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Data Descriptor

Genomic Insights into *Bacillus thuringiensis* V-CO3.3: Unveiling Its Genetic Potential against Nematodes

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Abstract: *Bacillus thuringiensis* (Bt) is a Gram-positive, spore-forming, and ubiquitous bacterium harboring plasmids encoding a variety of proteins with insecticidal activity, but also with activity against nematodes. The aim of this work was to perform the genome sequencing and analysis of a native Bt strain showing bipyramidal parasporal crystals and designated V-CO3.3, which was isolated from the dust of a grain storehouse in Córdoba (Spain). Its genome comprised 99 high-quality assembled contigs accounting for a total size of 5.2 Mb and 35.1% G + C. Phylogenetic analyses suggested that this strain should be renamed as *Bacillus cereus s.s.* biovar Thuringiensis. Gene annotation revealed a total of 5495 genes, among which, 1 was identified as encoding a Cry5Ba homolog protein with well-documented toxicity against nematodes. These results suggest that this Bt strain has interesting potential for nematode biocontrol.

Dataset: The BioSample data have been submitted under accession number SAMN41014059. The whole-genome sequences have been submitted to the NCBI with BioProject number PRJNA1102171. The assembled genome sequences are available under accession number JBCGZW000000000. The (Illumina) raw reads have been submitted to Sequence Read Archives (SRA) under accession number SRR28927673. The V-CO-3.3 strain has been stored in the Spanish Type Culture Collection (CECT) at the University of Valencia (Spain) under accession number CECT 31068.

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Keywords: delta endotoxin; nematicidal proteins; Cry5; genome sequence

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1. Summary

Bacillus thuringiensis (Bt) is a ubiquitous, Gram-positive, and spore-forming bacterium, capable of producing a variety of proteins with toxic activity against insect pests of different orders, including human-disease vectors (mosquitoes) [1]. Beyond this feature, Bt is also able to produce a sub-group of proteins with toxicity against nematodes of agronomic relevance (e.g., plant-pathogenic nematodes), but also against species of veterinary and human importance (e.g., parasitic disease-causing worms) [2–4]. Most of these proteins belong to a major group of proteins known as three-domain crystal proteins, namely Cry5, Cry12, Cry13, Cry14, Cry21, and Cry31 [5,6], plus others, belonging to the App6 class of alpha-helical pesticidal proteins (previously Cry6) and the latter to the Xpp55 class of proteins (previously Cry55), with yet uncharacterized structures [7]. Some of these proteins have shown remarkable nematicidal activities and have become promising tools for the biological control of harmful nematodes [5].

The mode of action of insecticidal Cry proteins, including Xpp proteins from the new nomenclature [7], is generally assumed to produce osmotic shock resulting from the

pore-forming action of the Cry protein. This process occurs after the protein is activated by insect midgut proteases and binds to receptors [8]. In the case of nematicidal Cry5 proteins, its mode of action is also generally accepted to behave similarly to that of insecticidal Cry proteins. Glycosphingolipids and the cadherin CDH-8 have been proposed as receptors for Cry5B, as the deletion of both genes in *Caenorabditis elegans* results in partial resistance to this toxin [9].

In this study, we conducted the whole-genome sequencing analysis of the bacterial strain V-CO3.3 to identify genes associated with biocidal activity and to determine the phylogenetic position of the bacterial strain into the *Bacillus cereus* group.

2. Data Description

2.1. Isolation and Morphological Characterization of V-CO3.3 Strain

The Bt strain V-CO3.3 was isolated in 1998 from dust from a grain storehouse in the province of Córdoba, Spain [10], and chosen after a comprehensive screening of strains carrying genes coding for nematicidal proteins [11]. The colony incubated for 72 h at 29 °C on CCY agar formed typical Bt colonies showing a matte white color and irregular borders (Figure 1A). The Bt vegetative cells displayed a rod-shaped morphology with subterminal spores along with bipyramidal parasporal crystals (Figure 1B).

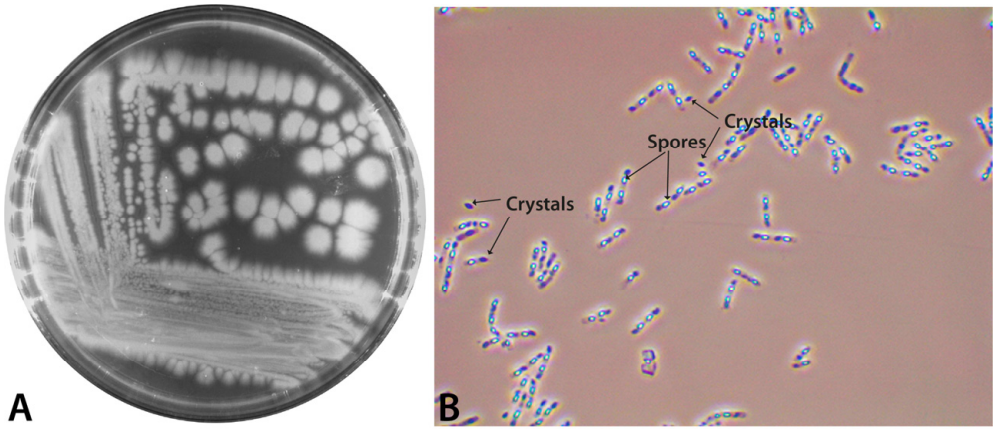


Figure 1. Morphological characterization of Bt strain V-CO3.3. (**A**) after 72 h of incubation on CCY agar at 29 °C and (**B**) sporulated colony material exhibiting spores and bipyramidal crystals under a phase-contrast microscope (1000×).

Based on macro- and microscopical morphological analysis, the strain was preliminarily identified as belonging to the *B. thuringiensis* species and was designated as V-CO3.3. This strain was included in 2024 in the Spanish Type Culture Collection at the University of Valencia (Spain) under accession number CECT 31068.

2.2. Genome Sequencing and Assembly Statistics

The genome sequencing procedure yielded 17,753,390 Illumina paired-end (raw) reads, which were assembled after trimming, generating 99 contig sequences (Table 1). The quality assessment of the genome sequence with ChekM v1.0.18 against *Bacillales* (Marker Lineage), including 139 genomes and 508 markers, showed a high-quality assembled genome with a completeness of 99.43% and no contamination.

Table 1. Summary statistics of the V-CO3.3 genome sequence.

Features	Value
Assembled genome size (bp)	5,265,864
Number of contigs	99
Largest contig (bp)	692,846
Shortest contig (bp)	206
Mean sequence size (bp)	53,190.5
Median sequence size	869
N50 (bp)	203,741
L50	8
G + C content (%)	35.1
Completion (%)	99.43
Contamination (%)	0.0
Estimated Coverage (×)	505

2.3. Phylogenetic Analysis and Species Classification Proposal

In order to better identify the species to which the V-CO3.3 strain belongs, a genome-based phylogenetic tree was built using the tool Insert Genome Into SpeciesTree v2.2.0 imbedded into The Department of Energy Systems Biology Knowledgebase (Kbase) [12] (Figure 2).

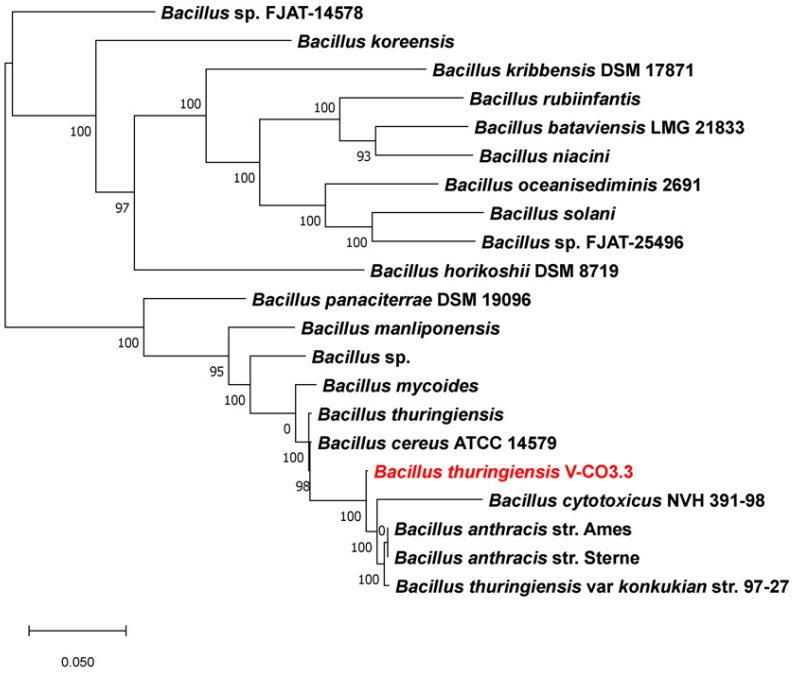


Figure 2. The genome-based phylogenetic tree showing the placement of the V-CO3.3 strain (red color) within *Bacillus* species.

As presented in Figure 2, the V-CO3.3 strain is closely related to the *B. cereus*-type strain, ATCC 14579, as well as to other species in the *B. cereus sensu lato* group. The Average Nucleotide Identity (ANI) threshold of 95–96% is commonly used as a reliable criterion for delineating species boundaries [13,14]. The FastANI v0.1.3 [15] software embedded in KBase was used to estimate %ANI relative to other closely related species genomes (Table 2). Since the ANI calculations between two strains retrieve two different reciprocal values, the mean value should be used for taxonomic purposes. The V-CO3.3 strain showed an ANI percentage greater than the established threshold for both *B. cereus* ATCC 14579 and *B. thuringiensis*, making species delineation inconsistent (Table 2).

Table 2. List of ten related assembled genomes from NCBI RefSeq database [16], in comparison with the genome of the V-CO3.3 strain based on estimated %ANI (average nucleotide identity) calculated using FastANI [15]. %ANI is the average of reciprocal values from two compared genome sequences (i.e., *B. cereus* ATCC 14579 vs. *B. thuringiensis* ATCC 10792 and *B. thuringiensis* ATCC 10792 vs. *B. cereus* ATCC 14579).

NCBI RefSeq Assembly	Species	Average %ANI
GCF_000007825	*B. cereus* ATCC 14579	98.9
GCF_001420855	*B. thuringiensis* ATCC 10792	96.9
GCF_000008505	*B. thuringiensis* var. *konkukian* str. 97-27	91.8
GCF_000008165	*B. anthracis* str. Sterne	91.8
GCF_000007845	*B. anthracis* str. Ames	91.7
GCF_000017425	*Bacillus cytotoxicus* NVH 391-98	82.6
GCF_000712595	*B. manliponensis*	80.3
GCF_000430785	*B. panaciterrae* DSM 19096	77.9
GCF_001648575	*B. horikoshii* DSM 8719	76.6
GCF_000752035	*B. rubiinfantis*	76.0

Additional comparisons were performed using the OAT program against the genomes of *B. cereus* ATCC 14579 and *B. thuringiensis* ATCC 10792, as well as other additional Bt strains (Figure 3). Both ANI and OrthoANI algorithms are comparable, sharing a species demarcation threshold of 95–96%. Similarly, V-CO3.3 showed OAT values greater than the species boundary value (95–96%), which did not allow consistent species delimitation, especially between highly related species such as *B. cereus* ATCC 14579 and *B. thuringiensis* ATCC 10792.

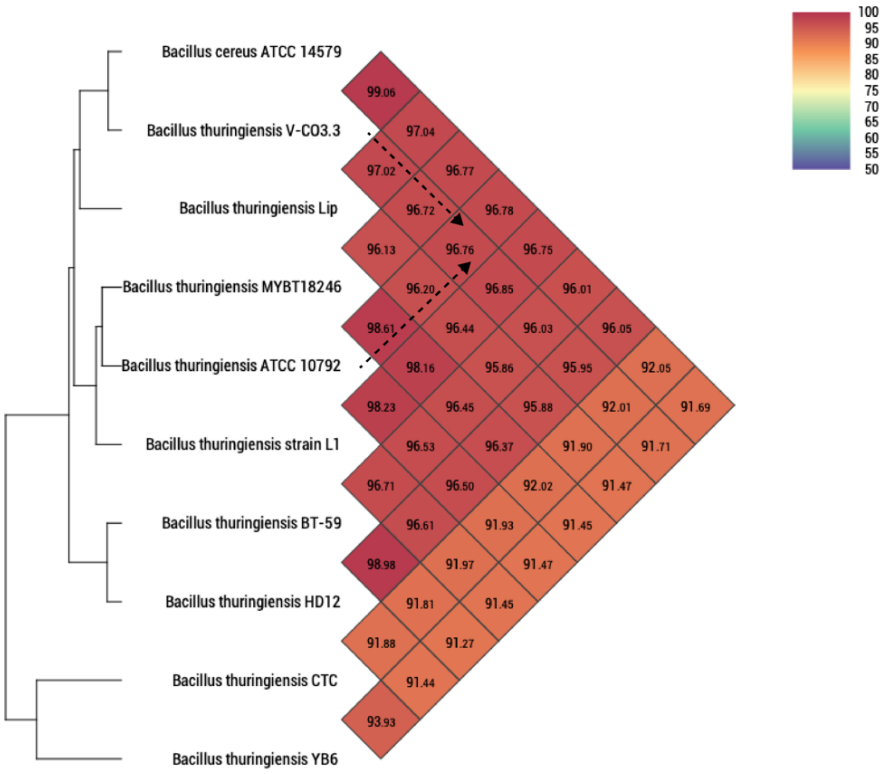


Figure 3. Heatmap displaying the OrthoANI values between the V-CO3.3 strain and closest relative species along with its UPGMA tree. The values are interpreted diagonally. For example, the V-CO3.3 strain showed a 96.76% OAT value against *B. thuringiensis* ATCC 10792 (indicated by black, arrowed dashed lines).

Finally, an additional phylogenetic study was performed using the Type Strain Genome Server (TYGS) [17], which showed that the V-CO3.3 strain is clustered along with the *B. cereus* type strain ATCC 14579 and the *B. thuringiensis* type strain ATCC 10792 in a unique branch (Figure 4).

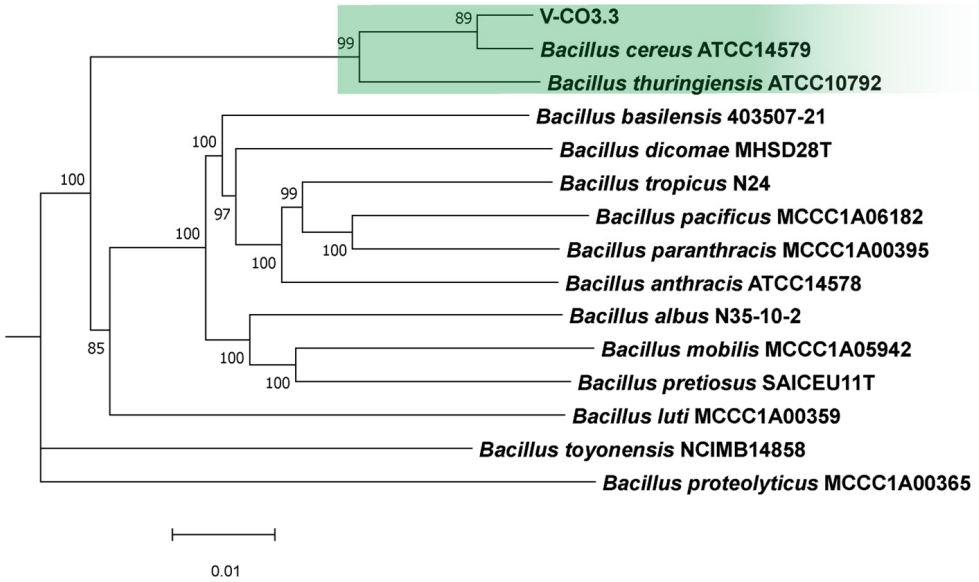


Figure 4. Whole-genome sequence-based tree showing the phylogenetic relationship of the V-CO3.3 strain with other type species strains (97.4% average branch support). Green color was used to highlight the V-CO3.3 strain, which clustered with closely related type strains.

The TYGS also showed a digital DNA-DNA hybridization (dDDH) value of 91% and a difference in the G + C content of 0.17% compared to the *B. cereus* ATCC 14579-type strain (Table 3). The dDDH value of 91% of our strain compared to *B. cereus* ATCC 14579 is above the 70% cut-off. This indicates that the V-CO3.3 strain is highly similar to *B. cereus* ATCC 14579 and likely belongs to the same species. Additionally, the G + C content difference of 0.17% supports this conclusion, as it is within the expected range of variation (no more than 1%) for strains of the same species.

Table 3. Pairwise dDDH (DNA-DNA hybridization) values between the V-CO3.3 genome and the type strain genomes selected by TYGS. The dDDH values are shown, including their confidence intervals (C.I.) for the d_4 formula (d_4 is independent of genome size and therefore robust when using draft genomes) [17].

Subject	d_4 (C.I.)	Diff. G + C %
B. cereus ATCC 14579	91 (88.8–92.8)	0.17
B. thuringiensis ATCC 10792	71 (68.0–73.8)	0.29
B. basilensis 403507-21	45.6 (43.0–48.1)	0.07
B. tropicus N24	45.6 (43.0–48.2)	0.08
B. dicomae MHSD28T	45.0 (42.5–47.6)	0.09
B. paranthracis MCCC 1A00395	44.9 (42.4–47.5)	0.07
B. toyonensis NCIMB 14858	44.8 (42.3–47.4)	0.44
B. anthracis ATCC 14578	44.7 (42.1–47.2)	0.13
B. pacificus MCCC 1A06182	44.4 (41.8–46.9)	0.09
B. albus N35-10-2	43.8 (41.2–46.3)	0.18
B. pretiosus SAICEU11T	43.7 (41.2–46.3)	0.18
B. luti MCCC 1A00359	43.3 (40.8–45.9)	0.33
B. mobilis MCCC 1A05942	42.9 (40.4–45.5)	0.16
B. proteolyticus MCCC 1A00365	39.4 (36.9–41.9)	0.03

This cluster is consistent with the new classification proposed by Carroll et al. (2020), which denoted that most of the biovar Thuringiensis isolates, including the type strain *B. thuringiensis* serovar Berliner ATCC 10792, belong to the *B. cereus sensu stricto* group. Therefore, based on these data, it was concluded that the V-CO3.3 strain should be renamed as *B. cereus s.s.* biovar Thuringiensis [18].

2.4. Genome Analysis and Annotation

The NCBI Prokaryotic Genome Annotation Pipeline (PGAP) found a total of 5234 protein-coding genes (Table 4). These findings are consistent with the size and % G + C already observed in other sequenced genomes of *B. thuringiensis* obtained from the GenBank database [19].

Table 4. Summary of the NCBI Prokaryotic Genome Annotation.

Features	
Genes (total)	5495
CDSs (total)	5372
Genes (coding)	5234
CDSs (with protein)	5234
Genes (RNA)	123
rRNAs	10, 5, 1 (5S, 16S, 23S)
Complete rRNAs	6, 1 (5S, 23S)
Partial rRNAs	4, 5 (5S, 16S)
tRNAs	102
ncRNAs	5
Pseudo Genes (total)	138
CDSs (without protein)	138
Pseudo Genes (ambiguous residues)	0 of 138
Pseudo Genes (frameshifted)	55 of 138
Pseudo Genes (incomplete)	89 of 138
Pseudo Genes (internal stop)	35 of 138
Pseudo Genes (multiple problems)	35 of 138
CRISPR Arrays	1

The genome was also annotated with the RAST server which allowed the detection of chitinase enzymes. These enzymes should play a role in insect pathogenesis because they hydrolyze chitin, a major component of the insect exoskeleton and nematode cuticle [20].

Custom searches of potentially invertebrate-active genes showed the presence of a single CDS encoding a protein with 100% identity with Cry5Ba2 at contig 64, highlighting that, interestingly, the V-CO3.3 strain is monogenic for *cry* genes. Cry5 proteins are well known for their nematicidal activity against mammalian parasitic worms (e.g., *Ascaris* sp.) and plant-parasitic nematodes (e.g., *Meloydogines incognita*) [5,21].

In addition, custom BLAST searches conducted to detect the *thuE* gene involved in the synthesis pathway of β-exotoxin failed, suggesting that the V-CO3.3 strain should not be able to produce β-exotoxin, a secretable, harmful, wide-spectrum toxin produced by some Bt strains [22].

The genome of the V-CO3.3 strain was also screened for clusters with the potential to produce secondary metabolites with biological activity using antiSMASH [23] (Table 5). Fengycin is a lipopeptide (a betalactone that contains a protease inhibitor) with antifungal activity [24], whereas bacillibactin has demonstrated antibiotic effects when produced by some strains of *Bacillus amyloliquefaciens* [25]. In contrast, petrobactin has been described to behave as a siderophore [26], while terpene has been described to possess both antagonistic and beneficial effects in different organisms [27]. No clusters potentially related to both insecticidal and nematicidal activity were found using antiSMASH.

Table 5. Predicted biosynthetic gene clusters in the V-CO3.3 genome and % similarity found by antiSMASH.

Contig	Activity	Similar Cluster	% Similarity
74	Beta-lactone	Fengycin	40
82	NI-siderophore	Petrobactin	100
85	NRP-metallophore	Bacillibactin	85
95	Terpene	Molybdenum cofactor	17

Genomic analysis of the *B. thuringiensis* V-CO3.3 strain has revealed its potential for nematode biocontrol, highlighting a single gene encoding a Cry5Ba homolog protein, which is known for its nematicidal properties. Despite these promising genetic findings, it is crucial to perform in vivo experiments to verify the effectiveness of this strain as active against nematodes. This step is essential for validating the findings of this genomic study and ultimately contributing to the development of sustainable pest management strategies.

3. Methods

3.1. Strain Isolation, Growing Conditions, and Strain Identification

The strain was isolated from dust from a grain storehouse in Córdoba province, Spain and grown in CCY medium [28] at 29 °C for 48–72 h until sporulation occurred to perform the macroscopic characterization [8]. The colony was also analyzed under a phase-contrast microscope, Leica DM 2000 LED (Leica, Wetzlar y Mannheim, Germany), to confirm the presence of parasporal crystals using the 1000× objective (Figure 1B).

3.2. DNA Extraction, Library Construction, and DNA Genome Sequencing

The strain was grown overnight in liquid LB medium (labkem, Barcelona, Spain) at 29 °C with shaking (200 rpm). Total DNA, including the chromosome and plasmids (if any), was extracted using the Wizard genomic DNA purification kit from Promega (Madison, WI, USA) following the manufacturer's protocol for Gram-positive bacterial DNA extraction. DNA quality and concentration were assessed using 1% agarose gel electrophoresis and a Nanodrop 2000 Spectrophotometer from Thermo Scientific (Waltham, MA, USA), respectively. The purified DNA sample (2.5 µg/mL, $A_{260/280}$ = 1.98) was then sent to Novogen (Cambridge, UK) to construct a pooled Illumina library by using the Novogene NGS DNA Library Prep Set kit (Cat No. PT004, Novogene, Cambridge, UK) and sequencing using a high-throughput Illumina sequencing platform (Illumina Sequencing PE150).

3.3. Genome Assembly, Sequence Analysis, and Annotation

The de novo genome assembly was conducted over previously trimmed (raw) reads, using the Velvet v1.2.10 assembler tool included in the Geneious R11 software suite (www.geneious.com, accessed on 7 May 2024) with default parameters. The completeness of the assembled genomic sequence and the presence of potential contaminating reads were assessed with CheckM v1.0.18 [29] included in Kbase [12].

Phylogenetic analyses and classification were performed by using three different approaches: (i) the application Insert Genome Into SpeciesTree v2.2.0 from KBase [12], (ii) the OAT (OrthoANI) tool [30], and (iii) the Type Strain Genome Server (TYGS) [17]. SpeciesTree v2.2.0 is useful for constructing a species tree by using a collection of 49 fundamental, universally acknowledged genes delineated by COG (Clusters of Orthologous Groups) gene families: The app merged the studied genome sequence with a curated assortment of closely related genome sequences from the public KBase genome collection imported from NCBI RefSeq database. OAT uses Orthologous Average Nucleotide Identity (OrthoANI) to estimate the overall similarity between two genome sequences. TYGS is a bioinformatics tool designed for the rapid and accurate determination of the taxonomic position of bacterial and archaeal genomes.

The genome was annotated using the automated NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (2024, release) [31] and the RAST server [32], but also by using an in-house custom BLAST [33] database, including known pesticidal proteins obtained and available from the Bacterial Pesticidal Protein Database (https://bpprc-db.org, accessed on 7 May 2024) [34].

A secondary metabolite cluster search was also performed to predict the potential presence of genes involved in bioactive compounds synthesis pathways using antiSMASH [23].

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Data Availability Statement: This whole-genome shotgun project has been deposited in DDBJ/ENA/GenBank under accession number JBCGZW000000000. The version described in this paper is the first version JBCGZW010000000. Illumina paired-end (raw) reads were deposited in the Sequence Read Archive (SRA) database under accession number SRR28927673.

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Conflicts of Interest: The authors declare no conflicts of interest.

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