



Genome Sequence of Lipopeptide- and Antioxidant-Producing Strain *Bacillus velezensis* NWUMFkBS10.5

 Adetomiwa A. Adeniji,^{a*}  Olubukola O. Babalola^a

^aFood Security and Safety Niche Area, Faculty of Natural and Agricultural Science, North-West University, Mmabatho, South Africa

ABSTRACT Candidate biocontrol agent *Bacillus velezensis* NWUMFkBS10.5 possesses unique genomic characteristics revealed by antiSMASH analysis and *in vitro* metabolomic elucidation. Besides its capability to produce antimicrobial lipopeptides, further *in silico* genome profiling predicted the presence of metabolic pathways for synthesizing antioxidants like lampranthin-2, miraxanthin V, and 2-decarboxybetanidin.

The agroindustrial relevance of *Bacillus velezensis* species has become prominent in recent years (1–3), and more significantly, several strains producing beneficial lipopeptides with broad antimicrobial properties that could be exploited *in planta* and biotechnologically have been documented (4–7). With a view to providing an indigenous biocontrol agent against the etiological agents (e.g., *Fusarium verticillioides*, *Fusarium culmorum*, and *Fusarium graminearum*) of fusariosis, a worldwide disease that affects the quality and production of major cereal grains, we report here the draft genome sequence of an anti-*Fusarium* and antioxidant-producing *B. velezensis* strain isolated from the maize rhizosphere in the North West region of South Africa (8, 9). A pure culture of isolate NWUMFkBS10.5 was obtained from a distinct colony after 5 g of rhizospheric soil from the maize farm was cultured on HiCrome (Oxoid) selective *Bacillus* commercial agar plates following the manufacturer's instructions. The isolate exhibited *in vitro* antifungal properties against *F. graminearum* and *F. culmorum* when grown on potato dextrose agar (Oxoid) (28°C for 7 days), and it produced active secondary metabolites (9).

The genome sequence conditions, protocols, assembly, annotation, data mining, and *in silico* analysis of this *B. velezensis* strain (NWUMFkBS10.5) have been previously documented by Adeniji et al. (9). Briefly, genomic DNA of the strain was extracted using the Zymo Research (ZR) soil microbe DNA miniprep genomic isolation kit. Sequencing of the DNA was done with the Illumina MiSeq reagent kit v2 microsystem at MR DNA (Shallowater, TX, USA), using in-house protocols. A Nextera DNA sample preparation kit (Illumina) was used in constructing the library, and thereafter, the Qubit double-stranded DNA (dsDNA) high-sensitivity (HS) assay kit (Life Technologies, Inc.) was used for the final library concentration. Determination of the average library size was done on an Agilent 2100 Bioanalyzer (Agilent Technologies). The library was then pooled and diluted to 12.0 pM, and the paired ends were finally sequenced using a 600-cycle v3 reagent kit (Illumina) with an average coverage of 50×. As previously reported by Adeniji et al. (9), the KBase platform (10) was employed for checking read quality (FastQC v1.0.1), read trimming (Trimmomatic v0.32), and adaptor sequence removal (Cutadapt v1.0.1). The assembly of reads into contigs was carried out on the KBase platform using Assemble with SPAdes v3.13.0. Contigs from KBase were then uploaded to the Rapid Annotations using Subsystems Technology v2.0 (RAST) and SEED Viewer v2.0 servers (11, 12), the PATRIC online server (v3.3.15) (13), and the NCBI Prokaryotic

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

* Present address: Adetomiwa A. Adeniji, Human Metabolomics, Faculty of Natural and Agricultural Science, North-West University, Potchefstroom, South Africa.

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Genome Automatic Annotation Pipeline (PGAAP v4.2) (14) for automated annotation and comparison. Default settings were used for all of the bioinformatics analysis.

From the sequencing results with an average read length of 151 bp, a total of 15,010,234 paired-end reads and 7,505,117 clusters were obtained. The size of the NWUMFkBS10.5 genome and number of contigs were 3,964,473 bp and 37, respectively, with a G+C content of 46.39%. The N_{50} value of the raw sequences was 195,609 bp, and a total of 3,918 genes were annotated with PGAAP, including 3,701 coding genes, 51 rRNAs, 42 tRNAs, 5 noncoding RNAs (ncRNAs), and 166 pseudogenes. AntiSMASH 4.0.0rc1 (default parameters) (15) was used to predict the biosynthetic clusters (16 clusters) and bioproducts (bacillibactin, bacilysin, fengycin, diffidin, iturin, macrolactin, mersacidin, surfactin, and bacillaene) present in NWUMFkBS10.5. Based on phylogenomic characterization and Kyoto Encyclopedia of Genes and Genomes (KEGG) platform metabolic modeling (KEGG map, default settings), the strain was also reported to possess unique biosynthetic pathways capable of producing biomolecules (lampranthin-2, miraxanthin V, gomphrenin-1, 2-decarboxybetanidin, and celosianin-2) not previously documented for *B. velezensis* (8). This genome sequence should provide further avenues for the biotechnological manipulation of NWUMFkBS10.5 for possible applications outside agroindustry.

Data availability. This whole-genome shotgun project was deposited at DDBJ/ENA/GenBank under the accession number [NZ_NITU01000037](https://ncbi.nlm.nih.gov/nucl/NZ_NITU01000037) and the assembly accession number [GCF_002204665](https://ncbi.nlm.nih.gov/nucl/GCF_002204665). The version described here is the first version, NZ_NITU01000037.1. The BioProject and BioSample designations for this project are [PRJNA388288](https://ncbi.nlm.nih.gov/bioproject/PRJNA388288) and [SAMN07174738](https://ncbi.nlm.nih.gov/biosample/SAMN07174738), respectively.

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REFERENCES

- Fan B, Blom J, Klenk H-P, Borriss R. 2017. *Bacillus amyloliquefaciens*, *Bacillus velezensis*, and *Bacillus siamensis* form an "operational group *B. amyloliquefaciens*" within the *B. subtilis* species complex. *Front Microbiol* 8:22. <https://doi.org/10.3389/fmicb.2017.00022>.
- Baptista JP, Sanches PP, Teixeira GM, Morey AT, Tavares ER, Yamada-Ogatta SF, da Rocha SPD, Hungria M, Ribeiro RA, Balbi-Peña MI, Chideroli RT, Pereira UP, de Oliveira AG. 2018. Complete genome sequence of *Bacillus velezensis* LABIM40, an effective antagonist of fungal plant pathogens. *Genome Announc* 6:e00595-18. <https://doi.org/10.1128/genomeA.00595-18>.
- Gao X-Y, Liu Y, Miao L-L, Li E-W, Sun G-x, Liu Y, Liu Z-P. 2017. Characterization and mechanism of anti-*Aeromonas salmonicida* activity of a marine probiotic strain, *Bacillus velezensis* V4. *Appl Microbiol Biotechnol* 101:3759–3768. <https://doi.org/10.1007/s00253-017-8095-x>.
- 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>.
- Yoo Y, Seo D-H, Lee H, Cho E-S, Song N-E, Nam TG, Nam Y-D, Seo M-J. 2019. Inhibitory effect of *Bacillus velezensis* on biofilm formation by *Streptococcus mutans*. *J Biotechnol* 298:57–63. <https://doi.org/10.1016/j.jbiotec.2019.04.009>.
- Rehman NU, Abed RMM, Hussain H, Khan HY, Khan A, Khan AL, Ali M, Al-Nasri A, Al-Harrasi K, Al-Rawahi AN, Wadood A, Al-Rawahi A, Al-Harrasi A. 2018. Anti-proliferative potential of cyclotrapeptides from *Bacillus velezensis* RA5401 and their molecular docking on G-protein-coupled receptors. *Microb Pathog* 123:419–425. <https://doi.org/10.1016/j.micpath.2018.07.043>.
- Cao Y, Pi H, Chandrangsou P, Li Y, Wang Y, Zhou H, Xiong H, Helmann JD, Cai Y. 2018. Antagonism of two plant-growth promoting *Bacillus velezensis* isolates against *Ralstonia solanacearum* and *Fusarium oxysporum*. *Sci Rep* 8:4360. <https://doi.org/10.1038/s41598-018-22782-z>.
- Adeniji AA, Loots DT, Babalola OO. 2019. *Bacillus velezensis*: phylogeny, useful applications, and avenues for exploitation. *Appl Microbiol Biotechnol* 103:3669. <https://doi.org/10.1007/s00253-019-09710-5>.
- Adeniji AA, Aremu OS, Babalola OO. 2018. Selecting lipopeptide-producing, *Fusarium*-suppressing *Bacillus* spp.: metabolomic and genomic probing of *Bacillus velezensis* NWUMFkBS10.5. *Microbiologyopen* 8:e00742. <https://doi.org/10.1002/mbo3.742>.
- Arkin AP, Cottingham RW, Henry CS, Harris NL, Stevens RL, Maslov S, Dehal P, Ware D, Perez F, Canon S, Sneddon MW, Henderson ML, Riehl WJ, Murphy-Olson D, Chan SY, Kamimura RT, Kumari S, Drake MM, Brettin TS, Glass EM, Chivian D, Gunter D, Weston DJ, Allen BH, Baumohl J, Best AA, Bowen B, Brenner SE, Bun CC, Chandonia JM, Chia JM, Colasanti R, Conrad N, Davis JJ, Davison BH, DeJongh M, Devoid S, Dietrich E, Dubchak I, Edirisinghe JN, Fang G, Faria JP, Frybarger PM, Gerlach W, Gerstein M, Greiner A, Gurtowski J, Haun HL, He F, Jain R, Joachimiak MP, Keegan KP, Kondo S, Kumar V, Land ML, Meyer F, Mills M, Novichkov PS, Oh T, Olsen GJ, Olson R, Parrello B, Pasternak S, Pearson E, Poon SS, Price GA, Ramakrishnan S, Ranjan P, Ronald PC, Schatz MC, Seaver SMD, Shukla M, Sutormin RA, Syed MH, Thomason J, Tintle NL, Wang D, Xia F, Yoo H, Yoo S, Yu D. 2018. The United States Department of Energy systems biology knowledgebase (KBBase). *Nat Biotechnol* 36:566–569. <https://doi.org/10.1038/nbt.4163>.
- Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST server: Rapid Annotations using Subsystems Technology. *BMC Genomics* 9:75. <https://doi.org/10.1186/1471-2164-9-75>.
- Overbeek R, Olson R, Pusch GD, Olsen GJ, Davis JJ, Disz T, Edwards RA, Gerdes S, Parrello B, Shukla M, Vonstein V, Wattam AR, Xia F, Stevens R. 2014. The SEED and the Rapid Annotation of microbial genomes using Subsystems Technology (RAST). *Nucleic Acids Res* 42:D206–D214. <https://doi.org/10.1093/nar/gkt1226>.

13. Wattam AR, Davis JJ, Assaf R, Boisvert S, Brettin T, Bun C, Conrad N, Dietrich EM, Disz T, Gabbard JL, Gerdes S, Henry CS, Kenyon RW, Machi D, Mao C, Nordberg EK, Olsen GJ, Murphy-Olson DE, Olson R, Overbeek R, Parrello B, Pusch GD, Shukla M, Vonstein V, Warren A, Xia F, Yoo H, Stevens RL. 2017. Improvements to PATRIC, the all-bacterial bioinformatics database and analysis resource center. *Nucleic Acids Res* 45: D535–D542. <https://doi.org/10.1093/nar/gkw1017>.
14. Pruitt KD, Tatusova T, Brown GR, Maglott DR. 2012. NCBI Reference Sequences (RefSeq): current status, new features and genome annotation policy. *Nucleic Acids Res* 40:D130–D135. <https://doi.org/10.1093/nar/gkr1079>.
15. Weber T, Blin K, Duddela S, Krug D, Kim HU, Bruccoleri R, Lee SY, Fischbach MA, Müller R, Wohlleben W, Breitling R, Takano E, Medema MH. 2015. antiSMASH 3.0—a comprehensive resource for the genome mining of biosynthetic gene clusters. *Nucleic Acids Res* 43:W237–W243. <https://doi.org/10.1093/nar/gkv437>.