1068>