

Whole-genome and Comparative Genomic Analysis Reveal the Biocontrol and Plant Growth-Promoting Potential of *Bacillus velezensis* JN.Y2 Against Oat Anthracnose

Wei Quan

Inner Mongolia Agricultural University

Ting Zhang

Inner Mongolia Autonomous Region General Station of Forestry and Grassland

Chen-Lu Liu

Inner Mongolia Agricultural University

Shao-Xuan Shi

Inner Mongolia Agricultural University

Xi-Xi Zhang

Inner Mongolia Agricultural University

Ke-Han Liu

Inner Mongolia Agricultural University

Chun-Yang Wang

Inner Mongolia Agricultural University

Ming-Min Zhao

Inner Mongolia Agricultural University

Bao-Zhu Dong

`dongbaozhu2020@imau.edu.cn`

Inner Mongolia Agricultural University

Hong-You Zhou

Inner Mongolia Agricultural University

Research Article

Keywords: *Bacillus velezensis*, Oat anthracnose, Biocontrol, Plant growth promotion, Whole-Genome Sequencing, Comparative genomics

Posted Date: April 3rd, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-9114671/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.
[Read Full License](#)

Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at Functional & Integrative Genomics on June 26th, 2026. See the published version at <https://doi.org/10.1007/s10142-026-01951-8>.

Abstract

Oat anthracnose, primarily caused by *Colletotrichum cereale*, represents a significant threat to oat production, necessitating the development of sustainable biocontrol alternatives. In this study, we characterized an oat endophytic bacterium, *Bacillus velezensis* JN.Y2, isolated from healthy oat leaves in Inner Mongolia. In vitro assays demonstrated that *B. velezensis* JN.Y2 possesses an inhibitory activity against *C. cereale* (74.49%) and exhibits a broad antifungal spectrum. Greenhouse and three-year multi-location field trials confirmed its beneficial biocontrol efficacy, which remained stable even under fluctuating climatic conditions and high disease pressure, consistently outperforming conventional chemical treatments. Beyond disease suppression, *B. velezensis* JN.Y2 significantly enhanced oat growth and grain yield, supported by its ability to produce IAA, solubilize nutrients, and secrete diverse hydrolytic enzymes. Complete genome sequencing revealed a 3.87 Mb circular chromosome containing 13 secondary metabolite BGCs and 4 AOs for RiPPs, including Amylocyclicin and LCI. Comparative genomic analysis highlighted an "open" pangenome and identified 411 unique genes associated with specialized metabolism and environmental sensing. While *B. velezensis* JN.Y2 shares high sequence synteny with *B. velezensis* CBMB205, distinct variations in the sporulation kinase *kinA* and secondary metabolite pathways suggest a fine-tuned adaptation to the oat endosphere. Furthermore, biosafety evaluations confirmed a relatively high level of genetic stability and a lack of active antibiotic resistance. Collectively, these findings provide a beneficial molecular foundation for the application of *B. velezensis* JN.Y2 as a reliable and secure biocontrol agent in sustainable agriculture.

Introduction

Oats (*Avena sativa*) are globally significant cereal crops renowned for their multifunctional properties and rich nutritional profile (Butt et al. 2008). In China, naked oats (*A. nuda*) are predominantly cultivated in regions such as Inner Mongolia, Hebei, and Ningxia, serving as both functional food and essential forage. However, oat cultivation is severely threatened by anthracnose, a destructive disease primarily caused by *Colletotrichum cereale* (Crouch and Beirn, 2009; Shi et al. 2023). Other species, including *C. americae-borealis* and *C. graminicola*, also pose significant risks to oat production (Politis, 1976; Wang et al. 2022). While chemical fungicides remain the conventional management strategy, their application is increasingly hampered by high costs, environmental impacts, and the emergence of pathogen resistance (Janisiewicz and Conway, 2010).

Biological control using beneficial plant endophytes offers a sustainable and eco-friendly alternative to chemical treatments (Khairullina et al. 2023; Wang et al. 2023). Endophytes are recruited by plants from the rhizosphere, often through chemotactic responses to root exudates, and play a critical role in enhancing host health and resilience (Ghimire et al. 2023; Malfanova et al. 2013). Among these, the genus *Bacillus* is distinguished by its robust and well-documented efficacy in promoting plant growth and suppressing pathogens (Miljaković et al. 2020). *Bacillus* strains facilitate plant health through nutrient solubilization, phytohormone synthesis, and the production of a diverse arsenal of secondary

metabolites, such as lipopeptides and cell wall-degrading enzymes (Alamri, 2015; Choub et al. 2021; Grahovac et al. 2023; Zhen et al. 2022).

Despite the recognized potential of *Bacillus* species as biocontrol agents, research on endophytic strains specifically adapted to the oat microenvironment remains limited. While certain strains like *B. subtilis* and *B. velezensis* have shown promise in controlling oat pathogens, there is still a lack of specific strains widely applied for targeted disease management. Understanding the regulatory mechanisms and colonization strategies of these bacteria is essential for their effective application (Nicholson et al. 2000; Ye et al. 2021; Zhang et al. 2023).

In this study, an endophytic strain, Bv. JN.Y2, was isolated from healthy oat leaves in Inner Mongolia. Its broad-spectrum antifungal potential and biocontrol efficacy against oat anthracnose were assessed through in vitro assays, greenhouse experiments, and systematic three-year multi-location field trials. Furthermore, integrated whole-genome and comparative genomic analyses were performed to characterize the genetic landscape of Bv. JN.Y2, specifically investigating evolutionary structural variations, secondary metabolite biosynthesis, and environmental sensing mechanisms. This research aims to elucidate the molecular determinants of the biocontrol and plant growth-promoting capacities of Bv. JN.Y2, providing a beneficial theoretical foundation for its stable colonization and sustainable application within the oat microenvironment.

Materials and methods

Strains, pathogens and culture condition

The strains used in this study are listed in Table 1. Specifically, the endophytic bacterial Bv. JN.Y2 was isolated from healthy oat leaves collected in Jining District, Ulanqab City, Inner Mongolia, China. A kanamycin-resistant mutant, Bv. JN.Y2-kan^R, which was previously constructed and maintained in our laboratory, was used for colonization assays. Stock cultures were maintained at -80 °C in Luria-Bertani (LB) broth (10 g/L tryptone, 10 g/L NaCl, 5 g/L yeast extract) supplemented with 30% (v/v) glycerol. For routine use, Bv. JN.Y2 was cultured on LB agar at 37 °C for 24 h. When culturing Bv. JN.Y2-kan^R, the medium was supplemented with 30 µg/mL kanamycin sulfate.

The fungal pathogens used in this study included *Colletotrichum cereale*, *Colletotrichum fructicola*, *Phyllosticta sorghina*, *Fusarium oxysporum*, *Fusarium graminearum*, *Alternaria alternata*, *Rhizoctonia solani*, and *Didymella glomerata*. All fungi were obtained from the Key Laboratory of Biopesticide Creation and Resource Utilization of Inner Mongolia Autonomous Region. Fungal stocks were stored at -80 °C in 25% (v/v) glycerol. For experiments, mycelial plugs from frozen stocks were inoculated onto the center of Potato Dextrose Agar (PDA, 200 g/L potato, 20 g/L glucose, 18 g/L agar) plates and incubated in the dark at 25 °C for 10–12 days.

Table 1
Bacterial, plasmids and fungal strains used in this study.

Strains/Plasmids	Description	Source
Strains		
Bv. JN.Y2	Wild-type biocontrol strain isolated from oat.	This laboratory
Bv. JN.Y2-kan ^R	Spontaneous kanamycin-resistant mutant (<i>kan^R</i>); used for colonization assays.	This laboratory
<i>B. amyloliquefaciens</i> YN.J3	Biocontrol strain; used as a positive control for biofilm formation assays.	This laboratory
Fungal Pathogens		
<i>Colletotrichum cereale</i>	Anthraxnose pathogen	This laboratory
<i>Colletotrichum fructicola</i>	Anthraxnose pathogen	This laboratory
<i>Phyllosticta sorghina</i>	Leaf spot pathogen	This laboratory
<i>Fusarium oxysporum</i>	Root rot pathogen	This laboratory
<i>Fusarium graminearum</i>	Head blight pathogen	This laboratory
<i>Alternaria alternata</i>	Leaf blotch pathogen	This laboratory
<i>Rhizoctonia solani</i>	Root rot pathogen	This laboratory
<i>Didymella glomerata</i>	Leaf spot pathogen	This laboratory

In Vitro antifungal activity assays

The antagonistic activity of Bv. JN.Y2 was evaluated using a dual-culture assay (Xu, 2020). A 5-mm mycelial plug of each pathogen was placed 2 cm from the edge of a PDA plate. Bv. JN.Y2 was streaked on the opposite side, 3 cm from the fungal plug. Plates inoculated only with the pathogen alone served as controls. After incubation at 25 °C for 5–10 days, the radial growth of the fungus in control (R_c) and treatment (R_t) plates was measured. Each treatment consisted of three independent biological replicates, with five technical replicates per biological replicate. The inhibition rate was calculated as:

$$\text{Inhibition (\%)} = \frac{R_c - R_t}{R_c} \times 100$$

Additionally, to assess the morphological impact, hyphae from the edge of the inhibition zone were observed under a microscope to detect potential abnormalities (Kim, 2022).

To evaluate the broad-spectrum antagonistic activity of Bv. JN.Y2, the strain was tested against seven additional phytopathogenic fungi: *C. fructicola*, *P. sorghina*, *F. oxysporum*, *F. graminearum*, *A. alternata*, *R. solani*, and *D. glomerata*. Each treatment consisted of three independent biological replicates, with five technical replicates per biological replicate. All plates were incubated under the same conditions, and the inhibition rates were calculated.

The biocontrol efficacy of Bv. JN.Y2 was evaluated in a greenhouse pot experiment (Meng et al. 2016). A spore suspension of *C. cereale* was prepared from 14-day-old PDA cultures by rinsing with 0.1 M PBS, with the concentration adjusted to 1×10^6 spores/mL using a hemocytometer. The bacterial inoculum was prepared by harvesting late-exponential phase Bv. JN.Y2 cells through centrifugation at 4°C and 6,000 rpm for 10 min, followed by washing and resuspension in 0.1 M PBS to a final concentration of 1×10^8 CFU/mL. Three treatments were established: (1) Bv. JN.Y2 treatment followed by *C. cereale* inoculation; (2) sterile 0.1 M PBS treatment followed by *C. cereale* inoculation as a negative control; (3) thiophanate-methyl (TM, 1,500-fold dilution) treatment followed by *C. cereale* inoculation as a positive control. Each treatment consisted of three independent biological replicates, with five technical replicates per biological replicate. The disease incidence, disease index and control efficacy were calculated as previously described: Grade 1, 0%; Grade 2, < 10%; Grade 3, 11%–25%; Grade 4, 26%–50%; and Grade 5, > 50% or necrotic (McKinney, 1923). The disease index and control efficacy were calculated as below:

$$DI = \frac{(\sum (\text{Grade} \times n))}{(N \times 5)} \times 100$$

$$\text{Controlefficacy (\%)} = \frac{(DI_{(\text{control})} - DI_{(\text{treatment})})}{DI_{(\text{control})}} \times 100$$

Field trials

Field trials were conducted during the 2023, 2024, and 2025 growing seasons at two distinct locations in Inner Mongolia: Jining District (40.92°N, 113.15°E) and Wuchuan County (41.20°N, 111.47°E). Both sites feature chestnut soil and are endemic regions for oat anthracnose, with severe annual outbreaks of *C. cereale*. During the trial period, annual precipitation increased significantly from approx. 340–360 mm in 2023 to over 500–600 mm in 2024 and 2025, providing a rigorous environment for evaluating biocontrol stability. The experiment followed a randomized complete block design with three treatments (five replicates each): (1) Untreated Control (CK); (2) TM, 1,500-fold dilution; and (3) Bv. JN.Y2 cell suspension (1×10^8 CFU/mL). Each plot was 30 m² (5 m × 6 m) with a 1 m buffer zone maintained between adjacent plots. The oat cultivar "Baiyan No. 2" was sown at a rate of 180 kg/ha. For the Bv. JN.Y2 treatment, seeds

were soaked in the bacterial suspension for 2 h prior to sowing, followed by two foliar sprays in booting stage and heading stage. The CK group seeds were soaked in sterile water for 2 h as a control for the soaking process. For the TM treatment, only foliar sprays were applied at the same time points, with no seed soaking performed. Standard local agronomic practices were followed uniformly across all plots. In October, the disease index and grain yield (kg/ ha) were recorded.

Identification and characterization of Bv. JN.Y2

The morphological characteristics of Bv. JN.Y2 were observed after incubation on LB agar at 37°C for 24 h. Colony morphology, including shape, color, margin, and surface texture, was recorded. Cell morphology, Gram reaction, and endospore formation were examined using light microscopy (Olympus, Tokyo, Japan), respectively (Zhou et al. 2022). Motility was assessed using semi-solid agar tubes.

Physiological and biochemical characteristics were evaluated according to Bergey's Manual of Systematic Bacteriology (Sneath et al. 1986). Catalase activity was determined by bubble formation in 3% H₂O₂, and oxidase activity was tested using tetramethyl-p-phenylenediamine. Hydrolytic activities were assessed on agar plates supplemented with specific substrates: starch (amylase), gelatin (gelatinase), skim milk (protease), and carboxymethyl cellulose (cellulase). The metabolic profile was further characterized through standard assays, including nitrate reduction, Voges-Proskauer (V-P), methyl red, indole production, H₂S production, urease activity, and citrate utilization. Carbon source utilization was tested in minimal medium supplemented with 1% (w/v) of glucose, sucrose, maltose, D-mannitol, or lactose as the sole carbon source.

Genomic DNA was extracted from overnight cultures of Bv. JN.Y2 using a bacterial genomic DNA extraction kit (Tiangen, Beijing, China). For taxonomic identification, the 16S rRNA gene was amplified using specific primers as detailed in Online Resource 1 (Table S1). The PCR amplification was performed under the following conditions: initial denaturation at 95°C for 3 min; followed by 33 cycles of denaturation at 94°C for 25 s, annealing at 57°C for 25 s, and extension at 72°C for 60 s; and a final extension at 72°C for 5 min. PCR products were purified and sequenced by Tsingke Biotechnology Co., Ltd. (Beijing, China). The resulting sequence was aligned with reference sequences of closely related *Bacillus* species retrieved from the NCBI database using ClustalW. A phylogenetic tree was constructed based on the sequences of 16S rRNA using the Neighbor-Joining method in MEGA 7.0 software. The reliability of the phylogenetic clusters was evaluated by bootstrap analysis with 1,000 replicates.

Characterization of enzymatic activity, biofilm formation, and plant colonization

Hydrolytic enzyme assays: Extracellular enzyme production was screened on agar plates supplemented with specific substrates: 1% (w/v) skim milk for protease, 1% (w/v) soluble starch for amylase (Guleria et al. 2016), 1% (w/v) sodium carboxymethyl cellulose for cellulase (Teather et al. 1982), 1% (w/v) apple

pectin for pectinase (Kuvvet et al. 2019), and 0.5% (w/v) colloidal chitin for chitinase (Agrawal et al. 2012). After incubation at 30 °C for 3–5 days, the presence of clear halos around colonies indicated positive enzymatic activity. To visualize halos for cellulase and pectinase, plates were stained with 0.1% Congo red for 15 min and destained with 1 M NaCl to 10 min.

Biofilm formation was quantified in MSgg medium using a 24-well plate assay as described previously (Liu et al. 2022). The hyper-biofilm-forming strain *B. amyloliquefaciens* YN.J3 served as a positive control. Briefly, 10 µL of an overnight culture (adjusted to OD₆₀₀ = 1.0) was inoculated into 2 mL MSgg medium and incubated statically at 37 °C for 48 h. Biofilms were stained with 0.1% (w/v) crystal violet, gently washed three times with 0.1 M PBS to remove planktonic cells, and subsequently solubilized with 30% (v/v) acetic acid. Biofilm biomass was quantified by measuring the absorbance at 570 nm (A₅₇₀).

The colonization ability of Bv. JN.Y2 was evaluated using the kanamycin-resistant mutant Bv. JN.Y2-kan^R (Fan et al. 2017). Thirty-day-old oat seedlings were inoculated via root drenched with 50 mL of a bacterial suspension (1.5×10^8 CFU/mL). At 1, 3, 7, 10, 20, and 30 days post-inoculation (dpi), the root, stem, and leaf tissues were harvested. Samples were surface-sterilized with 75% ethanol and 3% NaClO, rinsed five times with sterile water, and homogenized. The homogenates were serially diluted and plated on LB agar containing 30 µg/mL kanamycin. After incubation at 37°C for 24 h, the colonizing population was enumerated and expressed as CFU/g fresh weight.

Evaluation of plant growth-promoting traits and growth enhancement

The plant growth-promoting (PGP) potential of Bv. JN.Y2 was evaluated through multiple assays. Nitrogen metabolism and assimilation was assessed by observing bacterial growth on nitrogen-free Ashby's medium. Phosphate and potassium solubilization were determined by the formation of clear halos on Pikovskaya's agar and Aleksandrov agar, respectively. Siderophore production was detected using Chrome Azurol S (CAS) agar, where a change in color to an orange halo indicated a positive result (Ariyani, 2021; Qiu et al. 2025).

A greenhouse experiment was conducted to evaluate the effect of Bv. JN.Y2 on oat growth, following the protocol of Wang (Wang et al. 2018) with minor modifications. Oat seeds (cv. Baiyan No. 2) were surface-sterilized with 75% ethanol for 30 s and 3% NaClO for 5 min, followed by five rinses with sterile water. Germinated seedlings were transplanted into pots at the two-leaf stage. Ten-day-old seedlings were inoculated via root drenching with 50 mL of the Bv. JN.Y2 suspension (1.5×10^8 CFU/mL). Control plants were treated with an equal volume of sterile water. All plants were maintained in a greenhouse at 28°C, 60% relative humidity, with a 16 h/8 h (light/dark cycle). After 30 days, plant height, root length and root fresh weight were measured. To determine the root dry weight (biomass), samples were dried at 65°C for 72 h until a constant weight was achieved.

Genome sequencing, assembly, and annotation

High-quality genomic DNA of Bv. JN.Y2 was isolated using the SDS-CTAB protocol. A hybrid sequencing strategy was employed by Novogene Co., Ltd., utilizing both PacBio Sequel and Illumina NovaSeq PE150 platforms. The raw PacBio subreads were processed via SMRT Link (v8.0) and assembled *de novo* using Canu (v2.0) (Koren et al. 2017). To enhance sequence accuracy, the resulting assembly was polished with Illumina short reads using Pilon (v1.22), yielding a single circular chromosome.

Genome annotation was performed through an integrated pipeline. GeneMarkS (v4.17) was used for coding sequence prediction (Besemer et al. 2001), while repetitive elements were identified via RepeatMasker and Tandem Repeats Finder (TRF) (Benson, 1999). The non-coding RNA (ncRNA) fractions, including tRNAs and rRNAs, were detected using tRNAscan-SE and rRNAmmer, respectively (Lowe and Eddy, 1997; Lagesen et al. 2007; Kalvari et al. 2018). Furthermore, mobile genetic elements (MGEs), such as prophages and genomic islands, were localized using phiSpy and IslandPath-DIOMB(Hsiao et al. 2003; Akhter et al. 2012; Ge et al. 2016).

Functional characterization of the proteome was achieved by searching against multiple databases, including KEGG, GO, COG, and Swiss-Prot(Ashburner et al. 2000; Galperin et al. 2015; Kanehisa et al. 2004; Kanehisa et al. 2006; Cantarel et al. 2009). Carbohydrate-active enzymes were categorized using dbCAN. The final genomic landscape was visualized using a Circos map (Krzywinski et al. 2009).

Genome mining and comparative analysis of biosynthetic gene clusters

BGCs responsible for secondary metabolite production in Bv. JN.Y2 genome were predicted using antiSMASH (v8.0) (Blin et al. 2025). The analysis was performed using the "relaxed" detection strictness to maximize the identification of putative clusters. To characterize the genetic organization and evolutionary conservation of these clusters, a comparative analysis was performed between Bv. JN.Y2 and four closely related strains *B. velezensis* FZB42 (Bv. FZB42), *B. velezensis* SQR9 (Bv. SQR9), *B. velezensis* CBMB205 (Bv. CBMB205) and *B. amyloliquefaciens* GKT04 (Ba. GKT04). The ClusterBlast module integrated into antiSMASH was employed to identify homologous gene clusters and visualize synteny. The schematic diagrams of gene arrangement and orientation were generated directly based on the antiSMASH visualization outputs.

Genomic characterization and comparative analysis of bacteriocin biosynthetic gene clusters in Bv. JN.Y2

The whole-genome sequence of Bv. JN.Y2 was subjected to comprehensive genome mining using the BAGEL4 web server to identify potential bacteriocins and Ribosomally synthesized and Post-translationally modified Peptides (RiPPs) (Liu et al. 2020). The identification process integrated core peptide searches and context-based gene cluster detection. Based on genomic proximity and functional relevance, the identified loci were categorized into specific Areas of Interest (AOIs) for subsequent

detailed characterization. To evaluate the evolutionary conservation and structural features of the identified core peptides, representative homologous sequences were retrieved from the NCBI GenBank and BAGEL4 databases. Multiple sequence alignment was executed using the Clustal Omega algorithm with default parameters (Lugani and Sooch, 2017). The resulting alignment profile was professionally rendered using ESPript 3.2 to visualize conserved residues and physicochemical properties (Peng et al. 2024). Absolute conservation was defined by a 100% identity threshold across all compared strains, while similarity was assessed based on the equivalence of amino acid residues. The functional annotation of the core peptides was further validated through BLASTp searches against the non-redundant protein database at NCBI.

Genomic screening for acquired resistance genes and biosafety evaluation

To evaluate the genomic safety of Bv. JN.Y2 for potential agricultural applications, the presence of acquired antimicrobial resistance (AMR) genes was screened using the ResFinder (Version 4.7.2) database (Kim et al., 2025). The whole-genome sequence was analyzed with a minimum identity threshold of 80% and a minimum length coverage of 60%. To further assess the potential for horizontal gene transfer (HGT), the chromosomal coordinates of identified AMR homologs were cross-referenced against the boundaries of genomic islands and prophage regions, which were predicted using IslandPath-DIOMB program and PHAST, respectively. Phenotypic resistance predictions were derived from the ResFinder system to ensure a comprehensive evaluation of Bv. JN.Y2 safety.

Comparative genomic analysis between Bv. JN.Y2 and reference *Bacillus*

For the comparative genomic analysis, eight representative *Bacillus* strains were selected to serve as taxonomic and functional references (Table 2). These include species type strains to establish a precise phylogenetic framework, and established model biocontrol agents to provide a comparative context for evaluating the specific PGP and antagonistic mechanisms of Bv. JN.Y2. Furthermore, distantly related lineages were included to better delineate the core and strain-specific genomic features of the genus. Whole-genome alignments and synteny analyses were conducted using MUMmer (v3.23) and LASTZ (v1.03.54) (Kurtz, et al. 2004; Harris, 2007; Chiaromonte et al. 2001). These tools were employed to identify Single Nucleotide Polymorphisms (SNPs), small insertions/deletions (Indels), and structural variations (SVs) across the genomes. To evaluate the evolutionary relationships, a phylogenetic tree based on genome-wide SNPs was constructed using Treebest (v1.9.2) and PhyML (v3.0). Pan-genome analysis was performed via CD-HIT (v4.6.1) to classify the core, dispensable, and strain-specific gene sets.

Table 2
Strain information required for comparative genome analysis.

Strain name	Strain name in report	Genebank accession number
<i>B. velezensis</i> JN.Y2	Bv. JN.Y2	CP178220.1
<i>B. licheniformis</i> ATCC 14580	Bl.14580	CP140161.1
<i>B. mycoides</i> BPN36/3	Bm.BPN36.3	CP035997.1
<i>B. subtilis subsp. subtilis</i> str. 168	Bs.168	AL009126.3
<i>B.velezensis</i> SQR9	Bv.SQR9	CP006890.1
<i>B.velezensis</i> CBMB205	Bv.CBMB205	CP011937.1
<i>B.velezensis</i> FZB42	Bv.FZB42	CP000560.2
<i>B. thuringiensis</i> serovar <i>berliner</i> ATCC 10792	Bt.10792	CM000753.1
<i>B. amyloliquefaciens</i> GKT04	Ba.GKT04	CP072120.1

Statistical analysis

All experiments were conducted with three independent biological replicates to ensure the reproducibility and reliability of the data. Within each biological replicate, five technical replicates were performed. Data are presented as mean $\pm$ standard deviation (SD). Prior to analysis, the normality of the distribution and homogeneity of variance were confirmed using Shapiro-Wilk and Levene's tests, respectively. For the PGP experiment, an independent samples t-test was employed to evaluate the statistical significance of the differences. Statistical significance was determined using one-way analysis of variance (ANOVA). Significant differences between treatment means were evaluated using Duncan's multiple range test and the Least Significant Difference test at a significance level of $P < 0.05$. All statistical computations were performed using SPSS Statistics v26.0 (IBM Corp., Armonk, NY, USA). Graphical representations were generated using GraphPad Prism 9 (GraphPad Software, San Diego, CA, USA). The final multi-panel figures and illustrations were compiled and processed using Adobe Photoshop 2021 software (Adobe Systems Inc., San Jose, CA, USA).

Results

Bv. JN.Y2 exhibits broad-spectrum antifungal activity and effective control to oat anthracnose

An endophytic bacterium, designated as Bv. JN.Y2, was isolated from healthy oat plants. In dual-culture assays, Bv. JN.Y2 significantly suppressed the mycelial growth of *C. cereale*, achieving an inhibition rate of 74.49% (Table 3; Fig. 1A, 1B). Microscopic examination revealed that Bv. JN.Y2 induced swelling and

deformation of the hyphal tips, indicating a disruption of apical hyphal extension (Fig. 1C, 1D). In greenhouse pot experiments, Bv. JN.Y2 demonstrated a control efficacy of 62.40% (Fig. 1E-G), which is better than TM.

Table 3
Evaluation of the inhibitory effect of Bv. JN.Y2 on *C. cereale* under dual-culture and greenhouse conditions.

CK	Dual-culture inhibitory rate(%)	Greenhouse disease incidence (%)	Greenhouse disease index	Greenhouse control efficacy (%)
	-	100.00 ± 0.00 ^a	47.00 ± 1.73 ^a	-
Bv. JN.Y2	74.49 ± 2.73	56.67 ± 12.58 ^b	17.67 ± 3.51 ^b	62.40
Thiophanate-methyl	-	86.67 ± 7.64 ^c	30.67 ± 5.03 ^c	34.74
Note: Values are expressed as mean ± SD. Different lowercase letters (a, b, c) in the same column indicate significant differences according to Duncan's multiple range test (<i>P</i> < 0.05).				

During the 2023–2025 trial period, annual precipitation in Wuchuan and Jining showed a significant increasing trend, shifting from near-normal levels in 2023 (approximately 340–360 mm) to historical extreme rainfall in 2024 and 2025 (over 500–600 mm). Despite the increased disease pressure from surging humidity, Bv. JN.Y2 demonstrated stable biocontrol efficacy (Table 4). During the initial field trials in 2023, the control efficacy of Bv. JN.Y2 was recorded at 56.01% in Jining and 64.96% in Wuchuan. These values were comparatively higher than those observed for the chemical fungicide TM, which yielded control efficacy values of 42.69% and 53.94% at the respective sites. In 2024, as annual precipitation increased, Bv. JN.Y2 continued to demonstrate stable biocontrol performance, achieving control efficiencies of 63.41% in Jining and 60.55% in Wuchuan. During the same period, the CE of the chemical treatment was recorded at 54.75% and 50.58%, respectively. By 2025, a year characterized by historically high rainfall levels, the biocontrol stability of Bv. JN.Y2 remained evident. The strain maintained control efficiencies of 56.15% in Jining and 65.64% in Wuchuan, consistently exceeding the suppression levels provided by TM (46.53% and 50.08%, respectively). Collectively, the three-year monitoring data suggest that Bv. JN.Y2 possesses a balanced and reliable capacity to suppress oat anthracnose across fluctuating environmental conditions and varying levels of disease pressure.

Table 4

Control efficiency of Bv. JN.Y2 on oat anthracnose and yield in two experimental sites from year 2023 to 2025.

Year	Test site	Treatment	Disease index	Control efficiency	Yield (kg/ha)
2023	Ulanqab Agricultural Science Institute, Jining District, Ulanqab City, Inner Mongolia Autonomous Region(40.92506N, 113.15690E)	Control	70.00 ± 2.80 ^a	-	2940.00 ± 164.17 ^a
		Bv. JN.Y2	30.27 ± 0.61 ^c	56.01 ± 0.89	3391.35 ± 80.82 ^b
		Tm	39.45 ± 5.00 ^b	42.69 ± 7.27	3066.15 ± 104.19 ^a
	X030 private land in Keligeng Town, Wuchuan County, Hohhot City(41.20277N, 111.473127E)	Control	70.64 ± 2.01 ^a	-	2583.45 ± 93.07 ^a
		Bv. JN.Y2	24.11 ± 2.06 ^c	64.96 ± 2.30	3069.85 ± 79.43 ^b
		Tm	31.69 ± 1.15 ^b	53.94 ± 1.67	2729.35 ± 51.08 ^a
2024	Ulanqab Agricultural Science Institute, Jining District, Ulanqab City, Inner Mongolia Autonomous Region	Control	68.27 ± 2.20 ^a	-	3127.65 ± 54.98 ^a
		Bv. JN.Y2	25.20 ± 3.86 ^c	63.41 ± 5.63	3532.30 ± 70.99 ^b
		Tm	31.13 ± 0.90 ^b	54.75 ± 1.31	3234.30 ± 42.27 ^a
	X030 private land in Keligeng Town, Wuchuan County, Hohhot City	Control	60.17 ± 7.32 ^a	-	2732.10 ± 64.49 ^a
		Bv. JN.Y2	27.00 ± 2.51 ^c	60.55 ± 3.69	3016.60 ± 84.08 ^b
		Tm	34.00 ± 2.50 ^b	50.58 ± 3.63	2851.20 ± 62.50 ^a
2025	Ulanqab Agricultural Science Institute, Jining District, Ulanqab City, Inner Mongolia Autonomous Region	Control	71.87 ± 6.89 ^a	-	2980.65 ± 81.68 ^a
Note: Data are presented as mean ± SD. Different lowercase letters (a, b, c) within the same column for each location and year indicate significant differences according to Duncan's multiple range test ($P < 0.05$). Control: blank control; Bv. JN.Y2: <i>Bacillus velezensis</i> JN.Y2; Tm: Thiophanate-methyl.					

Year	Test site	Treatment	Disease index	Control efficiency	Yield (kg/ha)
		Bv. JN.Y2	31.33 ± 1.29 ^b	56.15 ± 4.27	3535.95 ± 41.93 ^b
		Tm	38.4 ± 3.82 ^b	46.53 ± 2.84	3065.65 ± 25.02 ^a
	X030 private land in Keligeng Town, Wuchuan County, Hohhot City	Control	70.27 ± 1.22 ^a	-	2768.75 ± 76.55 ^a
		Bv. JN.Y2	24.13 ± 1.80 ^c	65.64 ± 2.88	3151.50 ± 25.28 ^b
		Tm	35.07 ± 0.23 ^b	50.08 ± 1.18	2908.85 ± 51.72 ^c
Note: Data are presented as mean ± SD. Different lowercase letters (a, b, c) within the same column for each location and year indicate significant differences according to Duncan's multiple range test ($P < 0.05$). Control: blank control; Bv. JN.Y2: <i>Bacillus velezensis</i> JN.Y2; Tm: Thiophanate-methyl.					

To assess its antifungal spectrum, Bv. JN.Y2 was co-cultured with seven other common fungal phytopathogens. The strain exhibited significant inhibitory activity against all tested pathogens ($P < 0.05$) (Fig. 2). Inhibition rates ranged from 48.39% to 81.16%, with the highest activity observed against *P. sorghina* (81.16%). Inhibition rates against *C. fructicola*, *R. solani*, *D. glomerata*, and *A. alternata* exceeded 50%. While inhibition rates against *F. graminearum* and *F. oxysporum* were 48.39% and 48.53%, respectively. These findings confirm that Bv. JN.Y2 possesses broad-spectrum antifungal capacity, could be potentially applied for controlling multiple plant disease, caused by fungal pathogens.

Morphological, physiological, and molecular identification of Bv. JN.Y2

After incubation on LB agar at 37°C for 24 h, colonies of Bv. JN.Y2 exhibited a creamy-white, opaque appearance, characterized by irregular margins and a rugose (wrinkled) surface (Fig. 3A). Microscopic examination and physiological assays indicated that Bv. JN.Y2 is a Gram-positive, motile, rod-shaped bacterium (Fig. 3B). The formation of central ellipsoidal endospores was observed, which is a typical characteristic of the genus *Bacillus* (Fig. 3C). As detailed in Table 5, Bv. JN.Y2 demonstrated relatively diverse hydrolytic capabilities, testing positive for catalase and cellulase activities, as well as the hydrolysis of starch, gelatin, and casein. The strain also showed positive results for nitrate reduction, the Voges-Proskauer (V-P) reaction, and citrate utilization. In contrast, negative results were recorded for oxidase activity, methyl red test, indole production, H₂S production, and urease activity. Regarding carbon source utilization, the strain was capable of metabolizing glucose, sucrose, maltose, and D-mannitol, while it appeared unable to utilize lactose. These morphological and biochemical traits are consistent with the established descriptions of *Bacillus velezensis*.

Table 5
Physiological and biochemical characteristics of Bv. JN.Y2.

Characteristics	Results
Morphology	
Gram staining	+
Cell shape	Rod
Endospore	+
Motility	+
Enzyme Activity	
Catalase	+
Oxidase	-
Starch hydrolysis	+
Gelatin liquefaction	+
Casein hydrolysis	+
Cellulase	+
Biochemical Tests	
Nitrate reduction	+
Voges-Proskauer	+
Methyl Red	-
Indole production	-
Citrate utilization	+
H ₂ S production	-
Urease	-
Carbon Source	
Glucose	+
Sucrose	+
Maltose	+
D-Mannitol	+
Note: +, positive response or growth; -, negative response or no growth. Data represents the mean of three independent replicates.	

Characteristics	Results
Morphology	
Lactose	-
Note: +, positive response or growth; –, negative response or no growth. Data represents the mean of three independent replicates.	

For the preliminary taxonomic classification of Bv. JN.Y2, 16S rRNA gene sequence analysis was initially performed. PCR amplification of the 16S rRNA gene yielded a single, distinct band of the expected size upon agarose gel electrophoresis (Fig. 3D). Subsequent sequence analysis and the resulting phylogenetic tree indicated that Bv. JN.Y2 clustered within the same clade as *B. velezensis* GT3033 and CSQXZD26, with a bootstrap support of 94% (Fig. 3E). Although the discriminatory power of 16S rRNA sequences among closely related species within the genus *Bacillus* is relatively limited, this analysis provided foundational evidence for the genus-level assignment of Bv. JN.Y2. Given the high degree of genetic similarity among closely related species within this genus, a phylogenetic tree based on whole-genome SNPs was subsequently constructed to achieve a more robust identification. The SNP-based phylogenetic analysis revealed a close genetic affinity between Bv. JN.Y2 and Bv. CBMB205, supported by a bootstrap value of 99% (Fig. 3F). By integrating the morphological, physiological, and biochemical characteristics with this high-resolution genomic evidence, Bv. JN.Y2 was confirmed as *B. velezensis*.

Hydrolase secretion, biofilm formation and colonization

To further explore the potential biocontrol mechanisms of Bv. JN.Y2, its capacity for extracellular enzyme secretion and biofilm formation was evaluated. The secretion of hydrolytic enzymes is generally considered a relevant factor in both pathogen antagonism and successful endophytic colonization. In this study, distinct hydrolysis halos were observed on agar plates supplemented with protease, amylase, cellulase, and pectinase substrates (Fig. 4A–D). Specifically, proteases can participate in the degradation of structural proteins within fungal cell walls, while cellulases and pectinases may facilitate the entry and systemic spread of the endophytic bacterium within host plant tissues. Although no obvious hydrolysis halo was detected on colloidal chitin agar (Fig. 4E), Bv. JN.Y2 maintained the ability to grow using chitin as the sole carbon source, suggesting a basal chitin-utilizing capacity that might assist in fungal antagonism. Collectively, these results indicate that Bv. JN.Y2 can secrete a relatively diverse array of hydrolases, which may coordinately contribute to its biocontrol efficacy.

Furthermore, biofilm formation assays revealed that Bv. JN.Y2 was capable of developing structural biofilms in MSgg medium. Quantitative analysis using crystal violet staining showed an OD₅₇₀ value of 1.62 for Bv. JN.Y2. While this production was comparatively lower than that of the hyper-producing reference strain *B. amyloliquefaciens* YN.J3 (OD₅₇₀ = 2.17), it still demonstrated a favorable biofilm-forming capability relative to the control (Fig. 4F, Table 6).

To dynamically assess the spatial and temporal colonization patterns of Bv. JN.Y2 in oat plants, a kanamycin-resistant transformant (Bv. JN.Y2-kan^R), which was obtained from our laboratory collection, was utilized and tracked via plate counting. The results indicated that the bacterial population in the roots reached its peak at 1 dpi with a density of 1.21×10^5 CFU·g⁻¹, and subsequently showed a gradual decrease, maintaining a level of 2.85×10^4 CFU·g⁻¹ at 30 dpi. In the aerial parts, colonization in the stems and leaves peaked slightly later, reaching 8.20×10^4 CFU·g⁻¹ at 3 dpi and 5.73×10^4 CFU·g⁻¹ at 7 dpi, respectively, before experiencing a gradual decline (Fig. 4G). These findings suggest that Bv. JN.Y2 possesses the ability to systemically spread and establish a relatively stable population within oat tissues over an extended period.

PGP traits of Bv. JN.Y2 and its effect on oat yield enhancement

To comprehensively assess the PGP potential of Bv. JN.Y2, both laboratory assays and field trials were evaluated. In vitro biochemical assays revealed that Bv. JN.Y2 was capable of growing on nitrogen-free Ashby medium, and it produced clear halos on Pikovskaya and Aleksandrov agar plates, indicating its capacities for nitrogen metabolism or assimilation (Fig. 5A), and solubilization of phosphate and potassium (Fig. 5B, 5C). Furthermore, the distinct halo observed on the CAS agar medium suggested the production of siderophores (Fig. 5D). In seedling evaluations, treatment with Bv. JN.Y2 resulted in an increase in seedling height and shoot fresh weight by 19.73% and 36.73%, respectively, although the difference in dry weight was not statistically significant. Regarding root development, root length, root fresh weight, and root dry weight were enhanced by 43.03%, 40.21%, and 11.96%, respectively. Additionally, the chlorophyll SPAD value exhibited a 25.27% increase compared to the control group (Fig. 5E, Table 7).

Table 6
Quantitative analysis of biofilm formation
measured by crystal violet staining (OD₅₇₀)

Strain	OD ₅₇₀
<i>B. amyloliquefaciens</i> YN.J3	2.17 ± 0.07 ^c
Bv. JN.Y2	1.62 ± 0.04 ^b
CK (LB+Msgg media)	0.37 ± 0.07 ^a

Table 7
Effect of Bv. JN.Y2 on the growth parameters of oat seedlings.

Plant height (cm)	Sterile water	Bv. JN.Y2
	24.12 ± 0.17 ^a	28.88 ± 0.58 ^b
Root length (cm)	5.88 ± 0.24 ^a	8.41 ± 0.49 ^b
Shoot fresh weight (g)	0.49 ± 0.03 ^a	0.67 ± 0.04 ^b
Root fresh weight (g)	0.0475 ± 0.0029 ^a	0.0666 ± 0.0012 ^b
Shoot dry weight (g)	0.10 ± 0.01 ^a	0.11 ± 0.02 ^a
Root dry weight (g)	0.0184 ± 0.0015 ^a	0.0206 ± 0.0012 ^b
Chlorophyll content (SPAD)	27.70 ± 0.79 ^a	34.70 ± 0.56 ^b
Note: Data are presented as mean ± SD. Statistical analysis was performed using an independent samples <i>t</i> -test. Different lowercase letters within the same row indicate significant differences between treatments (<i>P</i> < 0.05).		

Across the three consecutive years of field trials (2023–2025) in Jining and Wuchuan, oat plants treated with Bv. JN.Y2 consistently exhibited higher grain yields compared to CK and TM groups (Table 4). In 2023, the application of Bv. JN.Y2 led to favorable yield increases, reaching 3391.35 kg/ha in Jining and 3069.85 kg/ha in Wuchuan. During 2024, as environmental precipitation increased, the yield-promoting effect remained relatively stable, with the Bv. JN.Y2 treatment achieving 3532.30 kg/ha and 3016.60 kg/ha at the two respective sites. In the multi-rain year of 2025, the yield in the Bv. JN.Y2-treated plots reached 3535.95 kg/ha in Jining and 3151.50 kg/ha in Wuchuan, maintaining a consistent advantage over the corresponding controls. Collectively, these findings suggest that Bv. JN.Y2 likely promotes oat growth and stably enhances crop yield across varying climatic conditions by facilitating nutrient absorption and improving overall physiological vigor.

Genomic features and functional gene mining of Bv. JN.Y2

To uncover the molecular basis underlying its beneficial traits, the complete genome of Bv. JN.Y2 was sequenced and annotated. The genome consists of a single circular chromosome of 3,871,731 bp with a GC content of 47.26% (Fig. 6, Table 8). No plasmid was detected. The genome contains 3,987 predicted protein-coding genes with an average length of 872 bp, along with 25 rRNA genes, 84 tRNA genes, and 8 sRNA genes. The complete genome sequence has been deposited in the NCBI database under the GenBank accession number CP178220.1. Functional annotation was performed across multiple databases, yielding the following assignments: COG (2,939 genes), NR (3,912), GO (2,667), KEGG (3,836), Pfam (2,667), Swiss-Prot (3,255), CAZy (155), and TCDB (516). Subsequent genomic screening revealed an array of genes associated with PGP activities. Specifically, gene clusters related to nitrogen metabolism and assimilation (*nifH*, *nifU*, *yutI*), Fe-S cluster assembly (*sufB*, *sufC*, *sufU*, *sufD*),

phosphate metabolism (*pstA/B1/B2/C/S*, *phoA*, *bglA*), potassium uptake (*ktrA/C/D*, *kdpD*), siderophore production (*fhu*, *feu*, *dhb*, *ent*), and indole-3-acetic acid (IAA) synthesis (the *trp* operon) were identified (Online Resource 1 Table S2). These findings provide a genetic explanation for the physiological PGP traits observed in vitro.

Table 8
The general genome feature of Bv. JN.Y2

Feature	Vaule
Genome size (bp)	3,871,731
G + C content	47.26%
Topology	Circular
Plasmid	0
Total size of protein-coding genes	3,478,611
Protein-coding genes	3987
Average CDs size (bp)	872
rRNA number (total)	25
tRNA number	84
sRNA number	8
Repetitive sequence(bp)	18131 (0.4683%)
CRISPR	7
Prophage	13
GIs	9
Gene cluster	12
Genes assigned to COG	2939
Genes assigned to NR	3912
Genes assigned to GO	2667
Genes assigned to KEGG	3836
Genes assigned to Pfam	2667
Genes assigned to Swiss-Prot	3255
Genes assigned to CAZy	155
Genes assigned to TCDB	516

Furthermore, a variety of genes encoding hydrolases were annotated, including amylases (*amyA*), proteases (*epr*, *aprE*, *vpr*), cellulases (*celA/B/C/F*, *xynB*), pectinases (*pel*, *pgdA*, *pgdB*), and chitin-related enzymes (*yxkH*, *yheN*). Additionally, a comprehensive set of genes essential for plant-microbe interactions and colonization was identified, encompassing those governing chemotaxis (*cheA/W/Y*, *mcpA/B/C*), flagellar motility (*fliG/M/N*), quorum sensing (*comA*, *comP*, *luxS*, *phrA*, *sinI/R*), and biofilm formation (*tasA*, *epsC*, *bslA*) (Online Resource 1 Table S3). Together, these genomic features establish a relatively solid genetic foundation for the biocontrol and growth-promoting capacities of Bv. JN.Y2.

Discovery and comparative analysis of secondary metabolite biosynthetic gene clusters

To further elucidate the molecular mechanisms underlying the biocontrol efficacy of Bv. JN.Y2, the antiSMASH tool was employed to mine its genome for BGCs. A total of 13 BGCs were identified, encompassing non-ribosomal peptide synthetases (NRPS), trans-AT polyketide synthases (transAT-PKS), hybrid PKS/NRPS systems, and terpenes (Table 9). Among these, seven clusters exhibited high similarity to known pathways and were predicted to direct the synthesis of surfactin, macrolactin H, bacillaene, fengycin, difficidin, bacillibactin, and bacilysin. Furthermore, Region 2 (PKS-like), Region 4 (Lanthipeptide-class-II), Region 9 (T3PKS), and multiple terpene gene clusters (Regions 3, 8, and 11) distributed at various genomic positions exhibited notably low similarity to entries in known databases. These findings suggest that Bv. JN.Y2 possesses a favorable potential for the synthesis of novel or strain-specific antimicrobial metabolites, which may relatively significantly assist in its environmental adaptation and biocontrol performance.

To characterize the genetic organization of these clusters, a comparative synteny analysis was performed between Bv. JN.Y2 and four biocontrol reference strains. Visualization revealed a remarkably high degree of collinearity and synteny between Bv. JN.Y2 and the model strain Bv. FZB42 (Fig. 7). The arrangement and orientation of core biosynthetic operons (e.g., *srfAA-AC*, *fenA-E*, *mInA-G*, *baeJ-R*, and *difA-L*) were highly conserved across the analyzed *B. velezensis* strains. This conservation not only confirms the genetic integrity of these antimicrobial pathways in Bv. JN.Y2, but supports its taxonomic identification at the genomic level, indicating its potential as a biocontrol agent.

Table 9

Predicted genes clusters involved in synthesis of secondary metabolites in Bv. JN.Y2 genome.

Region	Most similar known cluster	Type	Size	Similarity Confidence	Position
1	Surfactin	NRPs	65,407 bp	High	264,083–329,490
2	Butirosin	PKS-like	41,244 bp	-	865,948 – 907,192
3	-	Terpene	20,740 bp	-	989,236-1,009,976
4	-	Lanthipeptide-class-ii	28,888 bp	-	1,130,469-1,159,357
5	Macrolactin H	TranAT-PKS	88,233 bp	High	1,325,970-1,414,203
6	Bacillaene	transAT-PKS,T3PKS,NRPS	110,093 bp	High	1,632,894-1,742,987
7	Fengycin	NRPS, transAT-PKS,betalactone	137,801 bp	High	1,807,618-1,945,419
8	-	Terpene	21,883 bp	-	1,970,646-1,992,529
9	-	T3PKS	41,100 bp	-	2,055,847-2,096,947
10	Difficidin	TransAT-PKS	106,182 bp	High	2,211,933-2,318,115
11	-	Terpene-precursor	20,890 bp	-	2,341,409-2,362,299
12	Bacillibactin	NRP-metallophore,NRPS,Ripp-like	51,791 bp	High	2,942,817-2,994,608
13	Bacilysin	Other	41,418 bp	High	3,530,918-3,572,336

In silico mining of bacteriocins and RiPPs

The genomic architecture of Bv. JN.Y2 was explored using the BAGEL4 platform, which identified four distinct Areas of Interest (AOIs) dedicated to the synthesis of RiPPs (Fig. 8). Within the genomic architecture of Bv. JN.Y2, AOI_01 is situated between coordinates 2, 979, 272 and 2, 999, 461, encompassing 26 predicted open reading frames (ORFs). The primary biosynthetic product of this cluster is identified as Amylocyclicin, a circular bacteriocin recognized for its significant role in improving the competitive fitness of *Bacillus* species within their ecological niches. Distinct from the other synthesis-oriented clusters, AOI_02 (2, 929, 112–2, 949, 271) comprises 26 ORFs and is characterized as a regulatory locus centered on the signaling peptide ComX3. As a pivotal component of the Quorum Sensing system, ComX3 potentially modulates the secondary metabolic profile of Bv. JN.Y2, thereby orchestrating its adaptive responses to environmental stimuli without the requirement of a traditional core peptide synthesis template. Complementing these findings, AOI_03 is located at positions 243, 431 to 263, 566 and contains 19 ORFs, encoding the Linear Competition Inducer (LCI) as its core protein. LCI has been demonstrated to facilitate environmental dominance by exerting potent antimicrobial and antifungal activities against competing microorganisms. Lastly, AOI_04 (1, 132, 550–1, 158, 286) exhibits a sophisticated organizational structure with 28 ORFs and is classified as a class II lanthipeptide biosynthetic cluster. Notably, this cluster is characterized by the presence of two distinct core peptides exhibiting high homology to Haloduracin_beta, which are further associated with the Type A lanthibiotic (L_biotic_typeA) and Mersacidin-like families. This multi-component architecture is a hallmark of certain RiPPs that often exhibit enhanced bioactivity through synergistic mechanisms, potentially conferring a consistent defensive advantage to the host strain.

To validate the functional robustness of these systems, multiple sequence alignments were performed using Clustal Omega and visualized via ESPript 3.2 (Fig. 9). The resulting alignment profile confirmed a high degree of evolutionary conservation across the core peptides. Specifically, the Amylocyclicin sequence in Bv. JN.Y2 exhibits 100% identity with reference circular bacteriocins from *B. velezensis* FZB42 and DSYZ. This is further supported by BLASTp validation, showing 99.10% identity (99% coverage) to a circular bacteriocin from *B. inaquimensis* with a significant E-value of $2e-74$. For the LCI system, the core peptide shares 96.81% identity with unnamed *B. velezensis*, *B. velezensis* DSM 23117 and 98.94% with *B. siamensis* SCSIO 05746. Notably, Bv. JN.Y2 shared a significantly lower identity of only 53.33% with the model strain *B. velezensis* FZB42. This pronounced divergence in the LCI sequence, contrasted with the 100% identity observed for Amylocyclicin, highlights the unique evolutionary trajectory and the distinct antimicrobial profile of Bv. JN.Y2 compared to the classical model strain. Regarding the multi-component AOI_04, while AOI_04/1 shows varied homology (0-78.05%), AOI_04/2 exhibits absolute conservation with 100% identity to the homolog from *B. amyloliquefaciens* PM415. These molecular signatures underscore the sophisticated antimicrobial potential of the Bv. JN.Y2 genome.

Genomic screening for acquired resistance genes and biosafety evaluation

The genomic landscape of Bv. JN.Y2 is characterized by a relatively rich composition of MGEs, including 9 predicted genomic islands (GIs) and 13 prophage regions (Online Resource 1 Table S4, S5). This substantial dataset underscores an active evolutionary history driven by HGT, which likely contributes to the strain's genomic plasticity and its specialized adaptation to the oat endosphere. Notably, these GIs are hypothesized to harbor potential genes associated with environmental fitness, nutrient metabolism, and plant-growth promotion, which are essential for the endophytic lifestyle of Bv. JN.Y2. Consistent with this dynamic genomic architecture, ResFinder screening identified two acquired AMR homologs (Table 10): *cfr(B)* (identity: 88.61%) and *tet(L)* (identity: 86.87%). Spatial distribution analysis revealed that *cfr(B)* (52, 804–53, 839 bp) is localized within the Prophage 1 region (29, 352–103, 065 bp), providing a molecular record of an ancestral transduction event. In contrast, *tet(L)* (373, 529–374, 905 bp) resides within the stable chromosomal backbone, situated outside all predicted GIs and prophage sequences. Despite the historical acquisition of these elements, the ResFinder system predicted a "No resistance" phenotype across all tested antibiotic categories, including aminoglycosides and beta-lactams, for which no significant hits were found. Given the plasmid-free architecture of Bv. JN.Y2, the chromosomal localization and the "cryptic" nature of the prophage-associated *cfr(B)* suggest that these sequences likely represent non-functional evolutionary remnants rather than active resistance determinants. This sophisticated genomic compartmentalization appears to effectively control potential resistance risks while maintaining high genomic plasticity. Consequently, these findings underscore a relatively high level of biosafety and genetic stability for Bv. JN.Y2, supporting its potential as a sustainable and secure biocontrol agent in oat production.

Table 10
Summary of AMR genes and their spatial association with MGEs in Bv. JN.Y2

AMR Gene	Identity (%)	Chromosomal Position (bp)	MGE Association	Predicted Phenotype
<i>cfr(B)</i>	88.61	52, 804 – 53, 839	Prophage 1 (29, 352 – 103, 065 bp)	No resistance
<i>tet(L)</i>	86.87	373, 529 – 374, 905	None (Stable chromosomal backbone)	No resistance

Comparative genome analysis between Bv. JN.Y2 and eight reference Bacillus strains

To elucidate the genetic divergence, functional variations, and evolutionary adaptation of the tested strains relative to the reference strain Bv. JN.Y2, a systematic profiling of SNPs, InDels, and SVs was conducted across all genomes (Table 11, Fig. 10, Online Resource 1 Fig. S1). Among all investigated strains, Bv. CBMB205 exhibited a relatively high degree of genetic conservation compared to the reference genome. A genome-wide analysis revealed only 3 SNPs (including only 1 non-synonymous mutation) and 8 InDels within CDS regions (comprising 5 insertions and 3 deletions) (Table 11, Fig. 10A). The SNP-based phylogenetic tree (Fig. 3F) further illustrates these evolutionary relationships: closely related *B. velezensis*, such as Bv. CBMB205, Bv. SQR9 and Bv. FZB42, tend to accumulate higher densities of SNPs while maintaining robust sequence synteny. Conversely, distantly related lineages like

Bm. BPN36.3 and Bt. ATCC 10792 occupy basal positions in the tree, where their evolutionary divergence is primarily driven by complex structural rearrangements and poorer synteny, despite exhibiting relatively fewer detectable point mutations. This high level of sequence stability was further corroborated by the SV profiling. Similarly, other strains belonging to *B. velezensis* (Online Resource 1 Fig. S1D, E, F) demonstrated relatively low structural variation and maintained robust sequence synteny, reflecting their close intra-specific phylogenetic relationships. Notably, a divergent variation pattern was observed in Bm. BPN36.3 (Online Resource 1 Fig. S1B) and Bt. ATCC 10792 (Online Resource 1 Fig. S1G). While these strains showed comparatively low point mutation counts—with 2, 777 and 2, 152 SNPs, 9 and 11 InDels, respectively—their macro-scale structural profiles were characterized by dense, large-scale genomic clusters. This suggests that these lineages may have undergone relatively complex sequence rearrangements or recombinations during their evolutionary trajectories. In contrast, Bs. 168 exhibited significant genetic differentiation from the reference, with the total number of SNPs reaching 141, 090. Even within the *B. velezensis* group, Bv. FZB42 and Bv. SQR9 accumulated 55, 591 and 37, 721 SNPs, respectively, indicating a notable level of intra-specific genetic diversity. Collectively, the frequency of SVs across the strains fluctuated between 4 and 512 events, encompassing deletions, insertions, and complex translocations. The distribution of these structural features showed a relatively strong correlation with the taxonomic distance of each strain relative to Bv. JN.Y2. These multi-dimensional variation data not only elucidate the distinct genomic signatures formed during long-term evolution, but provide a relatively solid molecular foundation for subsequent investigations into functional gene expression and environmental adaptation across these diverse strains.

Furthermore, KEGG functional annotation was performed to investigate the impact of key genetic heterogeneities on the biocontrol properties and environmental fitness of Bv. JN.Y2, particularly from the dual perspectives of protein functional evolution and gene expression regulation. Bv. JN.Y2 exhibited relatively high structural stability relative to its close relative Bv. CBMB205, with key genetic divergence focused on non-synonymous SNPs in the sporulation kinase *kinA*. This variation suggests a potentially more sensitive regulatory strategy for sensing environmental stress. Meanwhile, the conserved core developmental framework and high sequence synteny provide a relatively solid genetic foundation for stable colonization. In comparisons with biocontrol strains such as Bv. FZB42, Bv. SQR9 and Ba. GKT04, non-synonymous SNPs and InDels were highly enriched within secondary metabolite clusters (e.g., Iturin, Fengycin, and Pks), driving the diversification of substrate specificity. In addition, localized SV-mediated rearrangements promoted the modular evolution of biocontrol genes, providing molecular evidence for its broad-spectrum antagonistic activity. Regarding environmental sensing, variations in coding regions of ComP and chemotaxis proteins, coupled with structural fine-tuning of upstream regulatory elements, imply a "precision-tuning" mechanism for responding to root exudates, thereby enhancing sensing sensitivity in soil. Compared to distant lineages like Bl. 14580 and Bm. BPN36.3, Bv. JN.Y2 underwent comprehensive differentiation ranging from large-scale rearrangements to core metabolic functions (e.g., *tuf*, *sufB*) and stress-resistance pathways (e.g., *spoII*P, *uvrA/B*). Collectively, these multi-level variation patterns reinforce the survival resilience and adaptive fitness of Bv. JN.Y2 under extreme environmental conditions.

Table 11
Statistics and annotation of SNPs in the genomes of eight *Bacillus* strains

Reference Strain	Sample Strain	Synonymous SNP	Nonsynonymous SNP	Total CDS SNP	Intergenic	Total SNP
Bv. JN.Y2	Bl.14580	21, 293	6, 428	27, 846	2, 100	29, 946
	Bm.BPN36.3	1, 546	803	2, 364	413	2, 777
	Bs.168	101, 661	28, 332	28, 332	130, 600	141, 090
	Bv.SQR9	24, 882	9, 148	34, 142	3, 579	37, 721
	Bv.CBMB205	0	1	3	0	3
	Bv.FZB42	37, 095	13, 014	50, 326	5, 265	55, 591
	Bt.10792	1, 164	611	1, 794	358	2, 152
	Ba.GKT04	37, 898	13, 216	51, 304	5, 273	56, 577

Core-Pan analysis between Bv. JN.Y2 and eight reference *Bacillus* strains

To characterize the evolutionary trajectories and functional divergence among nine *Bacillus* strains, including Bv. JN.Y2, a comparative pangenome analysis was conducted. From a total of 43, 274 identified protein-coding sequences, 12, 473 orthologous genes (pan-genes) were clustered to define the collective gene repertoire of the cohort. Within this framework, a core genome of 1, 086 genes shared across all strains was identified, likely representing the conserved genetic foundation of the group. Conversely, the dispensable genome, comprising 11, 387 genes distributed among subsets of strains, highlighted the considerable genomic plasticity of the lineage. Notably, Bv. JN.Y2 harbored 411 unique genes—a figure within a moderate range (13–1, 508) that reflects its distinct genetic identity. Dilution curve analysis further corroborated these dynamics, showing the core genome stabilizing at approximately 1,086 genes, while the pangenome expanded without reaching a saturation plateau. These findings indicate that this strain assembly possesses a typical "open" pangenome, suggesting a collective genetic repertoire with substantial potential for further expansion upon the inclusion of additional genomic data.

Among the 1, 086 core genes, 524 (48.2%) were successfully annotated via the KEGG database. At the primary functional categories, Metabolism was the most prominent category (cumulative frequency: 1, 485), followed by Genetic Information Processing (143) and Environmental Information Processing (78). These results suggest that the core genome primarily sustains basal physiological activities and signal transduction, providing a stable functional framework for Bv. JN.Y2 and its relatives. Further analysis at

the secondary functional categories highlighted a high prevalence of genes involved in Global and overview maps (817), Carbohydrate metabolism (158), and Amino acid metabolism (134). Specifically, specific metabolic pathways revealed 304 genes in Metabolic pathways, 60 in Carbon metabolism, and critical energy-related components like Glycolysis/Gluconeogenesis (25) and Oxidative phosphorylation (24). Regarding biocontrol and adaptation, genes for the biosynthesis of secondary metabolites (160) and antibiotics (120) exhibited high conservation, underscoring a relatively stable genetic basis for the antagonistic potential of the *B. velezensis* group. Additionally, the enrichment of Ribosome (45) genes supports efficient protein synthesis, while conserved Two-component systems (33) and ABC transporters (28) likely enhance environmental sensing and nutrient acquisition, collectively maintaining bacterial fitness across diverse ecological niches.

In contrast to the conserved core genome, the 411 strain-specific genes of Bv. JN.Y2 are characterized by specialized functions that likely facilitate its distinct ecological adaptation. KEGG annotation reveals a notable enrichment in secondary metabolism, particularly monobactam biosynthesis, suggesting a relatively good potential for the production of unique bioactive compounds. The identification of pathways such as lysine biosynthesis and butanoate metabolism further indicates enhanced metabolic flexibility. Furthermore, unique components of quorum sensing and two-component systems reflect a specialized capacity for environmental sensing and population-level coordination. Combined with specific ABC transporters and genes involved in sporulation and stress response, these genetic features collectively reinforce the resilience and competitive fitness of Bv. JN.Y2 within its ecological niche.

Discussion

Members of the genus *Bacillus* are widely regarded as promising biological control agents, a reputation primarily attributed to their capacity to establish mutualistic endophytic relationships with host plants and suppress pathogens through multifaceted mechanisms (Reva et al. 2004; Hashem et al. 2019). In this study, the endophytic strain Bv. JN.Y2, isolated from oat stems and taxonomically identified via both 16S rRNA sequences and whole-genome SNP-based phylogenetic trees, demonstrated relatively good dual functionality in anthracnose suppression and PGP. Comprehensive genome mining of Bv. JN.Y2 revealed a substantial repertoire of genes associated with secondary metabolite biosynthesis, nutrient utilization and cycling, environmental adaptation, as well as chemotaxis and colonization. Furthermore, a systematic comparative genomic analysis was conducted to elucidate the genetic architecture of Bv. JN.Y2. Our findings indicated that, in addition to the core genome, the strain has evolved 411 unique genes and exhibited a certain degree of specificity in terms of genomic variants, including SNPs, InDels, and SVs. These unique genes and sequence variations are notably enriched in key pathways such as secondary metabolism, quorum sensing, and stress response. In particular, the genetic divergence accumulated in the sporulation kinase kinA and secondary metabolite clusters (e.g., Iturin and Fengycin) provided a relatively good basis for the diversification of substrate specificity. Collectively, the abundant genetic variation within this "open" pan-genome defines the unique genetic identity of Bv. JN.Y2, establishing a relatively good foundation for its survival resilience and competitive adaptability within complex ecological niches.

The biocontrol potential of *Bacillus* species is generally attributed to the synergy of multiple traits, including direct antagonism facilitated by secondary metabolites from the NRPS, PKS, and RiPPs pathways (Jangir et al. 2018; Zhou et al. 2021), competition for finite resources (Kramer et al. 2020), and effective colonization of the rhizosphere and endophytic tissues, a process often facilitated by CWDEs and biofilm (Wang et al. 2024). Combined results from greenhouse assays and whole-genome annotation revealed that Bv. JN.Y2 possesses a diverse genetic repertoire encoding proteases, cellulases, and various BGCs including NRPS, PKS, and RiPPs. The co-existence of these multi-functional antimicrobial BGCs likely underpins the broad-spectrum inhibitory capacity of Bv. JN.Y2. As reported by Shen, the synergistic action of multiple secondary metabolites and CWDEs secreted by *B. velezensis* SH1471 effectively suppresses a wide range of pathogens, including *F. oxysporum* (Shen et al. 2023). This characteristic—simultaneous secretion of CWDEs and antimicrobial metabolites—underscores the potential of Bv. JN.Y2 as a versatile biocontrol agent for managing various plant diseases beyond oat anthracnose (Chowdhury et al. 2015). Furthermore, genomic mining and CAS assay results confirmed the potential of Bv. JN.Y2 to synthesize catecholate-type siderophores (e.g., bacillibactin) and its possession of sophisticated transport systems (*feu* and *fhu*) for mediating catecholate and hydroxamate iron uptake (Chen et al. 2007). This robust iron-sequestration capability likely facilitates the inhibition of pathogen proliferation under iron-limited conditions, thereby enhancing the strain's ecological fitness within the complex rhizosphere environment (Yu et al. 2011). In addition, longitudinal tracking of Bv. JN.Y2-kan^R demonstrated that the strain can stably colonize the roots, stems, and leaves of oat plants for at least 30 days. This persistent endophytic colonization is likely facilitated by a synergistic mechanism involving: (i) CWDEs (cellulases and pectinases) encoded by *celA/B/C/F*, *pel*, and *pgdA* genes, which assist in tissue penetration (Ku et al. 2018); (ii) *mcp*, *che*, motility-related and QS genes that orchestrate the recruitment process in response to root exudates (Zhou et al. 2016); and (iii) a relatively strong capacity for biofilm formation; (iv) the presence of a surfactin-encoding gene cluster, which is relatively effective in enhancing bacterial motility and promoting colonization (Gao et al. 2016). Our findings are consistent with those of Weng (Weng et al. 2013), who utilized GFP-labeling to show that *B. velezensis* SQR9 effectively responds to root exudates and establishes stable colonization in cucumber, subsequently protecting the host from *F. oxysporum* infection.

Microbes promoted plant growth by secreting IAA and facilitating nutrients absorption (Idris et al. 2007). The genome of Bv. JN.Y2 contains the complete *trp* operon (*trpABCDEF*), contributing to IAA synthesis. Bv. JN.Y2 grow normally on nitrogen-free medium and core genes (*nifH*, *nifU*), regulatory gene (*yutI*) are presented in genome of Bv. JN.Y2. Fan proved that genetic configurations of core genes and regulatory genes giving *B. velezensis* FZB42 nitrogen metabolism and assimilation trait (Fan et al. 2018). Moreover, genes related to phosphate metabolism and transport (*pstABCS*, *phoA*), potassium mobilization (*ktrACD*, *kdpD*) are also present in genome of Bv. JN.Y2, which collectively exert a relatively beneficial influence on plant growth promotion (Meena et al. 2016). Additionally, the genome harbors the *dhb* operon (*dhbACEBF*), which is responsible for the biosynthesis and transport of siderophores. This mechanism plays a relatively conducive role in chelating iron from the environment, and further supporting nutrient

acquisition under iron-limited conditions (Zhang et al. 2024). Overall, this multi-pronged genetic toolkit explains the enhanced biomass and yield observed in oats treated with Bv. JN.Y2.

Genome-wide analysis using antiSMASH and BAGEL4 identified a total of 17 BGCs. Notably, several clusters, such as those for Fengycin and Bacillaene, exhibit NRPS-PKS hybrid features, highlighting the robust potential of Bv. JN.Y2 to synthesize structurally sophisticated and functionally varied secondary metabolites (Zhao and Kuipers, 2016). Comparative genomic analysis with four closely related *Bacillus* strains further reveals that the arrangement and composition of core biosynthetic operons in Bv. JN.Y2 are highly syntenic with the model strain Bv. FZB42. This stands in contrast to Bv. CBMB205, which displays significant rearrangements within core clusters such as *fen*, *dhb*, and *dif*. Such high genetic conservation and structural stability in key metabolic pathways suggest that the biocontrol efficacy of Bv. JN.Y2 may be relatively predictable across diverse ecological environments (Rabbee et al. 2019; Wang et al. 2015; Xie et al. 2016;). Beyond conventional PKS/NRPS pathways, Bv. JN.Y2 exhibits unique characteristics in its RiPPs. The identification of four AOs via BAGEL4 underscores the strain's competitive advantage in complex ecological niches. These AOs are reported to encode products that regulate bacterial quorum sensing, induce membrane damage, or inhibit cell wall synthesis in pathogenic microorganisms (Scholz et al. 2014; Špacapan et al. 2020; Wang et al. 2024). Sequence alignment and characterization of core peptides reveal varying levels of conservation: while Amylocyclicin and LCI show high sequence identity with reference strains—indicating substantial evolutionary stability—the Class II lanthipeptide displays lower consistency. This divergence suggests that Bv. JN.Y2 may have undergone adaptive evolution under environmental pressures, potentially leading to the emergence of novel antimicrobial compounds with specialized diversity.

Given the dissemination risks associated with acquired resistance genes among environmental microbiota, systematic biosafety evaluation of functional strains is an indispensable prerequisite for safeguarding agro-ecological equilibrium and public health (Von et al. 2016). Three-year field trials across two distinct locations demonstrated that Bv. JN.Y2 consistently enhances oat growth. To assess its environmental release risks, comprehensive genomic mining of acquired resistance genes and MGEs were performed. Despite the identification of MGEs and homologs of *cfr(B)* and *tet(L)*, Bv. JN.Y2 exhibited a robust biosafety profile. Notably, its plasmid-free architecture—with all ARGs anchored exclusively within the chromosomal scaffold or prophage regions—significantly mitigates the potential for HGT (Panel, 2018). Furthermore, the "no resistance" phenotype predicted by ResFinder suggests that these sequences represent non-functional evolutionary remnants rather than active resistance determinants, further reinforcing the suitability and safety of Bv. JN.Y2 for sustainable oat production.

Comparative genomics serves as a sophisticated approach for elucidating evolutionary trajectories and functional variations (Alcaraz et al. 2010), providing a beneficial framework for identifying the superior traits of target strains. In this study, systematic comparative genomic analysis of Bv. JN.Y2 against both closely and distantly related strains revealed its distinct genetic differentiation and functional potential. Bv. JN.Y2 exhibits high sequence conservation and synteny with the rhizosphere-derived strain Bv. CBMB205. This observation aligns with the ecotype theory proposed by Cohan, which suggests that

strains occupying similar ecological niches may utilize "periodic selection" to maintain genomic consistency by purging genetic diversity (Cohan, 2002). Such conservation implies that Bv. JN.Y2 possesses a relatively high degree of environmental plasticity, allowing it to adapt to both rhizosphere and endophytic habitats—a trait that likely supports its broad-spectrum plant growth-promoting potential. Conversely, comparisons with distantly related strains indicate that large-scale SVs are more prevalent than point mutations. This supports the hypothesis that genomic rearrangement, rather than single-nucleotide polymorphism, serves as a primary driver of functional divergence in the long-term evolution of the *Bacillus* genus (Feulner and De-Kayne, 2017). Regarding its biocontrol mechanisms, Bv. JN.Y2 displays a metabolic profile distinct from the model strain Bv. FZB42. While Iturin is a well-known lipopeptide with antagonistic activity against various fungal pathogens (Yan et al. 2020), our analysis revealed a "genes-present, cluster-incomplete" phenomenon in Bv. JN.Y2. Specifically, although *ituABC* genes were identified, antiSMASH failed to detect a complete BGC, suggesting localized gene loss or functional recombination during evolution. This metabolic differentiation often reflects specialized niche adaptation (Steinke et al. 2021), indicating that Bv. JN.Y2 may rely on variations within other conserved clusters, such as Fengycin or Pks, to maintain its antagonistic efficacy and stabilize its biocontrol performance. The identification of non-synonymous mutations in the sporulation kinase *kinA* reflects the role of the natural variation of the phosphorelay system in environmental adaptation (Stephenson and Hoch, 2002). Similar to other stress-resistant *Bacillus* strains, Bv. JN.Y2 appears to employ fine-tuned regulation of core developmental genes to navigate fluctuating environments, establishing a molecular basis for effective colonization. Furthermore, sequence polymorphisms in ComP and chemotaxis proteins, coupled with structural variations in upstream regulatory elements, suggest a more precise root exudate sensing system. This likely drives the evolution of Bv. JN.Y2 from a "generalist" toward an "environment-specific" specialist, consistent with findings that signal transduction refinement provides a competitive advantage in soil colonization (Blake et al. 2021). Pangenome analysis characterized Bv. JN.Y2 as having an "open" genome. The presence of 411 unique genes—involved in monobactam synthesis, environmental signaling, transmembrane transport, and cellular stress response—suggests that HGT has facilitated the acquisition of novel metabolic features. This genetic uniqueness likely supports the strain's functional specificity and provides a solid molecular foundation for the targeted development of its beneficial traits (Olanrewaju et al. 2021). While these candidate variations offer valuable insights for future functional genomics, their specific biological effects require further experimental validation.

Conclusion

This study provides a comprehensive evaluation of Bv. JN.Y2 as a beneficial biocontrol and PGP agent tailored for oat cultivation. Through a combination of longitudinal field trials and high-resolution genomic mining, we demonstrated that Bv. JN.Y2 offers a relatively stable performance in suppressing *C. cereale* while simultaneously enhancing crop yields across diverse environmental conditions. The genomic architecture of Bv. JN.Y2, characterized by its "open" pangenome and diverse array of antimicrobial BGCs, underscores its favorable evolutionary adaptation to the plant endosphere. Our comparative

analysis revealed that Bv. JN.Y2 maintains a unique genetic identity through 411 strain-specific genes and key regulatory variations, such as those in the *kinA* and *comP* loci, which likely contribute to its efficient environmental sensing and colonization. Moreover, the plasmid-free nature and the absence of active resistance phenotypes establish a solid basis for its biosafety in agricultural applications. In summary, Bv. JN.Y2 represents a promising candidate for the development of targeted biopesticides and biofertilizers. While the identified candidate variations and unique metabolic pathways offer valuable insights, further functional studies will be beneficial to fully elucidate the specific biological effects of these genetic determinants in complex soil-plant ecosystems.

Declarations

Funding This work was supported by the Science and Technology Program of Inner Mongolia Autonomous Region [Grant No. 2025YFHH0165]; the Basic Research Fund for Universities Directly Affiliated with Inner Mongolia Autonomous Region [Grant No. BR251033]; the Central Government-Guided Local Science and Technology Development Fund [Grant Nos. 2022ZY0060 and 2022ZY0065]; the National Key Research and Development Program of China [Grant No. 2023YFD1600701-5]; and the China Agriculture Research System for Oat and Buckwheat [Grant No. CARS-07-C-3]. The support from these programs and organizations has been relatively helpful in the successful completion of this research.

Ethical approval Not applicable.

Informed Consent Not applicable.

Competing interests The authors declare that they have no competing interests or conflict of interest.

Author contribution W.Q. was responsible for conceptualization, methodology, investigation, formal analysis, writing – original draft, data curation, validation, and visualization. T.Z. contributed to investigation, methodology, validation, and resources. C.L.L. performed investigation, data curation, and formal analysis. S.X.S. contributed to investigation, resources, and validation. X.X.Z. was involved in investigation, methodology, and data curation. K.H.L. contributed to investigation, software, and visualization. C.Y.W. performed investigation, resources, and formal analysis. M.M.Z. contributed to formal analysis, validation, software, and writing – review & editing. B.Z.D. and H.Y.Z. were responsible for supervision, project administration, funding acquisition, conceptualization, and writing – review & editing. All authors reviewed and approved the final manuscript.

Data Availability The datasets generated during and analyzed during the current study are available from the corresponding author on reasonable request. The complete genome sequence has been deposited in the GenBank database under the accession numbers CP178220.1.

Acknowledgments We gratefully acknowledge the financial support provided by the following programs and organizations: the Science and Technology Program of Inner Mongolia Autonomous Region (Grant

No. 2025YFHH0165); the Basic Research Fund for Universities Directly Affiliated with Inner Mongolia Autonomous Region (Grant No. BR251033); the Central Government-Guided Local Science and Technology Development Fund (Grant Nos. 2022ZY0060 and 2022ZY0065); the National Key Research and Development Program of China (Grant No. 2023YFD1600701-5); and the China Agriculture Research System for Oat and Buckwheat (Grant No. CARS-07-C-3). The support from these funding sources has been relatively helpful in the successful completion of this research.

Supplementary Information The online version contains supplementary material available as Online Resource 1, which includes additional tables and figures supporting the findings of this study.

References

1. Butt MS, Tahir-Nadeem M, Khan M, K I et al (2008) Oat: unique among the cereals[J]. *Eur J Nutr* 47:68–79. <https://link.springer.com/article/10.1007/s00394-008-0698-7>
2. Crouch JA, Beirn LA (2009) Anthracnose of cereals and grasses[J]. *Fungal Divers* 39:19
3. Shi YH, Gao P, Fang ZH et al (2023) Evaluation of resistance to *Colletotrichum cereale* and analysis of loss in a field of fifteen imported oat cultivars[J]. *Acta Prataculturae Sinica* 32(9):130. <https://www.cabidigitallibrary.org/doi/full/10.5555/20231604593>
4. Politis DJ (1976) Ultrastructure of penetration by *Colletotrichum graminicola* of highly resistant oat leaves[J]. *Physiol Plant Pathol* 8(2):117–122. [https://doi.org/10.1016/0048-4059\(76\)90044-8](https://doi.org/10.1016/0048-4059(76)90044-8)
5. Wang Z, Chen J, Lee CJ (2022) First Report of Anthracnose Leaf Spot Caused by *Colletotrichum americae-borealis* on Oat in China[J]. *Plant Disease*, 106(7): 1997. <https://doi.org/10.1094/PDIS-10-21-2364-PDN>
6. Janisiewicz WJ, Conway WS (2010) Combining biological control with physical and chemical treatments to control fruit decay after harvest[J]. *Stewart Postharvest Rev* 6(1):1–16
7. Khairullina A, Micic N, Jørgensen HJL et al (2023) Biocontrol effect of *Clonostachys rosea* on *Fusarium graminearum* infection and mycotoxin detoxification in oat (*Avena sativa*)[J]. *Plants* 12(3):500
8. <https://doi.org/10.3390/plants12030500>
9. Wang J, Chen T, Wei X et al (2023) Evaluation of different antimicrobial agents for laboratory and field against *Pantoea agglomerans*, the causative agent of bacterial leaf blight disease on oat (*Avena sativa*)[J]. *Plant Pathol* 72(9):1585–1594. <https://doi.org/10.1111/ppa.13778>
10. Ghimire K, Peta V, Bücking H et al (2023) Effect of Non-Native Endophytic Bacteria on Oat (*Avena sativa* L.) Growth[J]. *Int J Plant Biology* 14(3):827–844. <https://doi.org/10.3390/ijpb14030062>
11. Malfanova N, Lugtenberg BJJ, Berg G (2013) Bacterial endophytes: who and where, and what are they doing there?[J]. *Mol Microb Ecol rhizosphere* 1:391–403. <https://doi.org/10.1002/9781118297674.ch36>
12. Miljaković D, Marinković J, Balešević-Tubić S (2020) The significance of *Bacillus* spp. *Microorganisms* 8(7):1037. <https://doi.org/10.3390/microorganisms8071037>. in disease

- suppression and growth promotion of field and vegetable crops[J]
13. Alamri SA (2015) Enhancing the efficiency of the bioagent *Bacillus subtilis* JF419701 against soil-borne phytopathogens by increasing the productivity of fungal cell wall degrading enzymes[J]. *Archives Phytopathol Plant Prot* 48(2):159–170. <https://doi.org/10.1080/03235408.2014.884671>
 14. Choub V, Ajuna HB, Won SJ et al (2021) Antifungal activity of *Bacillus velezensis* CE 100 against anthracnose disease (*Colletotrichum gloeosporioides*) and growth promotion of walnut (*Juglans regia* L.) trees[J]. *Int J Mol Sci* 22(19):10438. <https://doi.org/10.3390/ijms221910438>
 15. Grahovac J, Pajčin I, Vlajkov V (2023) *Bacillus* VOCs in the context of biological control[J]. *Antibiotics* 12(3):581. <https://doi.org/10.3390/antibiotics12030581>
 16. Zhen CY, Li WD, Wu SY et al (2022) Effects of *Bacillus subtilis* CF-3 volatile organic compounds on the transcriptome and proteome of *Monilinia fructicola* reveal a potential mechanism of action[J]. *Biol Control* 168:104872. <https://doi.org/10.1016/j.biocontrol.2022.104872>
 17. Nicholson WL, Munakata N, Horneck G et al (2000) Resistance of *Bacillus* endospores to extreme terrestrial and extraterrestrial environments[J]. *Microbiol Mol Biol Rev* 64(3):548–572. <https://doi.org/10.1128/membr.64.3.548-572.2000>
 18. YE F, MA W (2021) Control Effect of *Bacillus subtilis* Qh-618 on Oat Leaf Spot[J]. *Chin J Biol Control* 37(4):785. [10.16409/j.cnki.2095-039x.2021.04.006](https://doi.org/10.16409/j.cnki.2095-039x.2021.04.006)
 19. Zhang J, Yao T, Gong W et al (2023) Additive screening and formula optimization of microbial inhibitor having disease prevention and growth promotion effects on *Avena sativa*[J]. *Front Microbiol* 14:1208591. <https://doi.org/10.3389/fmicb.2023.1208591>
 20. Xu W, Zhang L, Goodwin PH et al (2020) Isolation, identification, and complete genome assembly of an endophytic *Bacillus velezensis* YB-130, potential biocontrol agent against *Fusarium graminearum*[J]. *Front Microbiol* 11:598285. <https://doi.org/10.3389/fmicb.2020.598285>
 21. Kim TY, Hwang SH, Noh JS et al (2022) Antifungal potential of *Bacillus velezensis* CE 100 for the control of different *Colletotrichum* species through isolation of active dipeptide, cyclo-(d-phenylalanyl-d-prolyl)[J]. *Int J Mol Sci* 23(14):7786. <https://doi.org/10.3390/ijms23147786>
 22. Meng Q, Jiang H, Hao JJ (2016) Effects of *Bacillus velezensis* strain BAC03 in promoting plant growth[J]. *Biol Control* 98:18–26. <https://doi.org/10.1016/j.biocontrol.2016.03.010>
 23. McKinney HH (1923) Influence of soil temperature and moisture on infection of wheat seedlings by *Helminthosporium sativum*[J]. <https://www.cabidigitallibrary.org/doi/full/10.5555/19241100488>
 24. Zhou J, Xie Y, Liao Y et al (2022) Characterization of a *Bacillus velezensis* strain isolated from *Bolbostemma Rhizoma* displaying strong antagonistic activities against a variety of rice pathogens[J]. *Front Microbiol* 13:983781. <https://doi.org/10.3389/fmicb.2022.983781>
 25. Sneath, Peter HA et al (1986) *Bergey's manual of systematic bacteriology. Volume 2*
 26. Guleria S, Walia A, Chauhan A et al (2016) Optimization of milk-clotting enzyme production by *Bacillus amyloliquefaciens* SP1 isolated from apple rhizosphere[J]. *Bioresources Bioprocess* 3(1):30. <https://link.springer.com/article/10.1186/s40643-016-0108-6>

27. Teather RM, Wood PJ (1982) Use of Congo red-polysaccharide interactions in enumeration and characterization of cellulolytic bacteria from the bovine rumen[J]. *Appl Environ Microbiol* 43(4):777–780. <https://doi.org/10.1128/aem.43.4.777-780.1982>
28. Kuvvet C, Uzuner S, Cekmecelioglu D (2019) Improvement of pectinase production by co-culture of *Bacillus* spp. using apple pomace as a carbon source[J]. *Waste Biomass Valoriz* 10(5):1241–1249. <https://link.springer.com/article/10.1007/s12649-017-0142-4>
29. Agrawal T, Kotasthane AS (2012) Chitinolytic assay of indigenous *Trichoderma* isolates collected from different geographical locations of Chhattisgarh in Central India[J]. *SpringerPlus* 1(1):73. <https://link.springer.com/article/10.1186/2193-1801-1-73>
30. Liu S, Huang J, Zhang C et al (2022) Probing the growth and mechanical properties of *Bacillus subtilis* biofilms through genetic mutation strategies[J]. *Synth Syst Biotechnol* 7(3):965–971. <https://doi.org/10.1016/j.synbio.2022.05.005>
31. Fan H, Zhang Z, Li Y et al (2017) Biocontrol of bacterial fruit blotch by *Bacillus subtilis* 9407 via surfactin-mediated antibacterial activity and colonization[J]. *Front Microbiol* 8:1973. <https://doi.org/10.3389/fmicb.2017.01973>
32. Ariyani MD, Dewi TK, Pujiyanto S et al (2021) Isolasi dan karakterisasi plant growth promoting rhizobacteria dari perakaran kelapa sawit pada lahan gambut[J]. *Bioma: Berkala Ilmiah Biologi* 23(2):159–171. <https://doi.org/10.14710/bioma.23.2.159-171>
33. Qiu J, Meng X, Li J et al (2025) *Bacillus megaterium* GXU087 secretes indole-3-lactic acid to promote soybean growth and nodulation[J]. *Front Plant Sci* 16:1560346. <https://doi.org/10.3389/fpls.2025.1560346>
34. Wang W, Wu Z, He Y et al (2018) Plant growth promotion and alleviation of salinity stress in *Capsicum annuum* L. by *Bacillus* isolated from saline soil in Xinjiang[J]. *Ecotoxicol Environ Saf* 164:520–529. <https://doi.org/10.1016/j.ecoenv.2018.08.070>
35. Koren S, Walenz BP, Berlin K et al (2017) Canu: scalable and accurate long-read assembly via adaptive k-mer weighting and repeat separation[J]. *Genome Res* 27(5):722–736. <http://www.genome.org/cgi/doi/10.1101/gr.215087.116>
36. Besemer J, Lomsadze A, Borodovsky M (2001) GeneMarkS: a self-training method for prediction of gene starts in microbial genomes. Implications for finding sequence motifs in regulatory regions[J]. *Nucleic Acids Res* 29(12):2607–2618. <https://doi.org/10.1093/nar/29.12.2607>
37. Lowe TM, Eddy SR (1997) tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence[J]. *Nucleic Acids Res* 25(5):955–964. <https://doi.org/10.1093/nar/25.5.955>
38. Lagesen K, Hallin P, Rødland EA et al (2007) RNAmmer: consistent and rapid annotation of ribosomal RNA genes[J]. *Nucleic Acids Res* 35(9):3100–3108. <https://doi.org/10.1093/nar/gkm160>
39. Kalvari I, Argasinska J, Quinones-Olvera N et al (2018) Rfam 13.0: shifting to a genome-centric resource for non-coding RNA families[J]. *Nucleic Acids Res* 46(D1):D335–D342. <https://doi.org/10.1093/nar/gkx1038>

40. Hsiao W, Wan I, Jones SJ et al (2003) IslandPath: aiding detection of genomic islands in prokaryotes[J]. *Bioinformatics* 19(3):418–420. <https://doi.org/10.1093/bioinformatics/btg004>
41. Akhter S, Aziz RK, Edwards RA (2012) PhiSpy: a novel algorithm for finding prophages in bacterial genomes that combines similarity-and composition-based strategies[J]. *Nucleic Acids Res* 40(16):e126–e126. <https://doi.org/10.1093/nar/gks406>
42. Ge R, Mai G, Wang P et al (2016) CRISPRdigger: detecting CRISPRs with better direct repeat annotations[J]. *Sci Rep* 6(1):32942. <https://doi.org/10.1038/srep32942>
43. Ashburner M, Ball CA, Blake JA et al (2000) Gene Ontology: tool for the unification of biology[J]. *Nat Genet* 25(1):25–29. <https://doi.org/10.1038/75556>
44. Kanehisa M, Goto S, Kawashima S et al (2004) The KEGG resource for deciphering the genome[J]. *Nucleic Acids Res* 32(1). <https://doi.org/10.1093/nar/gkh063>. D277-D280
45. Kanehisa M, Goto S, Hattori M et al (2006) From genomics to chemical genomics: new developments in KEGG[J]. *Nucleic Acids Res* 34(1):D354–D357. <https://doi.org/10.1093/nar/gkj102>
46. Galperin MY, Makarova KS, Wolf YI et al (2015) Expanded microbial genome coverage and improved protein family annotation in the COG database[J]. *Nucleic Acids Res* 43(Database issue):261–269. <https://doi.org/10.1093/nar/gku1223>
47. Cantarel BL, Coutinho PM, Rancurel C et al (2009) The Carbohydrate-Active EnZymes database (CAZy): an expert resource for glycogenomics[J]. *Nucleic Acids Res* 37(suppl 1):D233–D238. <https://doi.org/10.1093/nar/gkn663>
48. Krzywinski M, Schein J, Birol I et al (2009) Circos: an information aesthetic for comparative genomics[J]. *Genome Res* 19(9):1639–1645. <http://www.genome.org/cgi/doi/10.1101/gr.092759.109>
49. Blin K, Shaw S, Vader L et al (2025) antiSMASH 8.0: extended gene cluster detection capabilities and analyses of chemistry, enzymology, and regulation mode *pagerangestyle?*[J]. *Nucleic Acids Res* 53(W1):W32–W38. <https://doi.org/10.1093/nar/gkaf334>
50. Liu R, Zhang Y, Zhai G et al (2020) A cell-free platform based on nisin biosynthesis for discovering novel lanthipeptides and guiding their overproduction in vivo[J]. *Adv Sci* 7(17):2001616. <https://doi.org/10.1002/advs.202001616>Digital Object Identifier
51. Lugani Y, Sooch BS (2017) In silico characterization of cellulases from genus *Bacillus*[J]. *Int J Curr Res Rev* 9:30–37. <https://doi.org/10.7324/IJCRR.2017.9136>
52. Peng Y, Tang T, Li Q et al (2024) *Mycobacterium tuberculosis* FadD18 Promotes Proinflammatory Cytokine secretion to inhibit the intracellular survival of *Bacillus Calmette–Guérin*[J]. *Cells* 13(12):1019. <https://doi.org/10.3390/cells13121019>
53. Kim S, Jeong H, Lee NK et al (2025) Comprehensive Safety Assessment of *Lentilactobacillus buchneri* KU200793 as a Potential Probiotic[J]. *Microorganisms*, 13(9): 2067
54. Kurtz S, Phillippy A, Delcher AL et al (2004) Versatile and open software for comparing large genomes[J]. *Genome Biol* 5(2):R12. <https://link.springer.com/article/10.1186/gb-2004-5-2-r12>

55. Harris RS (2007) Improved pairwise alignment of genomic DNA[M]. ProQuest, ISBN:978-0-549-43170-1. Order Number:AAI3299002
56. Chiaromonte F, Yap VB, Miller W (2001) Scoring pairwise genomic sequence alignments[C]//Pacific Symposium on Biocomputing. 7: 115. https://doi.org/10.1142/9789812799623_0012
57. Reva ON, Dixelius C, Meijer J et al (2004) Taxonomic characterization and plant colonizing abilities of some bacteria related to *Bacillus amyloliquefaciens* and *Bacillus subtilis*[J]. FEMS microbiology ecology. 48(2):249–259. <https://doi.org/10.1016/j.femsec.2004.02.003>
58. Hashem A, Tabassum B, Abd_Allah EF (2019) *Bacillus subtilis*: A plant-growth promoting rhizobacterium that also impacts biotic stress[J]. Saudi J Biol Sci 26(6):1291–1297. <https://doi.org/10.1016/j.sjbs.2019.05.004>
59. Jangir M, Pathak R, Sharma S et al (2018) Biocontrol mechanisms of *Bacillus* sp., isolated from tomato rhizosphere, against *Fusarium oxysporum* f. sp. *lycopersici*[J]. Biol Control 123:60–70. <https://doi.org/10.1016/j.biocontrol.2018.04.018>
60. Zhou L, Song C, Li Z et al (2021) Antimicrobial activity screening of rhizosphere soil bacteria from tomato and genome-based analysis of their antimicrobial biosynthetic potential[J]. BMC Genomics 22(1):29. <https://link.springer.com/article/10.1186/s12864-020-07346-8>
61. Kramer J, Özkaya Ö, Kümmerli R (2020) Bacterial siderophores in community and host interactions[J]. Nat Rev Microbiol 18(3):152–163. <https://www.nature.com/articles/s41579-019-0284-4>
62. Wang L, Huang J, Chen S et al (2024) Endogenous cell wall degrading enzyme LytD is important for the biocontrol activity of *Bacillus subtilis*[J]. Front Plant Sci 15:1381018. <https://doi.org/10.3389/fpls.2024.1381018>
63. Shen Y, Shi Z, Zhao J et al (2023) Whole genome sequencing provides evidence for *Bacillus velezensis* SH-1471 as a beneficial rhizosphere bacterium in plants[J]. Scientific Reports, 13(1): 20929. <https://www.nature.com/articles/s41598-023-48171-9>
64. Chowdhury SP, Hartmann A, Gao XW et al (2015) Biocontrol mechanism by root-associated *Bacillus amyloliquefaciens* FZB42—a review[J]. Front Microbiol 6:780. <https://doi.org/10.3389/fmicb.2015.00780>
65. Chen XH, Koumoutsis A, Scholz R et al (2007) Comparative analysis of the complete genome sequence of the plant growth–promoting bacterium *Bacillus amyloliquefaciens* FZB42[J]. Nat Biotechnol 25(9):1007–1014. <https://doi.org/doi/10.1038/nbt1325>
66. Yu X, Ai C, Xin L et al (2011) The siderophore-producing bacterium, *Bacillus subtilis* CAS15, has a biocontrol effect on *Fusarium* wilt and promotes the growth of pepper[J]. Eur J Soil Biol 47(2):138–145. <https://doi.org/10.1016/j.ejsobi.2010.11.001>
67. Ku Y, Xu G, Tian X et al (2018) Root colonization and growth promotion of soybean, wheat and Chinese cabbage by *Bacillus cereus* YL6[J]. PLoS ONE 13(11):e0200181. <https://doi.org/10.1371/journal.pone.0200181>

68. Zhou D, Huang XF, Chaparro JM et al (2016) Root and bacterial secretions regulate the interaction between plants and PGPR leading to distinct plant growth promotion effects[J]. *Plant Soil* 401(1):259–272. <https://link.springer.com/article/10.1007/s11104-015-2743-7>
69. Gao S, Wu H, Yu X et al (2016) Swarming motility plays the major role in migration during tomato root colonization by *Bacillus subtilis* SWR01[J]. *Biol Control* 98:11–17
70. Weng J, Wang Y, Li J et al (2013) Enhanced root colonization and biocontrol activity of *Bacillus amyloliquefaciens* SQR9 by *abrB* gene disruption[J]. *Appl Microbiol Biotechnol* 97(19):8823–8830. <https://link.springer.com/article/10.1007/s00253-012-4572-4>
71. Idris ESE, Iglesias DJ, Talon M et al (2007) Tryptophan-dependent production of indole-3-acetic acid (IAA) affects level of plant growth promotion by *Bacillus amyloliquefaciens* FZB42[J]. *Molecular plant-microbe interactions*. 20(6):619–626. <https://doi.org/10.1094/MPMI-20-6-0619>
72. Fan B, Wang C, Song X et al (2018) *Bacillus velezensis* FZB42 in 2018: the gram-positive model strain for plant growth promotion and biocontrol[J]. *Front Microbiol* 9:2491. <https://doi.org/10.3389/fmicb.2018.02491>
73. Meena V, Singh et al (eds) (2016) *Potassium solubilizing microorganisms for sustainable agriculture*. Vol. 331. New Delhi: Springer, ISBN: 978-81-322-2774-8
74. Zhang Y, Zhang N, Bi X et al (2024) Growth promotion on maize and whole-genome sequence analysis of *Bacillus velezensis* D103[J]. *Microbiol Spectr* 12(12):e01147–e01124. <https://doi.org/10.1128/spectrum.01147-24>
75. Zhao X, Kuipers OP (2016) Identification and classification of known and putative antimicrobial compounds produced by a wide variety of *Bacillales* species[J]. *BMC Genomics* 17(1):882. <https://link.springer.com/article/10.1186/s12864-016-3224-y>
76. Xie J, Shi H, Du Z et al (2016) Comparative genomic and functional analysis reveal conservation of plant growth promoting traits in *Paenibacillus polymyxa* and its closely related species[J]. *Sci Rep* 6(1):21329. <https://doi.org/10.1038/srep21329>
77. Wang H, Sivonen K, Fewer DP (2015) Genomic insights into the distribution, genetic diversity and evolution of polyketide synthases and nonribosomal peptide synthetases[J]. *Curr Opin Genet Dev* 35:79–85. <https://doi.org/10.1016/j.gde.2015.10.004>
78. Rabbee MF, Ali MS, Choi J et al (2019) *Bacillus velezensis*: a valuable member of bioactive molecules within plant microbiomes[J]. *Molecules* 24(6):1046. <https://doi.org/10.3390/molecules24061046>
79. Scholz R, Vater J, Budiharjo A et al (2014) Amylocyclicin, a novel circular bacteriocin produced by *Bacillus amyloliquefaciens* FZB42[J]. *J Bacteriol* 196(10):1842–1852. <https://doi.org/10.1128/jb.01474-14>
80. Špacapan M, Danevčič T, Štefanič P et al (2020) The ComX quorum sensing peptide of *Bacillus subtilis* affects biofilm formation negatively and sporulation positively[J]. *Microorganisms* 8(8):1131. <https://doi.org/10.3390/microorganisms8081131>

81. Wang Z, Zhang W, Wang Z et al (2024) Analysis of antimicrobial biological activity of a marine *Bacillus velezensis* NDB[J]. Arch Microbiol 206(3):131. <https://link.springer.com/article/10.1007/s00203-024-03861-4>
82. Von Wintersdorff CJH, Penders J, Van Niekerk JM et al (2016) Dissemination of antimicrobial resistance in microbial ecosystems through horizontal gene transfer[J]. Front Microbiol 7:173. <https://doi.org/10.3389/fmicb.2016.00173>
83. on Additives EP, Rychen G, Aquilina G et al (2018) Guidance on the characterisation of microorganisms used as feed additives or as production organisms[J]. Efsa J 16(3):e05206. <https://doi.org/10.2903/j.efsa.2018.5206>
84. Alcaraz LD, Moreno-Hagelsieb G, Eguiarte LE et al (2010) Understanding the evolutionary relationships and major traits of *Bacillus* through comparative genomics[J]. BMC Genomics 11(1):332. <https://link.springer.com/article/10.1186/1471-2164-11-332>
85. Cohan FM (2002) What are bacterial species?[J]. Annual Reviews Microbiol 56(1):457–487. <https://doi.org/10.1146/annurev.micro.56.012302.160634>
86. Feulner PGD, De-Kayne R (2017) Genome evolution, structural rearrangements and speciation[J]. J Evol Biol 30(8):1488–1490. <https://doi.org/10.1111/jeb.13101>
87. Yan F, Li C, Ye X et al (2020) Antifungal activity of lipopeptides from *Bacillus amyloliquefaciens* MG3 against *Colletotrichum gloeosporioides* in loquat fruits[J]. Biol Control 146:104281. <https://doi.org/10.1016/j.biocontrol.2020.104281>
88. Steinke K, Mohite OS, Weber T et al (2021) Phylogenetic distribution of secondary metabolites in the *Bacillus subtilis* species complex[J]. Msystems, 6(2): 10.1128/msystems. 00057 – 21. <https://doi.org/10.1128/msystems.00057-21>
89. Stephenson K, Hoch JA (2002) Evolution of signalling in the sporulation phosphorelay[J]. Mol Microbiol 46(2):297–304. <https://doi.org/10.1046/j.1365-2958.2002.03186.x>
90. Blake C, Nordgaard M, Maróti G et al (2021) Diversification of *Bacillus subtilis* during experimental evolution on *A. rabidopsis thaliana* and the complementarity in root colonization of evolved subpopulations[J]. Environ Microbiol 23(10):6122–6136. <https://doi.org/10.1111/1462-2920.15680>
91. Olanrewaju OS, Ayilara MS, Ayangbenro AS et al (2021) Genome mining of three plant growth-promoting *Bacillus* species from maize rhizosphere[J]. Appl Biochem Biotechnol 193(12):3949–3969. <https://link.springer.com/article/10.1007/s12010-021-03660-3>

Figures

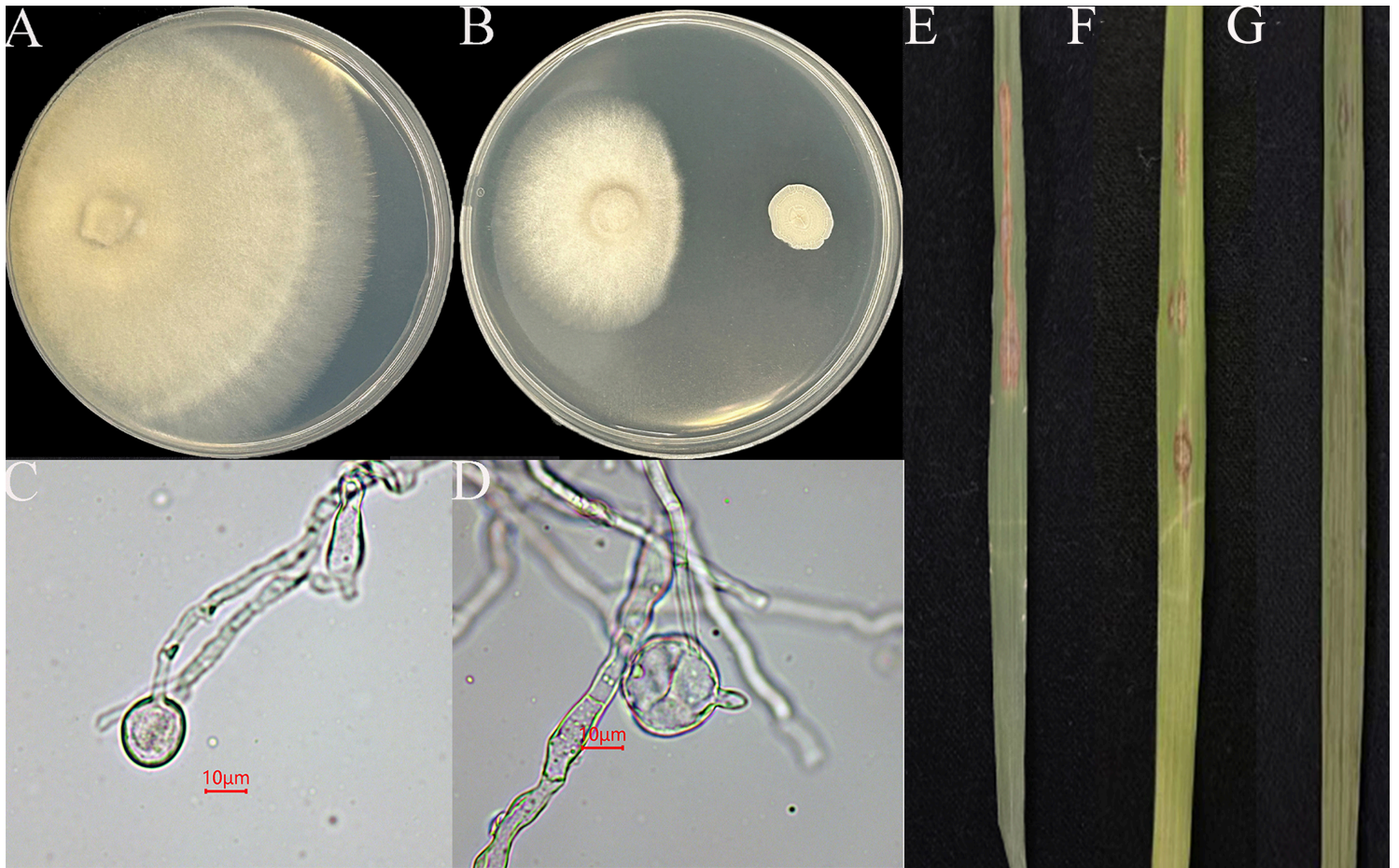


Figure 1

The inhibitory effect of Bv. JN.Y2 on *C. cereale* under dual-culture and greenhouse conditions.

Note: (A) Colony morphology of the pathogen *C. cereale* on PDA medium (Control). (B) Dual-culture assay showing the inhibition zone produced by strain JN.Y2 against the pathogen. (C–D) Microscopic observations of hyphal abnormalities induced by Bv. JN.Y2. Images show cellular swelling, deformation, and vacuolization of the fungal mycelia and spores. Scale bar = 10 μm . (E–G) Biocontrol efficacy assay on detached leaves. Leaves were inoculated with *C. cereale* mycelial spore suspension. (E) Negative control (CK), treated with sterile water. (F) Positive control, treated with the chemical fungicide thiophanate-methyl. (G) Treatment with the cell suspension of Bv. JN.Y2.

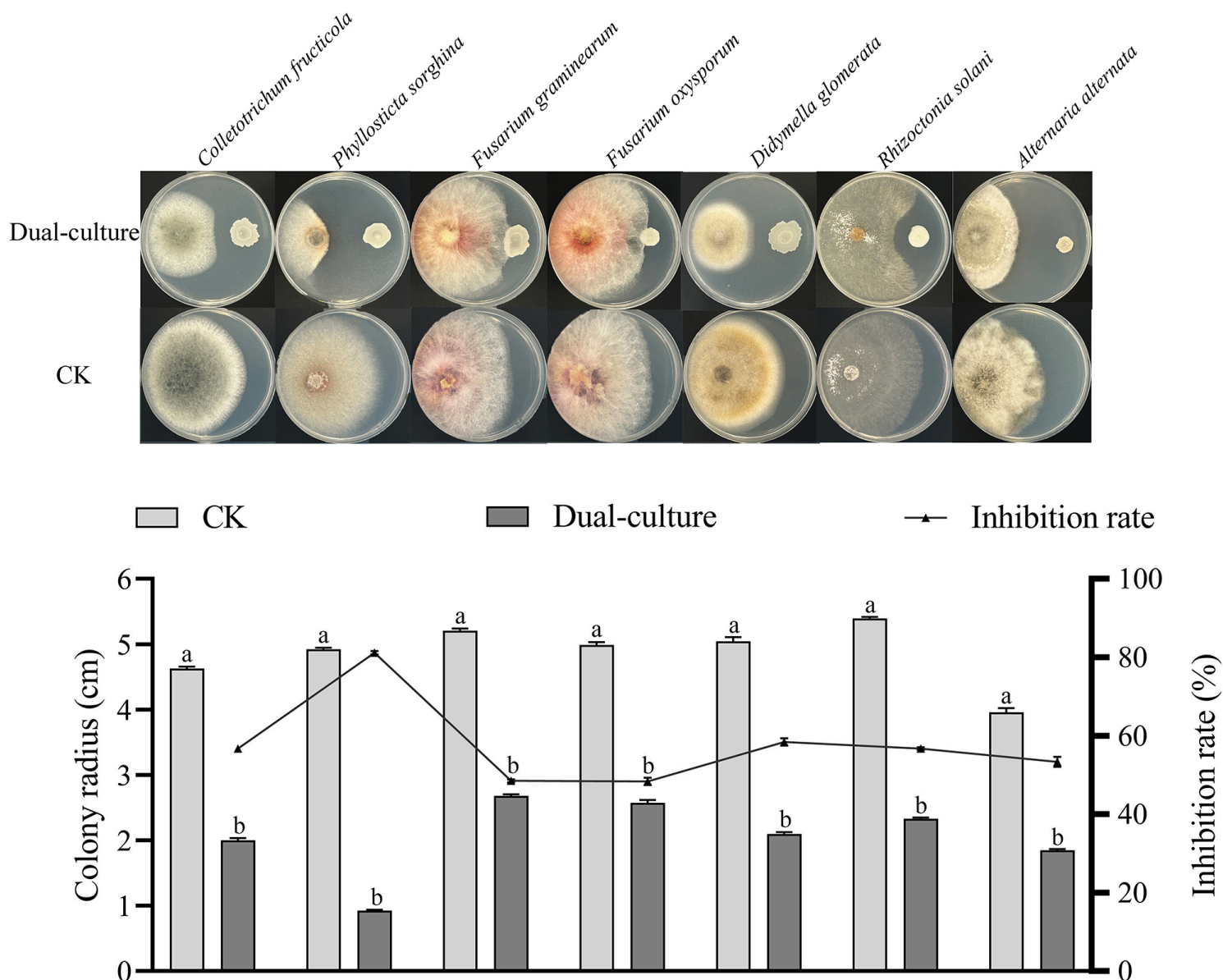


Figure 2

Determination of the inhibitory ability of Bv. JN.Y2 against different pathogens.

Note: The upper panel presents the dual-culture confrontation between Bv. JN.Y2 and various fungal pathogens. Data in the lower panel represent the mean $\pm$ SD. Different lowercase letters (a, b) above the bars for each pathogen denote significant differences between CK and the Dual-culture treatment, according to an independent samples *t*-test ($P < 0.05$). CK: blank control; Dual-culture: treatment with Bv. JN.Y2.

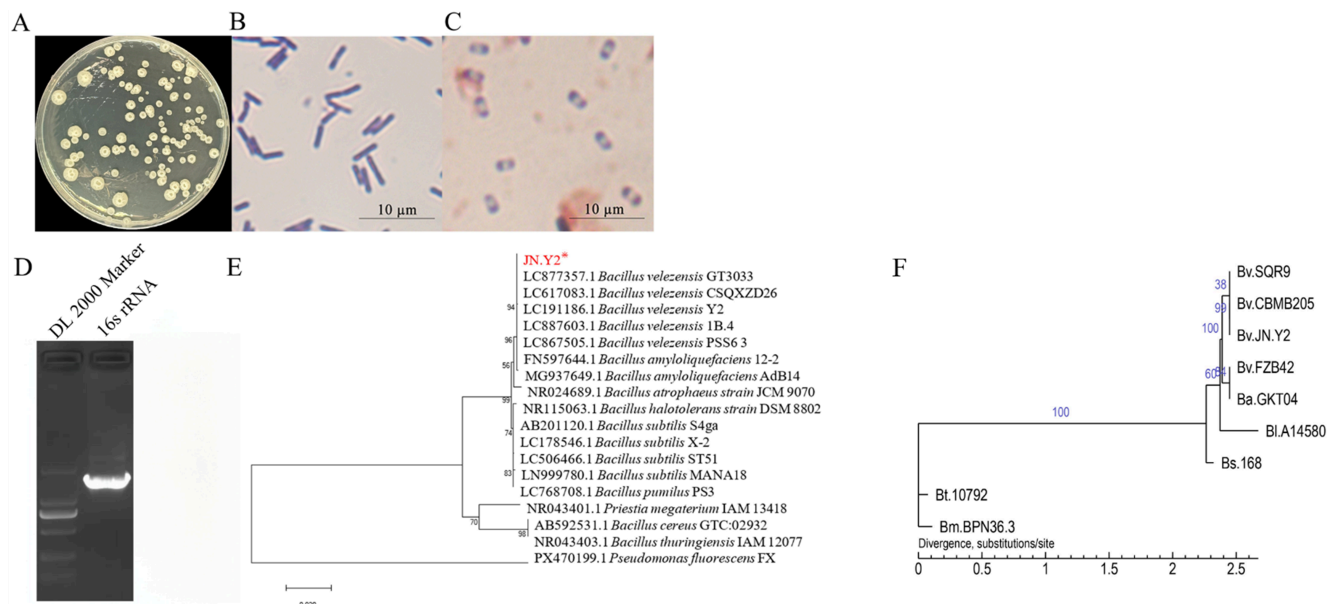


Figure 3

Morphological and molecular identification of Bv. JN.Y2.

Note: **(A)** Colony morphology of strain Bv. JN.Y2 grown on LB agar for 24 h. **(B)** Gram staining showing Gram-positive rod-shaped cells. Scale bar = 10 μ m. **(C)** Endospore staining showing central or subterminal ellipsoidal spores. Scale bar = 10 μ m. **(D)** Agarose gel electrophoresis of PCR products for 16S rRNA. **(E)** Phylogenetic tree constructed based on the sequences of 16S rRNA gene using the Neighbor-Joining method. Bootstrap values (>50%) are indicated at the nodes. *Bv. JN.Y2* is highlighted in red. **(F)** Phylogenomic tree constructed based on whole-genome SNPs. The scale bar represents the number of substitutions per site.

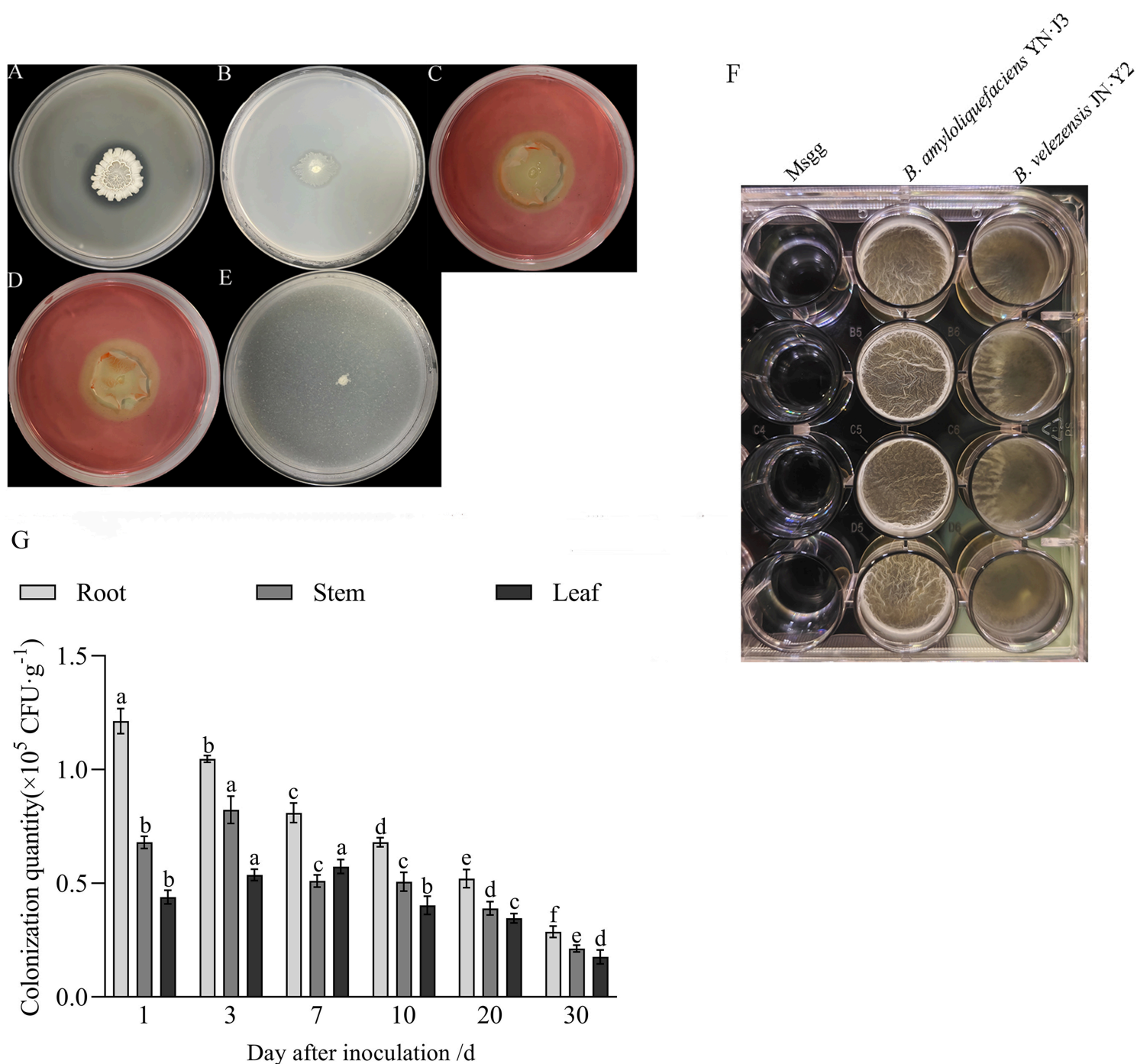


Figure 4

Evaluation of potential biocontrol characteristics of *Bv. JN.Y2*.

Note: (A–E) Qualitative detection of extracellular hydrolytic enzymes on agar plates. Clear halos around the colonies indicate positive activities for protease, amylase, cellulase, pectinase and chitinase (A–E). (F) Biofilm phenotype of strains *B. amyloliquefaciens* YN.J3 and *Bv. JN.Y2* at the air-liquid interface in MSgg medium compared to the control (MSgg+LB) after 48 h. (G) Colonization dynamics of *Bv. JN.Y2* in the root, stem, and leaf tissues of oats. Samples were collected at 1, 3, 7, 10, 20, and 30 dpi. Data are

presented as mean $\pm$ SD. Different lowercase letters (a, b, c) in the same column indicate significant differences according to Duncan's multiple range test ($P < 0.05$).

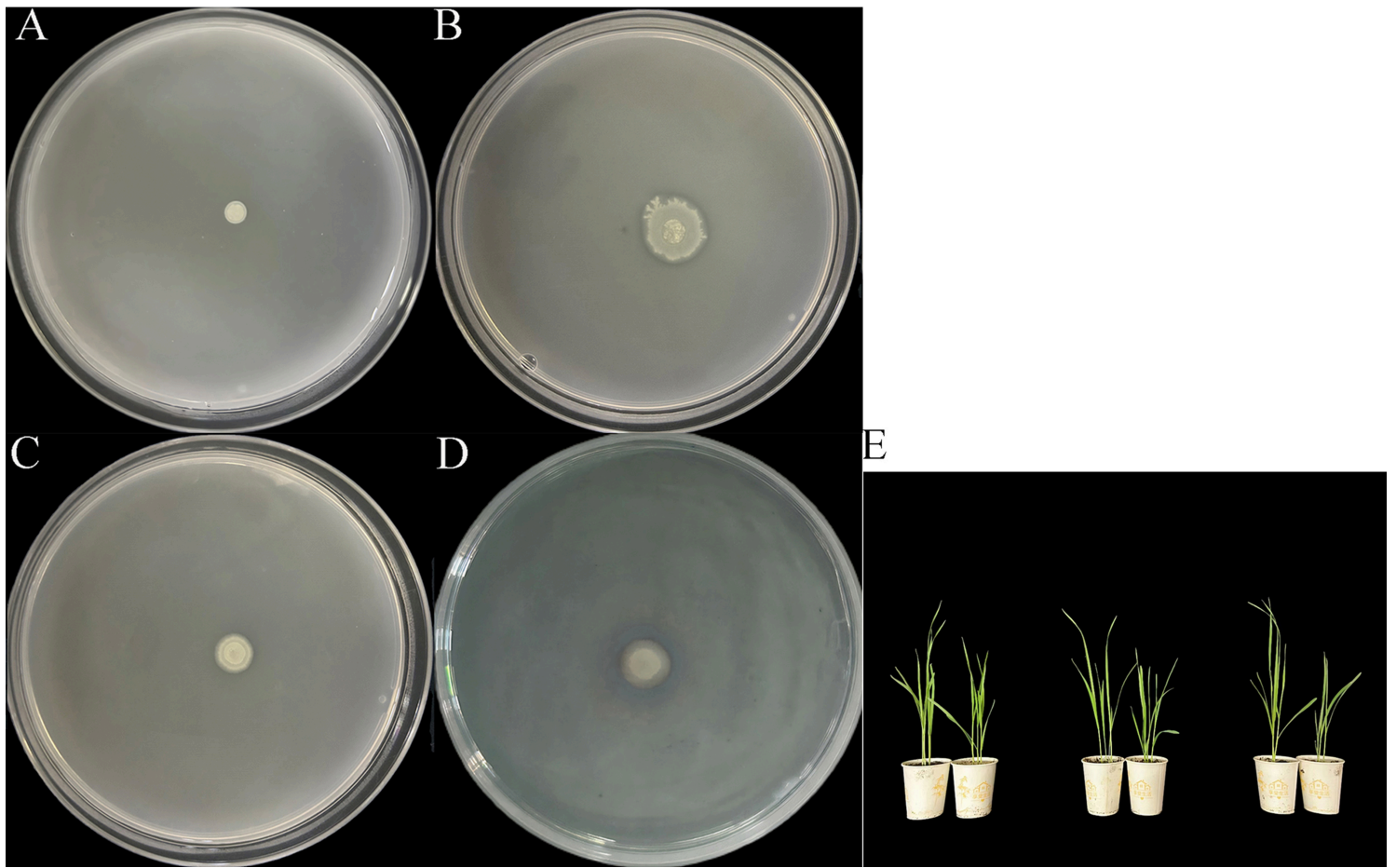


Figure 5

Potential PGP properties of Bv. JN.Y2 and its effects on oat growth.

Note: (A–D) Qualitative assays of PGP traits *in vitro*. (A) Nitrogen metabolism and assimilation capacity; (B) Phosphate solubilization; (C) Potassium solubilization; (D) Siderophore production. (E) Phenotypic comparison of seedlings treated with Bv. JN.Y2 suspension compared to the sterile water treatment.

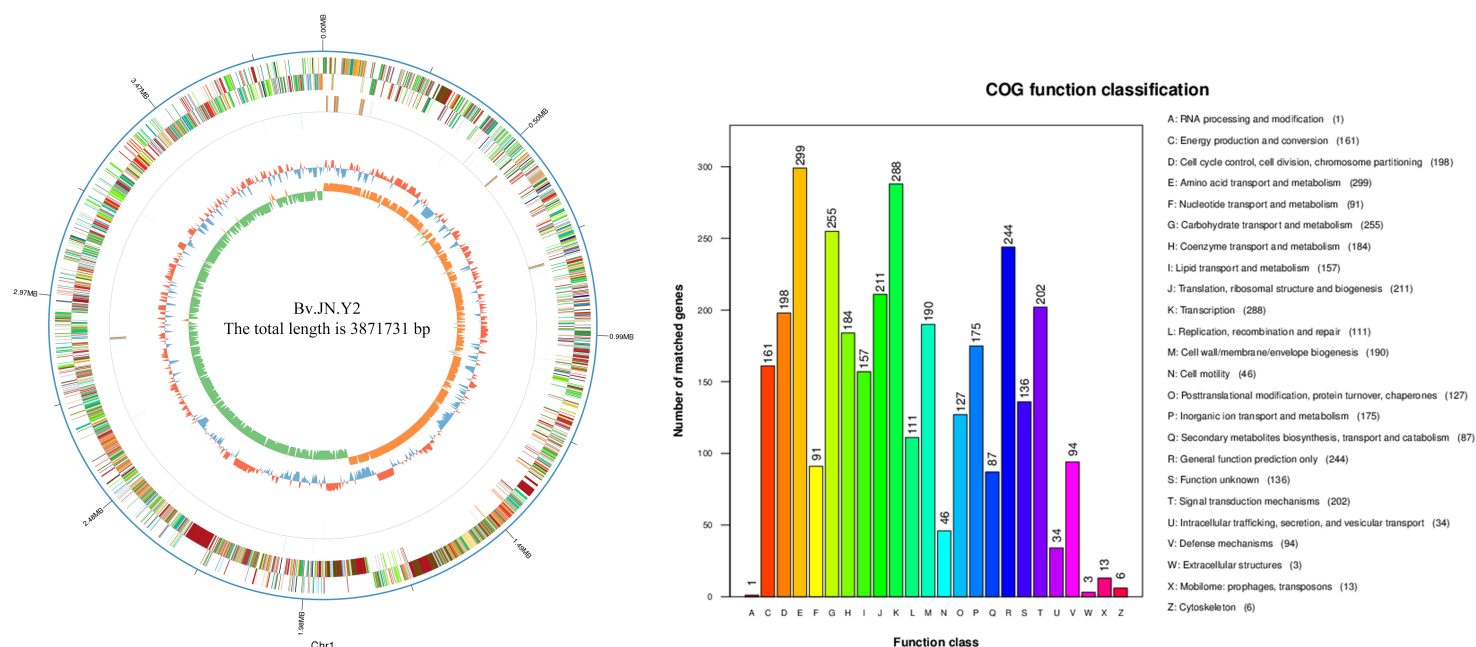


Figure 6

Circular genome map of Bv. JN.Y2 and its COG function classification.

Note: The outermost circle represents the genomic coordinates. Moving from the outside inward, the tracks display: gene functional annotation results (COG categories), ncRNA locations, GC content, and GC skew. GC content was calculated using a window and step size of (chromosome length/1000) bp. Blue regions pointing inward indicate that the GC content is lower than the genome-wide average, while red regions pointing outward indicate the opposite; the peak height represents the magnitude of the difference from the average. GC skew was calculated using the formula $(G-C)/(G+C)$ with a window and step size of (chromosome length/1000) bp. Green regions pointing inward indicate that the G content is lower than C, while orange regions pointing outward indicate the opposite. The x-axis represents the COG functional categories, and the y-axis represents the number of annotated genes.

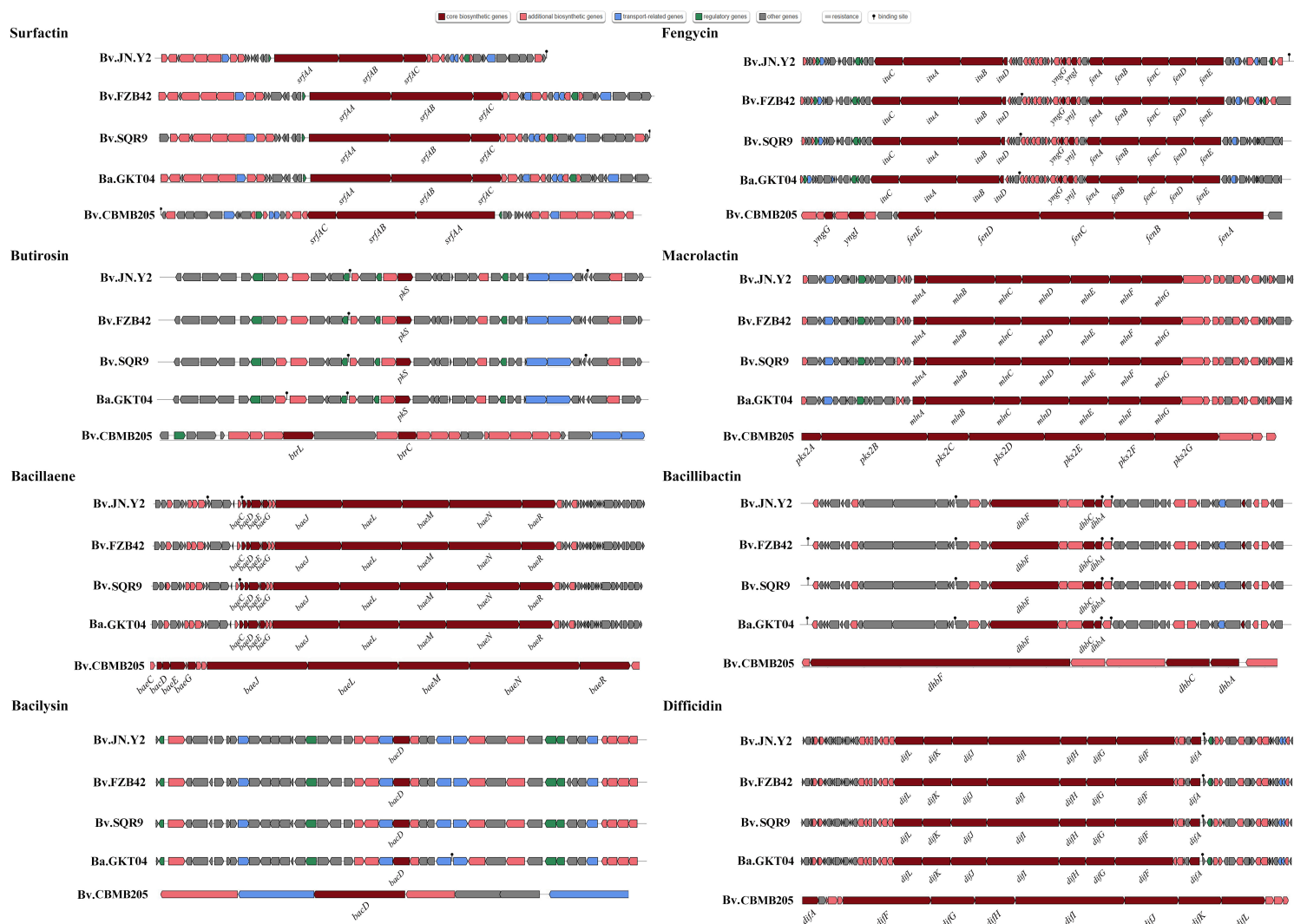


Figure 7

Comparisons of secondary metabolites synthesis clusters in Bv. JN.Y2, Bv. FZB42, Bv. SQR9, Bv. CBMB205 and Ba. GKT04.

Note: The genetic organization of eight predicted gene clusters is shown. Genes are represented by arrows, with the arrowhead indicating the direction of transcription. Color coding is based on predicted function: Dark red indicates core biosynthetic genes; light red/pink indicates additional biosynthetic genes; blue indicates transport-related genes; green indicates regulatory genes; and grey indicates other genes. Key biosynthetic genes are labeled with their standard gene names, including *srf*(surfactin), *fen* (fengycin), *btr/pks* (butirosin), *mln*(macrolactin), *bae* (bacillaene), *dhb* (bacillibactin), *bac*(bacilysin), and *dif* (difficidin) operons. Abbreviations: **Bv**, *Bacillus velezensis*; **Ba**, *Bacillus amyloliquefaciens*.

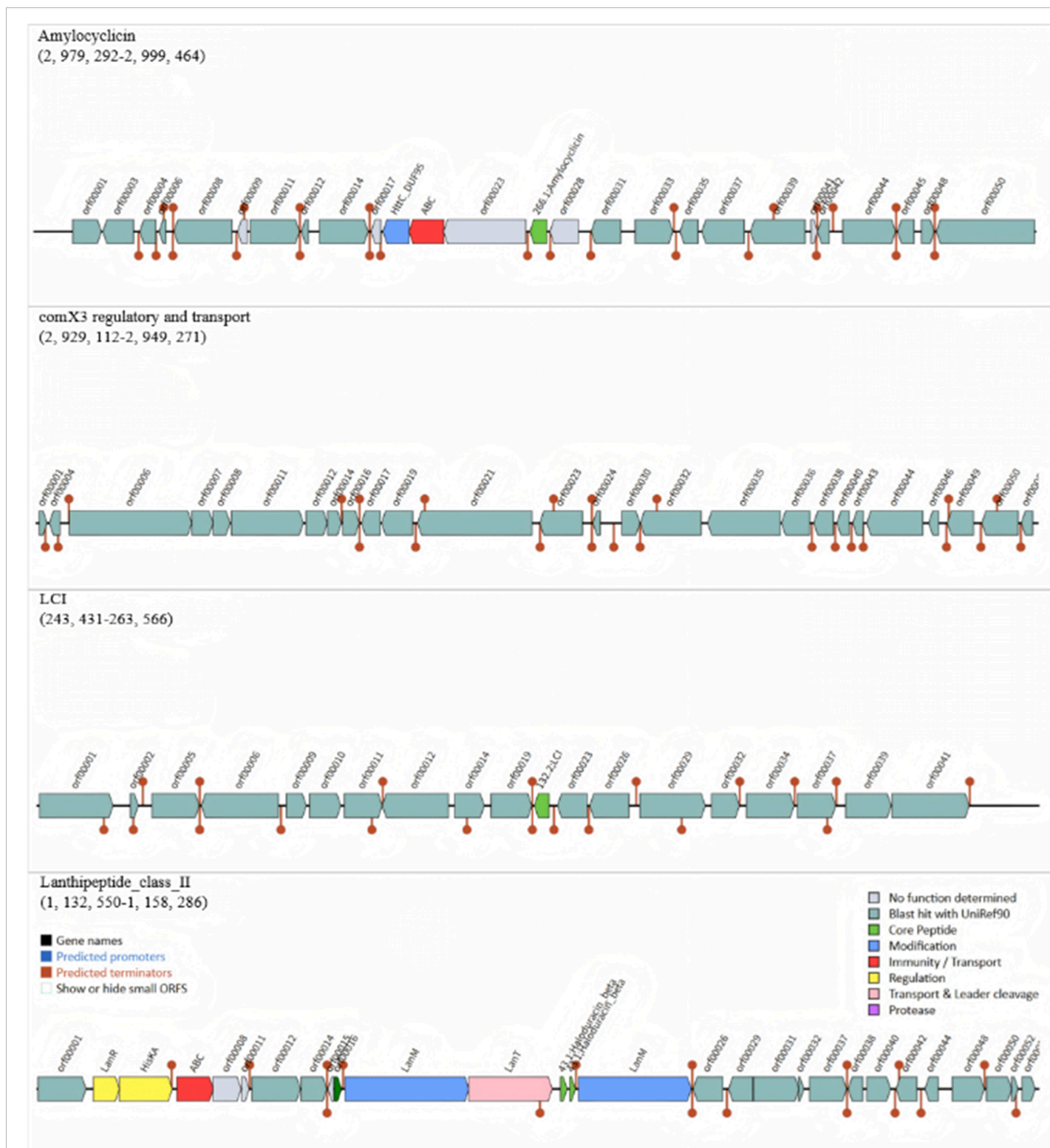


Figure 8

Genomic organization and putative functional assignment in Bv. JN.Y2 genome.

Note: The diagram illustrates four AOIs identified via the BAGEL4 platform within the Bv. JN.Y2 genome. Horizontal tracks represent specific genomic regions, with numerical coordinates in parentheses indicating their precise locations on the chromosome.

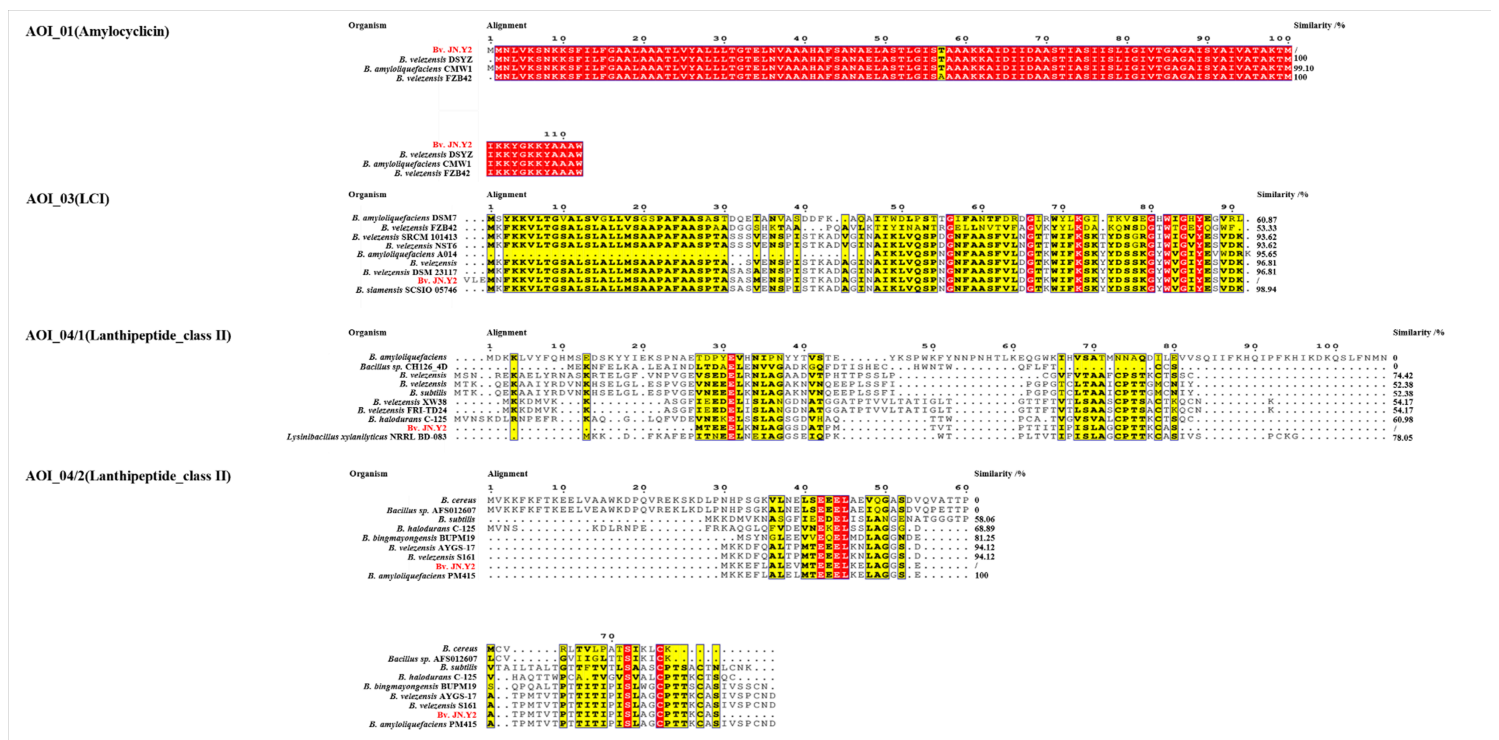


Figure 9

Comparative sequence analysis and conservation profiling of core antimicrobial peptides from Bv. JN.Y2.

Note: The figure presents the multiple sequence alignment of four core antimicrobial peptides identified within the Bv. JN.Y2 genome (corresponding to AOI_01, AOI_03, and the two subunits of AOI_04) compared with their representative homologs. The sequences were initially aligned using Clustal Omega, and the resulting genomic architecture was professionally visualized through ESPrnt 3.2. In this alignment profile, white characters on a red background signify absolute conservation (100% identity) across all analyzed strains, while yellow-boxed characters represent residues with similar physicochemical properties.

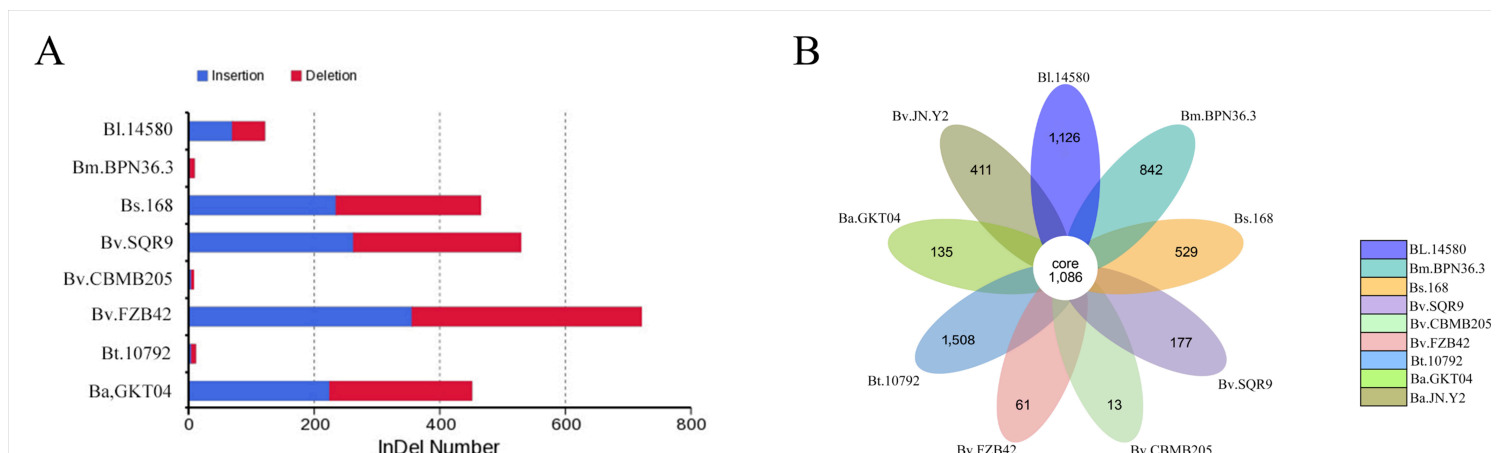


Figure 10

Comparative genomic analysis of Bv. JN.Y2 and eight reference strains.

Note: (A) Statistics of InDels detected in the genomes of reference strains compared to Bv. JN.Y2. Blue bars represent insertions, and red bars represent deletions. **(B)** Flower plot illustrating the core and pan-genome analysis. The central circle represents the number of core genes shared by all nine analyzed strains (1,086 genes), while the petals indicate the number of strain-specific (unique) genes for each strain. Abbreviations: **Bv**, *B. velezensis*; **Ba**, *B. amyloliquefaciens*; **Bs**, *B. subtilis*; **Bl**, *B. licheniformis*; **Bm**, *B. megaterium*; **Bt**, *B. thuringiensis*.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Supplementarymaterial.docx](#)
- [FullUncroppedGellImages.docx](#)