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Draft Genome Sequence of *Bacillus thuringiensis* serovar aizawai AS23, Isolated from the Rhizosphere of Korean Melon (*Cucumis melo* L.)

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We report the draft genome sequence of *Bacillus thuringiensis* serovar aizawai AS23, an insecticidal strain targeting lepidopteran pests, which was isolated from the rhizosphere of Korean melon (*Cucumis melo* L.). The genome of strain AS23 comprising 6,846,584 bp with a G + C content of 34.83% was assembled to 11 contigs obtained using hybrid assembly. Additionally, we mined the genome for pesticidal genes, identifying several insecticidal genes, including *Cry1Aa3*, *Cry1Ca9*, *Cry1Da2*, *Cry1Ia44*, *Cry2Ab41*, *Cry9Ea9*, *Spp1Aa1*, and *Vip3Aa86*.

Keywords: ***Bacillus thuringiensis*** **serovar aizawai, draft genome, Korean melon, biopesticide**

Bacillus thuringiensis is a Gram-positive, spore-forming bacterium that belongs to the *Bacillus* genus. It is widely recognized for its insecticidal properties, making it valuable in agricultural and biological pest control applications [1, 2]. *B. thuringiensis* produces insecticidal proteins, known as *Cry* and *Cyt* toxins (also called delta-endotoxins), which are toxic to a variety of insect pests, certain orders like Lepidoptera (moths and butterflies), Diptera (flies and mosquitoes), and Coleoptera (beetles) [3–5]. In particular, *B. thuringiensis* serovar aizawai AS23 is a subspecies that has an insecticidal effect on lepidopteran pests.

In this study, *B. thuringiensis* serovar aizawai AS23 was isolated from the rhizosphere soil of Korean melon (*Cucumis melo* L.), which was sampled from the greenhouse at the Seongju Korean Melon Fruit and Vegetable Research Institute (Seongju, Republic of Korea). The draft genome sequence of the strain is reported.

The genomic DNA of strain AS23 was extracted using the Wizard genomic DNA purification kit (Promega, USA) following the manufacturer's instructions. The quality and quantity of the extracted DNA were assessed using the Qubit Flex Fluorometer (Thermo Fisher Scientific, USA) and NanoDrop One Microvolume UV-Vis Spectrophotometer (Thermo Fisher Scientific). Sequencing was performed on two platforms: MinION (Oxford Nanopore Technologies [ONT], UK) and DNBSEQ-G400RS (MGI Tech, China), at the NGS Core Facility (Kyungpook National University, Republic of Korea).

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Long-read sequencing was carried out using the ONT MinION platform with the sequencing library prepared using a ligation sequencing kit SQK-LSK109 (ONT) and the NEBNext companion module (New England Biolabs, USA). The library was sequenced for 48 h on a FLO-MIN111 flow cell R10.4.1 (ONT, USA). Guppy v4.4.1 software was employed in high-accuracy mode to perform base calling and generate FASTQ files. For quality trimming, sequences with Phred scores below 7 were excluded from further analyses.

For short-read sequencing, the sequencing library was prepared using the MGIEasy FS DNA Library Prep Kit and DNBSEQ-G400RS High-throughout Sequencing Kit PE100 (MGI Tech., China) following the manufacturer's instructions. The same batched genomic DNA was sheared to approximately 100 bp using the Frag enzyme II and then end-repaired using the ERAT enzyme provided by the manufacturer. DNA Nano Ball (DNB) was constructed after circularization of the single-stranded DNA. The final DNB library was loaded into the flow cell and 2 ×100-bp pair-end sequenced for 72 h using a DNBSEQ-G400RS sequencer (MGI Tech.).

The hybrid assembly of long- and short-read sequences was performed using MaSuRCA (Maryland Super Read Cabog Assembler) version 4.0.9 with default settings [6]. Scaffolding was carried out using the assembled contigs as input in CSAR version 1.1.1 [7] with reference genome, i.e., *B. thuringiensis* serovar *berliner* ATCC 10792 (GenBank accession no. CM000753.1). The scaffolds generated by CSAR were subsequently polished using Polypolish version 0.5.0 [8].

Table 1. Genome feature of *B. thuringiensis* serovar aizawai AS23.

Feature	Value
Genome size (bp)	6,846,584
Number of contigs	11
G + C ratio (%)	34.83
Total number of genes	7,120
Number of protein-coding genes	6,639
Total number of RNA genes	155
rRNA genes (5S, 16S, 23S)	14, 14, 14
tRNA genes	108
ncRNA genes	5
Pseudo genes	326

The final assemblies were annotated using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP), and summarized results are presented in Table 1.

For mining pesticidal genes, protein-coding sequences (CDS) were predicted using Prodigal version 2.6.3 with the "-p meta" option [9]. Insecticidal toxic gene database was downloaded from the Bacterial Pesticidal Protein Resource Center (https://www.bpprc-db.org [10]), and predicted CDSs were annotated using blastp with a minimum of 80% identity and only the top alignment score. In total, the following insecticidal genes were identified: *Cry1Aa3*, *Cry1Ca9*, *Cry1Da2*, *Cry1Ia44*, *Cry2Ab41*, *Cry9Ea9*, *Spp1Aa1*, and *Vip3Aa86*.

Data Availability

The draft genome sequence of strain AS23 has been deposited in GeneBank under accession number PRJNA399840 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA399840).

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Conflict of Interest

The authors have no financial conflicts of interest to declare.

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