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GENOME SEQUENCES

# Complete Genome Sequences of Two *Bacillus velezensis* Strains Isolated from California Raisin Vineyard Soils

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**ABSTRACT** *Bacillus velezensis* strains JP3042 and JP3144 were isolated from California raisin vineyard soils and were selected for further study of *in vitro* antifungal activity. Here, we present the complete genome sequences of these strains to aid in the understanding of their antifungal activity and diversity within the species.

*Bacillus velezensis* is a Gram-positive soil and rhizosphere bacterium within "operational group *Bacillus amyloliquefaciens*" of the *Bacillus subtilis* species complex (1). This group exhibits antibacterial (2–4), antifungal (5, 6), antinematodal (7), and plant growth-promoting activities (8). *B. velezensis* strains JP3042 and JP3144 were isolated from cultivated vineyard soil in Fresno, California, by spreading soil suspensions in sterile 0.05% Tween 80 on 10% tryptic soy agar containing 100 mg/L cycloheximide, with incubation for 48 h at 28°C. Whole-genome comparisons via the Genome Taxonomy Database (GTDB) (9), calculated with FastANI v1.3 (10), classified both strains as *B. velezensis* (average nucleotide identity [ANI] of 98.08% for strain JP3042 and ANI of 99.10% for strain JP3144 with respect to *B. velezensis* type strain NRRL B-41580 [GenBank assembly accession number GCA_001461825.1]). Bacterial cultures were grown for 16 h at 28°C in 50% tryptic soy broth for genomic DNA extraction (sucrose-Tris, with phenol-chloroform cleanup [11]).

Sequencing was performed using Oxford Nanopore Technologies (ONT) and Illumina platforms. ONT libraries were prepared with the ligation sequencing kit (SQK-LSK109) and native barcoding expansions 1 to 12, following the manufacturer's protocols. Genomic DNA was sheared using g-TUBES (Covaris) and size selected (>15 kb) using BluePippin High Pass Plus (Sage Sciences). DNA repair and end preparation were performed for 30 min at 20°C and then for 30 min at 65°C. Libraries were loaded on a R9.4.1 flow cell for 21-h runs at 22 fmol and 28 fmol on a MinION sequencer (MIN-101B), with a MinIT v19.05.2 controller running MinKNOW v3.3.2 and Guppy v3.0.3 with settings for high-accuracy base calling and Q scores of ≥7. Illumina libraries were prepared using the KAPA LTP library preparation kit (Kapa Biosystems, Wilmington, MA). Libraries were loaded into the MiSeq system and sequenced using MiSeq reagent kit v2 with 2 × 250 cycles (Illumina, Inc.).

*De novo* assembly of ONT reads was conducted with Canu v1.8 (12) with default settings and a genome size of 4.2 Mb. Coverage parameters are listed in Table 1. Assemblies created 1 chromosomal contig for each strain. Chromosomes were circularized manually by finding overlap repeats at the contig ends. Chromosomes were rotated manually so that *dnaA* was the first gene, in agreement with previous genomes (13). MiSeq reads (listed in Table 1) were trimmed to a quality score of >Q20 and minimum read length of 50 nucleotides [nt] using the BBDuk v38.84 plug-in Geneious Prime v2021.2.2 (Biomatters, Ltd., Auckland, New Zealand) and were assembled to the respective Canu-based genomes with the reference assembler within Geneious Prime to validate ONT base calling. Final base calls were determined using the Geneious Prime Find Variation module with a minimum coverage of 20× and minimum variant frequency of 0.8. Protein-, rRNA-, and tRNA-coding genes in each genome were annotated using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) v5.2 (14).

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**TABLE 1** Sequencing and genome statistics for the *B. velezensis* strains in this study

| Parameter | Data for: Strain JP3042 | Strain JP3144 |
|---|---|---|
| ONT sequencing | | |
| No. of raw reads | 110,622 | 133,172 |
| $N_{50}$ (nt) | 16,935 | 15,778 |
| Fold coverage | 429 | 476 |
| Illumina sequencing | | |
| No. of raw reads | 938,016 | 1,249,238 |
| Genome size (nt) | 4,066,227 | 4,155,652 |
| GC content (%) | 46.3 | 46.1 |
| No. of predicted coding sequences | 3,849 | 3,993 |
| RNA | | |
| No. of rRNA operons | 9 | 9 |
| No. of tRNA genes | 86 | 86 |
| No. of predicted prophages | 3 | 5 |

Genome statistics are listed in Table 1. Using PHASTER (upgrade 6) (http://phaster.ca) (15, 16), one prophage region was found to be common to strain JP3042 (positions 1240251 to 1272153) and strain JP3144 (positions 1221512 to 1253885). Additional prophage regions were identified in strain JP3042 (positions 1737025 to 1792263 and 2165798 to 2189239) and strain JP3144 (positions 1168176 to 1180549, 1822239 to 1963515, 2687353 to 2722233, and 2724825 to 2736208).

**Data availability.** Genome sequences were deposited in GenBank under accession number CP082243 for *B. velezensis* strain JP3042 chromosome (BioProject accession number PRJNA758083, BioSample accession number SAMN20999035, and SRA accession numbers SRR16873780, SRR18143749, SRR18439287, SRR18439288, and SRR18439289) and accession number CP082283 for *B. velezensis* strain JP3144 chromosome (BioProject accession number PRJNA758085, BioSample accession number SAMN21001928, and SRA accession numbers SRR16872929, SRR18142940, SRR18435833, SRR18435834, SRR18438238, and SRR18438239).

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