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Complete genome sequence of *Bacillus velezensis* Tcba05, a biocontrol strain for multiple plant diseases in Taiwan

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ABSTRACT In this work, we report the complete genome sequence of *Bacillus velezensis* strain Tcba05. This strain is a potent biocontrol agent against multiple plant diseases in Taiwan, including asparagus bean *Fusarium* wilt and cabbage black rot. The assembly consists of a single circular chromosome of 4,027,462 bp with no plasmids.

KEYWORDS genome, *Bacillus velezensis*, *Bacillus amyloliquefaciens*, biological control agent (BCA), plant growth-promoting bacteria (PGPB)

The strain Tcba05 was isolated from the rhizospheric soil of a citrus tree in Taiwan (coordinates: 23.725745, 120.852890) by the Taichung District Agricultural Research and Extension Station on 20 June 2012 (1). It was initially identified as *Bacillus amyloliquefaciens* and exhibited biological control activity against *Fusarium oxysporum* and *Xanthomonas campestris* (1, 2). Here, we report its genome and revise its taxonomic classification.

A hybrid *de novo* assembly strategy was employed, following previous studies (3–6). Unless otherwise stated, kits were used per manufacturer instructions, and bioinformatics tools were applied with default settings.

The strain was revived from a −80°C glycerol stock in Chieh-Chen Huang's laboratory by streaking onto LB (279110; BD Difco, USA) agar and incubated at 30°C for 48 h. A single colony was picked and grown in LB broth at 30°C with shaking (200 rpm) for 48 h. The same inoculum was used for both sequencing platforms. For Illumina, DNA was extracted using the Wizard Genomic DNA Purification Kit (A1120; Promega, USA) and processed using the NEB Library Preparation Kit (07961880001; Roche, Switzerland). Sequencing on NovaSeq X Plus produced 13,589,230 151 bp paired-reads (Q30: 91.82%); no trimming or filtering was conducted. For Oxford Nanopore Technologies (ONT), DNA was extracted using the Nanobind CBB Kit (102-301-900; PacBio, USA). The ONT Ligation Sequencing Kit (SQK-LSK109) and Expansion Kit (EXP-NBD104) were used without shearing for library construction. Sequencing based on MinION (R9.4.1 flow cell; FLO-MIN106) and basecalling with Guppy v6.5.7 in super-accurate mode produced 23,700 raw reads (N50: 33,990 bp; total: 567.5 Mb). Applying a 30,000 bp length cutoff yielded 6,961 filtered reads (N50: 47,236 bp; total: 326.8 Mb).

Assembly using Unicycler v0.5.0 (7) with all Illumina reads and filtered ONT reads produced one closed circular chromosomal contig (4,027,462 bp; 46.5% GC), starting at *dnaA*. Validation involved read mapping with BWA v0.7.17 (8) and Minimap2 v2.15 (9), automated checking with SAMtools v1.9 (10), and manual inspection in IGV v2.11.1 (11). Four mismatches were corrected, yielding an error rate below 10^{-6}. The Illumina and filtered ONT reads provided 509- and 81-fold coverage, respectively.

Annotation was performed by the National Center for Biotechnology Information (NCBI) using the Prokaryotic Genome Annotation Pipeline (PGAP) v6.9 (12). Nine complete rRNA operons, 86 tRNA genes, five ncRNAs, and 3,825 protein-coding genes were annotated. Based on average nucleotide identity (ANI) analysis using fastANI v1.34

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(13), Tcba05 shared 92.31% alignment fraction and 97.48% ANI with *Bacillus velezensis* KCTC 13012^T (GCF_001267695.1), which belongs to the operational group *B. amyloliquefaciens* (14). According to the 95%–96% ANI threshold suggested for prokaryotic species delineation (15, 16), Tcba05 was reclassified as *B. velezensis*.

The benchmarking universal single-copy ortholog (BUSCO) analysis v5.1.2 (17) against the Bacillales data set was performed using gVolante v2.0.0 (18). The result identified 449 complete BUSCOs (99.8%), one fragmented (0.2%), and none missing. This assessment, together with the chromosomal contig being circular, supported that this genome assembly is complete.

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DATA AVAILABILITY

This genome project has been deposited in NCBI under the BioProject accession number PRJNA1191524. Metadata associated with this strain have been provided in BioSample SAMN45085276. The Illumina and ONT raw reads have been deposited in NCBI Sequence Read Archive under accession numbers SRX27637767 and SRX27637766, respectively. The genome sequence has been deposited in GenBank under accession number CP175541.

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