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METHODOLOGY

Antagonism of *Bacillus velezensis* ZGE166 Against the Pathogenic Fungi Causing Corm Rot Disease in Saffron (*Crocus sativus* L.)

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Abstract

Saffron can be infected with pathogenic fungi that cause corm rot as it grows and multiplies, which can reduce the quality and yield of saffron. Corm rot has become one of the most serious diseases of saffron. In this study, rhizosphere bacteria were isolated from saffron rhizosphere soil, and bacteria exhibiting antagonistic effects against corm rot pathogenic fungi were screened using in vitro plate co-culture assays and dual-compartment agar plate systems. Selected strains were further evaluated for hydrolase activity determination and PGP potential assessment. Among them, *Bacillus velezensis* showed the best disease resistance activity. The degradative enzyme production and some beneficial characteristics of *Bacillus velezensis* for plant growth promotion were evaluated. It was found that *Bacillus velezensis* possesses nitrogen fixing, NH_3-producing, IAA production, and ACC-deaminating enzymes. The whole genome sequence of this strain was annotated and analyzed. The genome of *Bacillus velezensis* consists of a circular chromosome of 3,908,025 bp base pairs, with a guanine and cytosine content of 46.64%. There are 3737 protein-coding genes, including 86 tRNA genes, 27 rRNA genes, and 85 sRNA genes. The genome also contains four genomic islands, two pre-phages, and one transposon. The prediction of the secondary metabolic accumulation gene cluster demonstrated that the genome sequence of ZGE166 encodes 12 gene clusters involved in the synthesis of secondary metabolites, including macrolactin H, bacillaene, fengycin, difficidin, and bacillibactin. In summary, strain ZGE166 *Bacillus velezensis* has the potential to be developed as a biological agent.

Keywords Saffron (*Crocus sativus* L.) · Corm rot · *Bacillus velezensis* · Antagonistic activity · Whole-genome sequencing

Introduction

Saffron is an ancient spice derived from the dried stigma of *Crocus sativus* L.; it has a long history of use as a dye spice with an important role in religion and customs, according to documents proving that the earliest use as medicine was documented in ancient Egypt [1]. Studies have found anti-anxiety, inhibition of Alzheimer's disease metabolic pathways, anti-diabetic, anti-inflammatory, and anti-atherosclerotic properties, etc. [2]. The demand for saffron has been increasing in recent years due to its medicinal properties.

Saffron is a triploid species, with a $3n = 24$ number of chromosomes [3]. Clonal reproduction by means of corm propagation is easy to cause soil-borne diseases, and saffron corm rot is caused by a variety of soil-borne pathogens. In recent years, the continuous decline in saffron yield has caused serious economic losses in saffron-growing regions worldwide. One of the major contributing factors is the prevalence of corm rot [4]. One of the main production areas of saffron in China, in Chongming, Shanghai, experienced

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corm rot mainly caused by four pathogenic fungi (*Fusarium oxysporum*, *Fusarium solani*, *Penicillium citreosulfuratum*, *and Penicillium citrinum*) [5].

Current preventive management measures for corms rely on the use of chemical fungicides and the development of disease-resistant varieties, as well as crop rotation [4]. However, improper use of chemical fungicides leads to pathogen resistance, stressing the environment and influencing soil microbial community (bacteria and fungi) diversity [6, 7]. Instead, biocontrol agents are cost-effective, eco-friendly, renewable, and have natural ways to tackle plant diseases and improve agricultural yields [8]. Biological control inhibits pests and diseases by introducing natural enemies, microorganisms, and their metabolites [9]. Biocontrol bacteria act on plants through both direct and indirect mechanisms, including competition for space and nutrition, fungal hyperparasitism, antagonism, and induction of systemic resistance (ISR) [10–12]. The rhizosphere represents one of the most diverse habitats on the planet and is central to ecosystem functioning [13–15]. The roots of plants have thousands of species referred to as the second genome of the plant, and they have a stake in the health of plants. The rhizosphere bacteria produce antibiotic compounds or extracellular enzymes to support plant growth and systemically boost the defensive capacity of the plant [16].

The genus *Bacillus* is an important microbial population of soil, plant surfaces, and the rhizosphere. Its nutrient requirements, isolation, and culture are simple, and it has a broad spectrum of antibacterial activity, which is a very valuable microorganism. *Bacillus brevis*, *Bacillus amyloliquefaciens*, *Bacillus tequilensis*, *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus thuringiensis*, and *Bacillus pumilus*, etc., can not only inhibit or kill plant pathogens to control diseases but also promote plant growth and enhance the tolerance of plants to abiotic stress through various mechanisms [17–20]. *Bacillus velezensis* has been proposed as a later heterotypic synonym of *Bacillus amyloliquefaciens* in previous studies based mainly on DNA-DNA relatedness values. A later study sequenced a draft genome of *B. velezensis*, compared genomics and DNA-DNA relatedness calculations, and found that the strain was not a synonym of *B. amyloliquefaciens*; it is instead synonymous with *Bacillus methylotrophicus* [21]. *Bacillus velezensis* was officially named in November 2005, so named because the strain was first obtained from the river named *Vélez in Málaga*, southern Spain [22]. *B. velezensis* has strong antimicrobial activity, and the control effect of *B. velezensis* on *Fusarium* head blight (FHB) in wheat caused by *Fusarium graminearum* could reach 72.3% in the field test [23]. *Botrytis cinerea* is a pathogen causing gray mold, which can cause a variety of plant diseases. *B. velezensis* biocontrol efficacy was above 50% [16]. For *Phytophthora sojae*, which causes diseases in crops such as soybeans and potatoes, and *Phytophthora* species, which causes other plant diseases, *B. velezensis* showed high antibacterial activity and inhibition rates of more than 50% [24]. Marta Torres et al. [25] concluded in their study that *B. velezensis* not only has a strong antibacterial effect but also promotes the growth of plants. In the experiment, the fresh weight of the applied XT1 increased by even more than 100% compared with the control group; the fresh weight and dry weight of roots also increased in both [26].

In this study, we aimed to screen bacteria capable of inhibiting pathogenic fungi responsible for saffron corm rot disease. By effectively suppressing the growth of pathogenic fungi, the incidence of corm rot can be significantly reduced. We isolated a strain of *Bacillus* ZGE166 from rhizosphere soil in the Chongming area of Shanghai, which exhibited strong antagonistic effects against four major pathogenic fungi causing corm rot. This strain was identified as *B. velezensis*. Through whole-genome sequencing analysis, we investigated gene clusters potentially associated with its antagonistic and growth-promoting properties and predicted corresponding bioactive secondary metabolites in this strain. These findings laid the foundation for its application as a biocontrol agent against saffron corm rot disease.

Materials and Methods

Indicative Pathogenic Fungi

In 2020, four pathogenic fungi isolated from the rotten corms of saffron by our research group, *Fusarium oxysporum*, *Fusarium solani*, *Penicillium citreosulfuratum*, and *Penicillium citrinum*, were preserved in a refrigerator at 4 °C.

Soil Collection and Isolation and Purification of Bacterial

The rhizosphere soil samples of *Crocus sativus* L. were collected from Chongming, Shanghai, China (longitude: 121.349092, latitude: 31.712459). The rhizosphere soil was naturally dried and crushed with a mortar and pestle, then passed through a 20-mesh sieve. Weighed 5 g of the soil sample in a triangular flask containing 45 mL of sterile water, and the conical flask was placed on a shaker at 30 °C and shaken at 150 rpm for 2 h [27]. After serial dilution, 200 µL of different concentrations of suspensions were spread on TWYE medium, LB medium, Beef Extract Peptone Medium, and TSB medium, each containing cycloheximide (50 mg/L), in triplicate. The plates were then inverted and incubated at 30 °C after spreading uniformly. The plates were incubated continuously for 14 days, and the growth of colonies was observed daily. Bacterial colonies were

promptly picked and transferred to LB medium for purification, then inoculated onto the corresponding slant medium and stored at 4 °C for further use.

Antibacterial Activity of Antagonistic Bacteria

In vitro screening of isolated bacteria using the plate co-culture assay showed that the colony edges of *F. oxysporum* and *F. solanii* were cultured for 7 days, and single colonies of *P. citreosulfuratum and P. citrinum were* cultured for 2 days. These were punched with a hole punch, and then the pathogen plugs were placed in the center of a PDA plate. The bacteria were inoculated in a criss-cross pattern around 2.5 cm away from the pathogen plugs, and only pathogen plugs were used as a control. The plate was incubated at 28 °C for a duration of 5 to 10 days, and it was observed whether antagonistic bands appeared on the plate of the experimental group. The experiment was repeated three times, and the inhibition rate was calculated to get the optimal antagonistic bacteria. Inhibition rate = (control colony diameter − treatment colony diameter)/control colony diameter × 100%.

Antibacterial Activity of Volatile Gas

Antibacterial activity of volatile gas was determined by the double-dish-to-deduction method, which involves dual culture by sealing two agar base plates together. [28, 29]. Empty PDA agar medium coupled with pathogen-containing PDA agar medium was used as a control. The plate was placed in an incubator at 30 °C for 7 days, the diameter of the pathogen was recorded, and the inhibition rate was calculated as described previously and repeated three times for each experimental group.

Morphological Characteristics, Physiology and Biochemistry Tests

Single bacterial colonies were inoculated onto LB medium for 5 days, and the shape, color, and texture of the colony were observed. Square agar blocks of bacteria with a side length of about 0.5 cm were cut from the medium, and SEM samples were prepared by referring to Miloslav Kaláb et al. [30]. The samples were placed in a critical point dryer for drying, and the dried samples were attached to the special sample table with double-sided conductive glue. Finally, the sample table was placed in the ion sputtering instrument to complete the surface gold plating treatment, and further observation was performed under SEM. According to the Berger Handbook of Bacteria Identification [31], physiological and biochemical tests were performed on strain ZGE166.

Molecular Identification of Strain ZGE166

PCR amplification of the 16S rRNA gene in ZGE166 was achieved using universal primers 27 F and 1492R [32]. The amplified PCR products were then purified and Sanger sequenced at Paishengnuo Biotech Co. LTD. (Shanghai, China). The bacterial 16S rRNA sequences obtained were checked on the National Center for Biotechnology Information website using the BLAST algorithm through the NCBI GenBank database [33]. The phylogenetic tree was constructed using the neighbor-joining method in MEGA 11.0.

Detection of Antagonism-Related Lytic Enzymes

Antagonistic bacteria secrete two primary categories of antimicrobial substances: small molecule antibiotics and big molecular weight antimicrobial proteins or cell wall hydrolases [34]. Cell wall hydrolysis plays an important role in inhibiting the growth of plant pathogens. A single colony of the activated biocontrol bacteria was selected and connected to glucanase assay plates, protease assay plates, cellulase assay plates, and chitin assay plates, respectively, and incubated at 30 °C for 5 days [35]. Observe whether there is a transparent circle on the chitin, dextranase, protease, and cellulase assay plate, which should be stained by adding an appropriate amount of 1 mg/mL Congo red solution to the petri dish for 1 h, pour off the staining solution, and then decolorize the plate by adding an appropriate amount of 1 mol/L NaCl solution for 1 h to observe whether a transparent circle is formed around it.

Assessment of Plant Growth Promotion (PGP) Traits

According to the method of Suarez-Moreno et al. [36], ZGE166 was inoculated in LB liquid medium containing 100 mg/L *L*-tryptophan. And after 3 days of incubation, 100 µL of the bacterial solution was placed in a test tube, an equal volume of Salkowski's colorimetric solution (50 mL of 35% $HClO_4$ + 1 mL of 0.5 mol/L $FeCl_3$) was added, and the tube was protected from light. After 30 min of incubation, the ability of the strain to produce indoleacetic acid IAA was observed. The ammonia production capacity of the strain was determined using peptone water liquid medium and Nessler's reagent [37]. The ability to utilize phosphate was determined using the National Botanical Research Institute's phosphate growth medium (NBRIP) [38]. The strains were inoculated on NBRIP and incubated for 5 days, and it was observed whether the medium near the colonies produced hyaline rings. For nitrogen fixation capacity of the strain, the strain was inoculated on Ashby's N-Free medium, incubated for 5 days, and observed for growth. The strain was inoculated on silicate medium, incubated at 30 °C for 5 days, and observed for the appearance of hyaline circles near the

medium to verify whether strain ZGE1666 has the ability to solubilize potassium [39]. ACC deaminase activity was determined by the method of Dubey et al. [40]. The strain was inoculated in CAS medium for 5 days to observe the iron carrier production ability of the strain [41].

Whole Genome Sequencing of ZGE166

Sample Preparation

Single colonies were picked up using an inoculating needle and inoculated into 200 mL of LB liquid medium. The cultures were then incubated at 30 °C with shaking at 220 r/min until they reached the logarithmic growth phase. Centrifugation was carried out at 12,000 rpm for 10 min at 4 °C, the supernatant was discarded, and the organisms were collected, washed off the medium components in the organisms with sterile water, and centrifuged again; the supernatant was discarded, and the organisms were collected. The organisms were quickly frozen with liquid nitrogen and immediately transported on dry ice to MGE Biological Medicine Technology Co., Ltd. (Shanghai, China).

DNA Extraction, Genome Sequencing, and Assembly

Genomic DNA was extracted using the bacterial/fungal DNA extraction kit (magnetic beads) (Majorbio, Shanghai, China), according to the manufacturer's protocol. Purified genomic DNA was quantified, and high-quality DNA was used to do further research.

Genome was sequenced using a combination of PacBio Sequel IIe and Illumina sequencing platforms. For Illumina sequencing, genomic DNA was used for each strain in sequencing library construction. DNA samples were sheared into ~400 bp fragments using Covaris M220 Focused Acoustic Shearer following the manufacturer's protocol. Illumina sequencing libraries were prepared from the sheared fragments using the NEXTFLEX Rapid DNA-Seq Kit. Briefly speaking, 5′ prime ends were first end-repaired and phosphorylated. Next, the 3′ ends were A-tailed and ligated to sequencing adapters. The next step is to enrich the adapters-ligated through the process of PCR. The prepared libraries were subsequently utilized for Illumina NovaSeq™ X Plus (Illumina Inc., San Diego, CA, USA). For PacBio sequencing, genomic DNA was fragmented at ~ 10 kb. DNA fragments were subsequently purified, end-repaired, and ligated with SMRT bell sequencing adapters in accordance with the manufacturer's recommendations (Pacific Biosciences, CA). Subsequently, the PacBio library underwent preparation and was sequenced on SMRT cell following standard methods.

The data generated from PacBio Sequel IIe and Illumina NovaSeq™ X Plus platform were used for bioinformatics analysis. All analyses were performed using the Majorbio Cloud Platform (cloud.majorbio.com), a free online platform provided by Shanghai Majorbio Bio-pharm Technology Co., Ltd. The detailed procedures are as follows.

Gene Prediction and Annotation

For Illumina sequence data, the raw data of Illumina sequencing was stored in fastq format. In order to make subsequent assembly more accurate, software fastp [42] v0.20.0 was used to control the quality of the raw data. Reads with low sequencing quality, high N content ratio, and small length after quality trimming were removed to obtain high-quality clean data. They were then used for subsequent genomic evaluation.The HiFi reads were assembled into Scaffolds using hifiasm (version0.19.5).

The prediction of open-reading frames (ORFs) was conducted utilizing Maker2, while tRNA-scan-SE [43] was employed for tRNA prediction, and Barrnap was utilized for rRNA prediction. The anticipated ORFs were annotated utilizing NR, Swiss-Prot, Pfam, GO, COG, KEGG, and CAZY databases through sequence alignment tools including BLAST, Diamond, and HMMER. In summary, each group of query proteins was aligned with the databases, and annotations for the best-matched subjects (E-value $< 10^{-5}$) were acquired for gene annotation purposes.

Result

Screening of Rhizosphere Bacteria

In this experiment, a total of 165 bacteria were isolated from the rhizosphere soil, and the antagonistic effect that was most effective was ZGE166, which was finally obtained after several screenings. ZGE166 inhibition rates of *Fusarium oxysporum*, *Fusarium solani*, P*enicillium citreosulfuratum*, and *Penicillium citrinum* were 82.9% ±0.6%, 73.4% ±0.3%, 73.8% ±1.3%, and 25.8% ±0.3% (Fig. 1), respectively. The volatile gas inhibition rates of *Bacillus velezensis* were 69.6% ±1.2%, 66.9% ±4.1%, 79.7% ±1.4%, and 79.3% ±1.4%, respectively (Fig. 2).

Identification of Rhizosphere Bacteria ZGE166

The single bacterial colony of strain ZGE166 was suborbicular, milky white, opaque, non-pigmented, with an irregular colony shape and radial edges. The surface of the colony was slimy, with wrinkled edges, a central bulge, and sticky, and it was a rod-shaped gram-positive bacterium (Fig. 3). Based on the above-described morphological characteristics and physiological and biochemical tests (Table 1), strain ZGE166 was initially identified as a *Bacillus* genus. Subsequently, the 16S rRNA

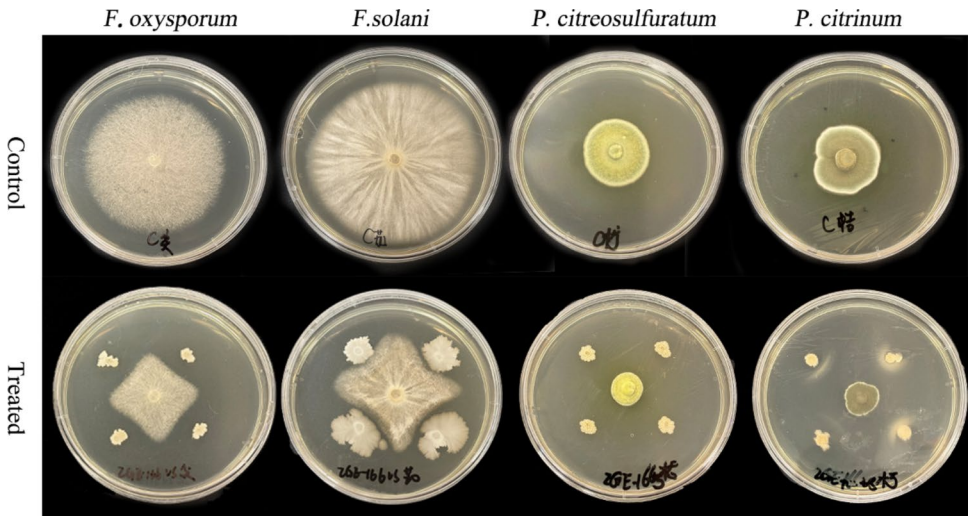

Fig. 1 Antagonism of the strain ZGE166 on four pathogenic fungi in PDA culture

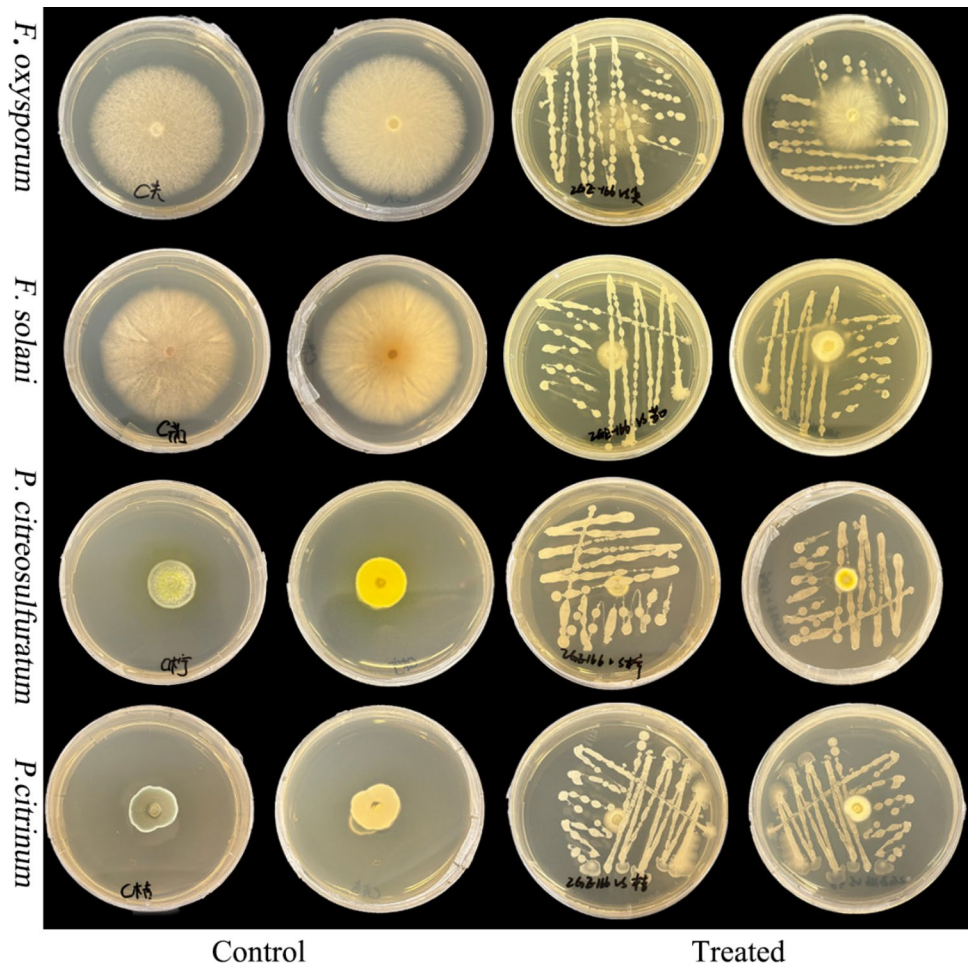

Fig. 2 Antagonism of strain ZGE166 volatile gas on four pathogens cultivated on PDA

gene sequences were compared with the NCBI database, which proved that strain ZGE166 was *Bacillus* spp. At the molecular biology level. Based on the 16S rRNA sequences, the 19 strains that were closest to each other at the genus level were selected to construct a phylogenetic tree using the NJ (Neighbor-Joining) method with MEGA 6.0 software (Fig. 4). The final identification was *Bacillus velezensis.*

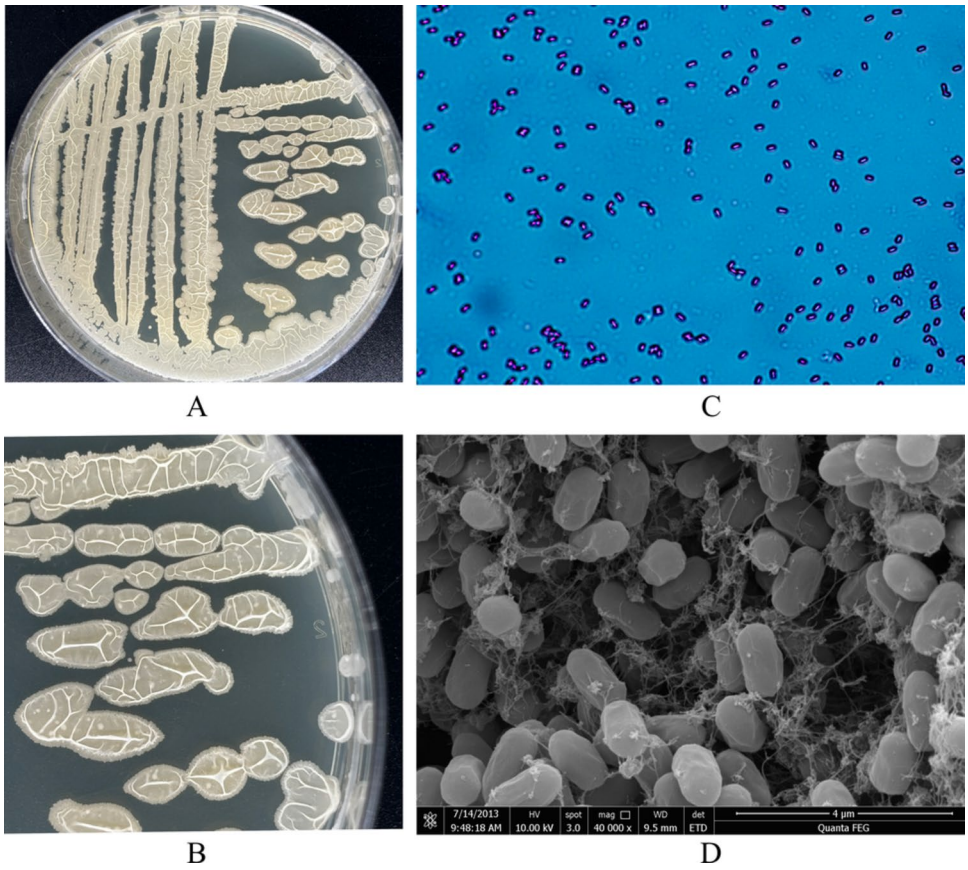

Fig. 3 Strain ZGE166 identification. **A**, **B** Colony morphological. **C** Gram staining. **D** Microscopic morphology

Table 1 Physiological and biochemical tests of strain ZGE166

Physiological and biochemical characterization	Result
Gram reaction	+
Growth at 4 °C	−
Growth at 40 °C	+
Oxidase	−
Catalase	+
Urease	+
Lipase	−
MR	−
V-P	+
Gelatin liquefaction	+
Starch hydrolysis	−
Milk coagulation	+
H2S production	−
Nitrate reduction	−
Citrate utilization	+

+, positive for test; −, negative for test

Hydrolytic Enzyme Activities and PGP Attributes of Strain ZGE166

According to the experimental results, strain ZGE166 lacked the activity of degrading enzymes. However, strain ZGE166 can perform nitrogen fixation, produce ACC deaminase enzyme, IAA and NH3, but it does not dissolve phosphorus and potassium (Table 2).

Genome Feature Analysis and Function Annotation

The genome of *B. velezensis* ZGE166 consists of a circular chromosome of 3,908,025 bp base pairs, with a guanine and cytosine (GC) content of 46.64% (Fig. 5). There are 3737 protein-coding genes (CDSs), which encompass 86 tRNA genes, 27 rRNA genes (16S, 23S, 5S), and 85 sRNA genes. The total length of the coding region is 3,908,025 bp, constituting 88.59% of the entire genome length; the average gene length is 926.42 bp, with four genomic islands, two pre-phages, and one transposon.

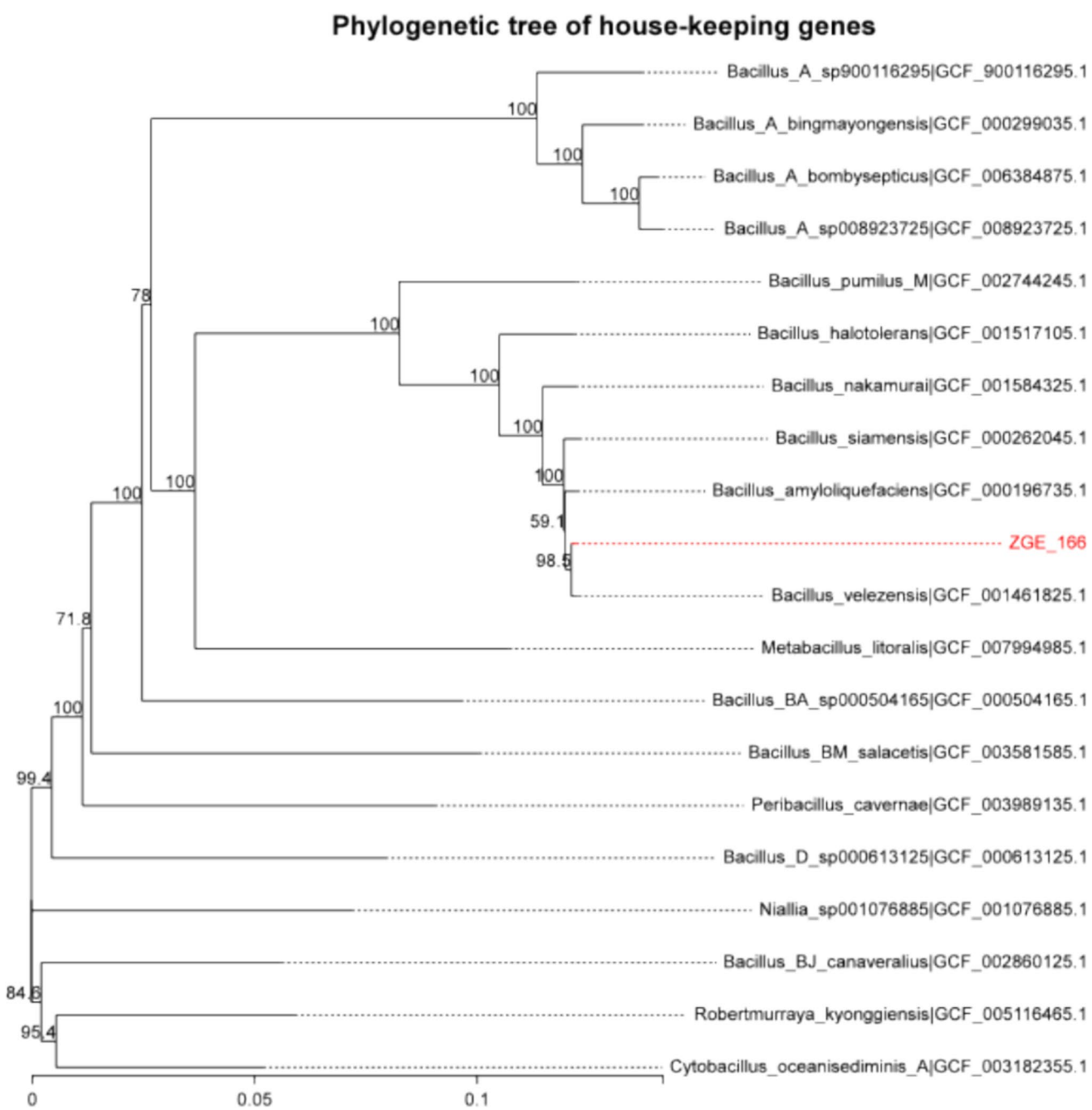

Fig. 4 Construction of strain ZGE166 phylogenetic tree based on housekeeping genes

The whole genome of *B. velezensis* ZGE166 was functionally annotated using five gene annotation databases: Pfam, COG, GO, KEGG, and CAZy. In the Pfam database, 3332 annotations were identified, representing 89.61% of the total annotations. The protein-coding sequences of the predicted genome of *B. velezensis* ZGE166 were analyzed by originators to using the COG database. And we found that 3052 protein-coding genes could be functionally annotated, accounting for 81.67% of the total protein sequence. These include genes involved in transcription (291), transcription (291), amino acid transport and metabolism (305), carbohydrate transport and metabolism (271), translation, and ribosomal structure and biogenesis (231) (Fig. 6). The biocontrol capacity of *Bacillus velezensis* correlates with COG functional categories: defense mechanisms (V) (COG0577, COG1131), secondary metabolite biosynthesis (Q) (NRPS, COG1020; PKS, COG3321), cell wall biogenesis (M) (COG0860, COG0463), intracellular

Table 2 PGB and degradative enzyme producing strain ZGE166

Hydrolytic enzyme activities and PGP attributes	ZGE166
Protease	−
Cellulase	−
Chitinase	−
Glucanase	−
Nitrogen fixation	+
Phosphate solubilization	−
Potassium dissolution	−
Siderophore production	−
NH3 production	+
IAA production	+
ACC deaminase enzyme	+

+, positive for test; −, negative for test

secretion (U) (COG0653, COG0805), and signal transduction (T) (COG0831, COG0832).

A comparison with the GO database showed that a total of 1737 annotations were included, accounting for 46.48% of all protein-coding gene sequences (Fig. 7). In the protein-coding genes of strain ZGE166, there are 427 types of genes associated with biological activities, with the highest quantity pertaining to translation (GO:0006412) and phosphorylation (GO:0016310). There are 64 genes associated with cellular component, the majority of which pertain to the membrane (GO:0016020), followed by cytoplasm (GO:0005737), plasma membrane (GO:0005886), ribosome (GO:0005840), and the genes related to ribonucleoprotein complex (GO:1,990,904). There are 743 genes related to molecular function, the most numerous of which are the genes related to ATP binding (GO:0005524), DNA binding (GO:0003677), metal ion binding (GO:0046872), hydrolase activity (GO:0016787), DNA-binding transcription factor activity (GO:0003700), ATP hydrolysis activity (GO:0016887), transmembrane transporter activity (GO:0022857), structural constituent of ribosome (GO:0003735), and oxidoreductase activity(GO:0016491). In GO annotations, antagonistic effects are associated with DNA binding activity (GO:0003677), ATP binding (GO:0005524), transferase activity (GO:0016740), and

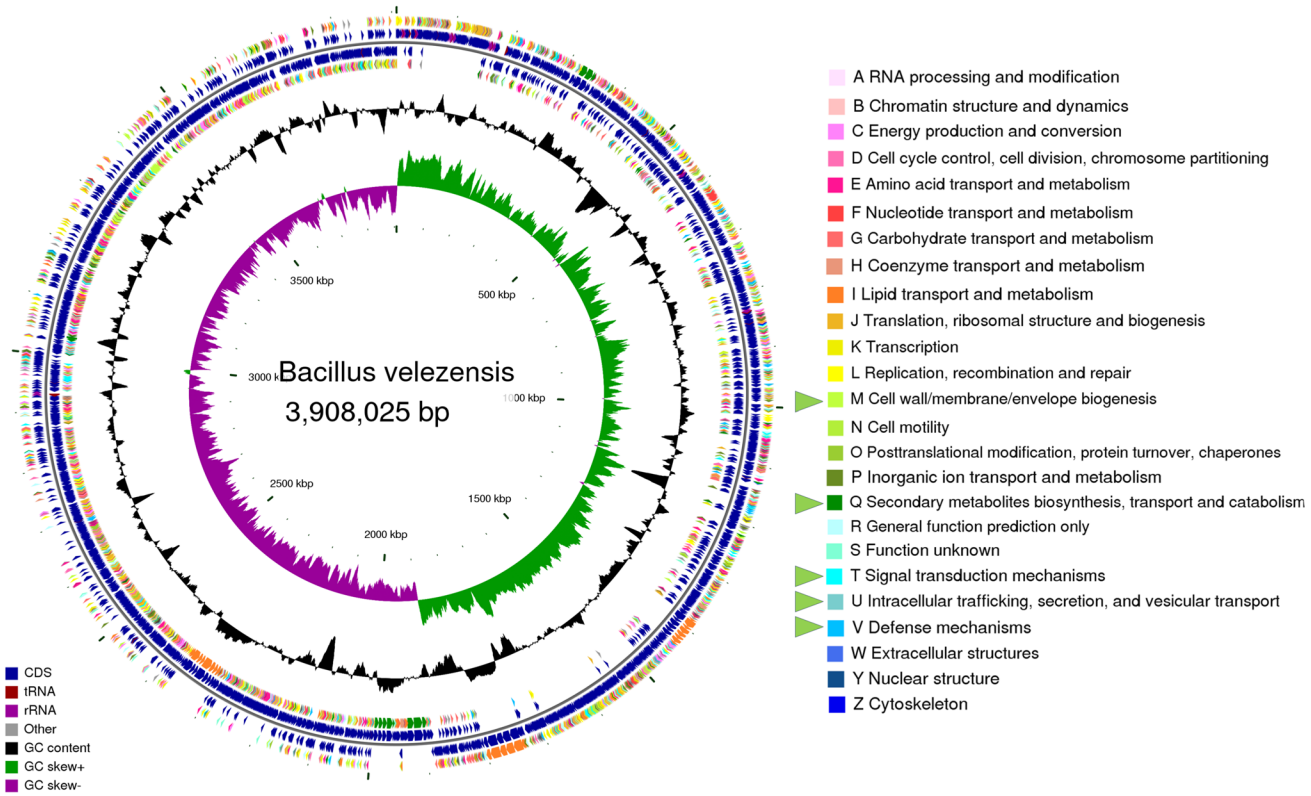

Fig. 5 *B. velezensis* ZGE166 genome circle diagram. From the outside to the inside, the first circle and the fourth circle are CDS on the positive chain and negative chain, and different colors indicate different COG functional classifications. The second circle and the third circle are CDS, tRNA, and rRNA on the positive chain and negative chain, respectively. The fifth circle is GC content, and the sixth circle is GC-skew value

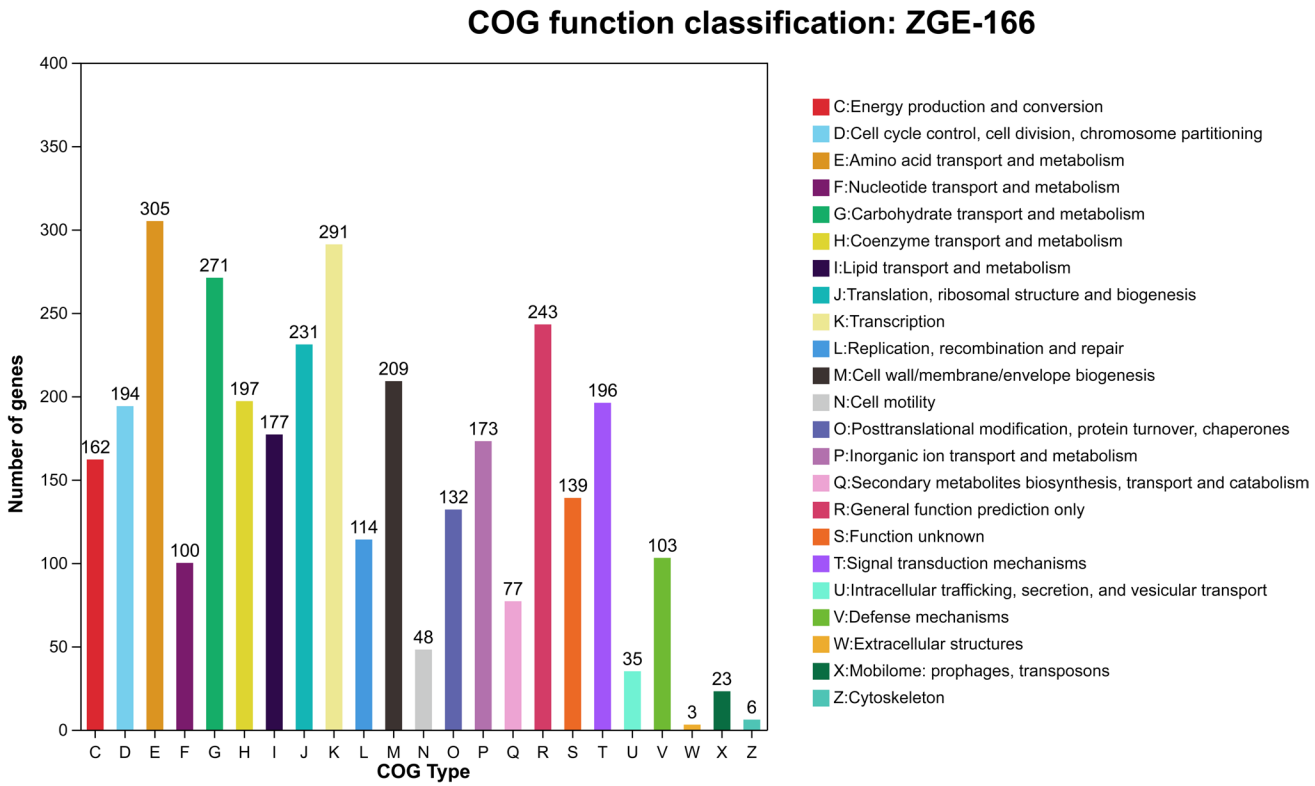

Fig. 6 COG function classification of strain ZGE166

hydrolase activity (GO:0016787) in molecular function; they are also linked to the regulation of DNA-template-dependent transcription (GO:0006351) in biological process.

The number of genes annotated in the KEGG database was 2808, representing 75.14% of the estimated gene count. Gene functions are categorized into 42 functional entries in six classes; of these, metabolism has the highest number of 2165 related genes, including global and overview maps (843), carbohydrate metabolism (278), amino acid metabolism (229), metabolism of cofactors and vitamins (213), and energy metabolism (118), respectively. The number of genes annotated to environmental information processing, genetic information processing, cellular processes, human diseases, and organismal systems was 307, 191, 149, 148, and 65, respectively (Fig. 8). Through annotation, it was found that biofilm formation (ko02025, ko02026, ko05111) and quorum sensing (ko02024) are related to antagonism. Biofilm formation provides bacteria with protection against environmental stress and antimicrobial agents, thereby enhancing their survival in competitive environments.

Upon comparison of the genome sequence with the CAZy database, it was found that the *B. velezensis* ZGE166 genome contains 130 protein-coding domains classified within the CAZy family: glycosyltransferases (43), glycoside hydrolases (42), carbohydrate esterases (32), auxiliary activities (9), polysaccharide lyases (3), and carbohydrate-binding modules (1) (Fig. 9).

The gene cluster of secondary metabolite synthesis in strain ZGE166 was predicted. Identify the gene clusters associated with the synthesis of secondary metabolites in strain ZGE166, which possesses 12 secondary metabolite biosynthetic gene clusters (BGCs). NRPS (non-ribosomal peptide synthetase) clusters, transAT-PKS clusters, and PKS-like clusters are present. Macrolactin H, bacillaene, fengycin, difficidin, bacillibactin, and bacilysin were identified as bioactive compounds with a predicted similarity of 100%. Strain ZGE166 contained four gene clusters with unknown functions, comprising two terpene clusters, one lanthipeptide-class-iicluster, and 0ne T3PKS(Type III PKS) cluster (Table 3). Macrolactin is a macrolide antibacterial substance. It can damage the integrity of bacterial cell membranes, inhibit the formation of biofilms, and interfere with bacterial protein synthesis [44]. Bacillaene is a kind of linear polyketide compound. It interferes with the growth of pathogenic bacteria by interacting with lipids in the cell membrane and disrupting the integrity of the cell membrane [45]. Fengycin is a kind of cyclic lipopeptide antibiotic. It can disrupt the cell membrane

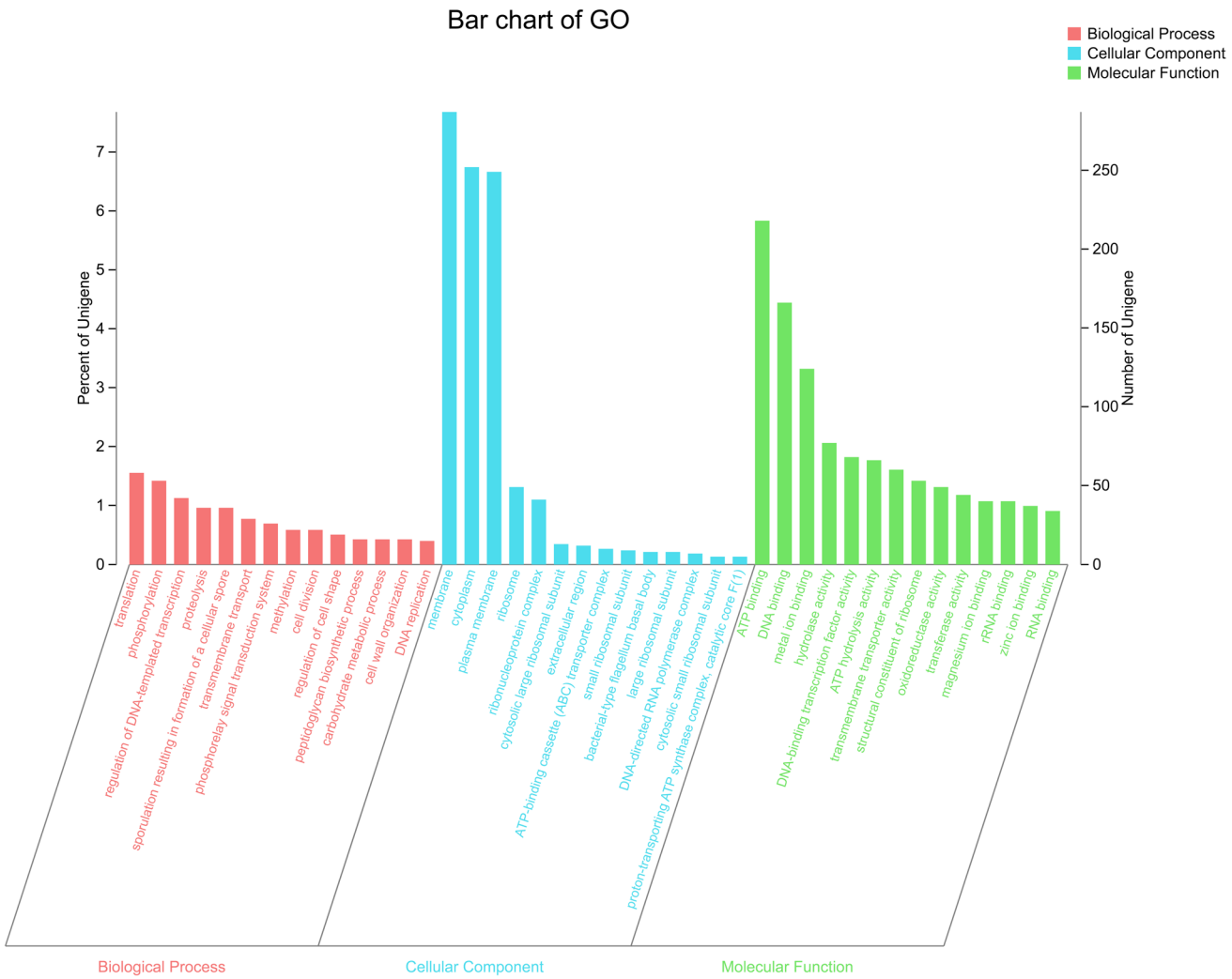

Fig. 7 GO function classification of strain ZGE166

of fungi, causing leakage of cytoplasm [46]. Difficidin is a macrolide antibiotic that causes the cell wall to become loose, porous, distorted, and even ruptured, leading to the efflux of intracellular components. Bacillibactin is a catechol-based iron carrier that can chelate iron ions ($Fe3^{+}$) from the surrounding environment. By sequestering iron, it restricts other microorganisms' access to this essential nutrient, thereby inhibiting their growth [47]. Bacilysin is a dipeptide antibiotic compound, and it causes cell lysis in bacteria and fungi [48].

Discussion

The way saffron is produced, as well as its medicinal parts, leads to its high price. It takes between 150,000 and 200,000 flowers to produce 1 kg of saffron [49], and corms directly affect flower production. Saffron corm rot is caused globally by a variety of pathogenic bacteria [50–53]. The extensive presence of corm rot significantly impacts saffron yield, serving as a primary limiting factor in saffron production regions. Methods such as crop rotation and chemical management have been applied to solve the saffron disease. Compared with chemical management methods, biological control has become a better solution [4]. *Bacillus* species are widespread in nature, easy to isolate and cultivate, non-pathogenic, and possess broad-spectrum antimicrobial properties [54]. Additionally, they can promote plant growth, making them widely used in biological control.

Through whole gene sequencing, we further understood the antagonistic effect of *B. velezensis*; surfactin and fengycin are both lipopeptide antibiotics. They not only exhibit broad-spectrum antibacterial activity but also induce systemic resistance (ISR) in plants through interaction with plant cell membranes, thereby enhancing the plants'

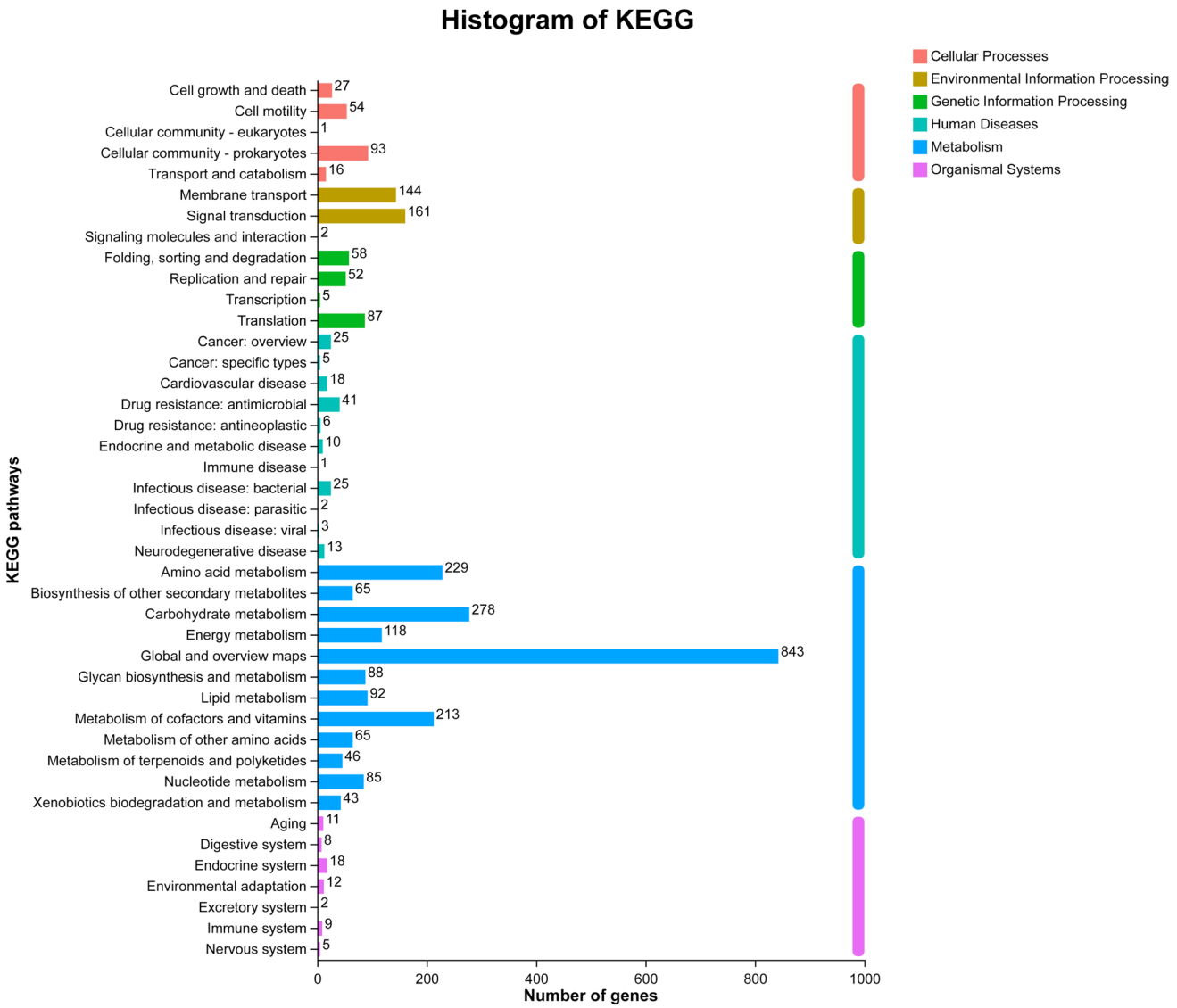

Fig. 8 KEGG function classification of strain ZGE166

defense capabilities against pathogenic bacteria [55]. Surfactin is regulated by the *SrfA* operon, which consists of four adjacent genes: *SrfAA*, *SrfAB*, *SrfAC*, and *SrfAD*. Genome sequence analysis showed that strain ZGE166 contains *SrfAA*, *SrfAC*, and *SrfAD* [56]. Bacillibactin is annotated in the whole-genome sequencing annotation, but it is negative in the CAS medium. In the synthesis of bacillibactin, the complete gene cluster (including *dhbA*, *dhbB*, *dhbC*, *dhbD*, *dhbE*, and *dhbF*) is crucial for efficient synthesis and secretion of bacillibactin. However, in strain ZGE166, only *dhbF* is present, which may lead to the obstruction of the synthesis process and the inability to effectively synthesize and secrete bacillibactin. Another possibility is attributed to the secretion quantity of bacillibactin being lower than the sensitivity threshold of the CAS detection method, leading to a false-negative result [57]. Another possibility is that the strains used in this experiment exhibit differences in their synthetic capabilities.

Compared with *B. velezensis* LS69, strain ZGE166 does not possess the iturin, amycolysin, and amylocyclicin-related gene clusters. In the bacillibactin-related gene cluster, *dhbABCDEF* are typically included, while strain ZGE166 only has *dhbF* [58]. Compared with *B. velezensis* LGMB12, LGMB319, LGMB426, and FZB42, strain ZGE166 has more genes related to carbohydrate esterases (CEs) and auxiliary activities (AAs), with a higher number of CEs [59]. This usually indicates that it can more effectively degrade complex polysaccharides in plant cell walls, such as xylan, pectin, and arabinogalactan [60]. Moreover, a higher number of AAs may indicate a stronger ability to

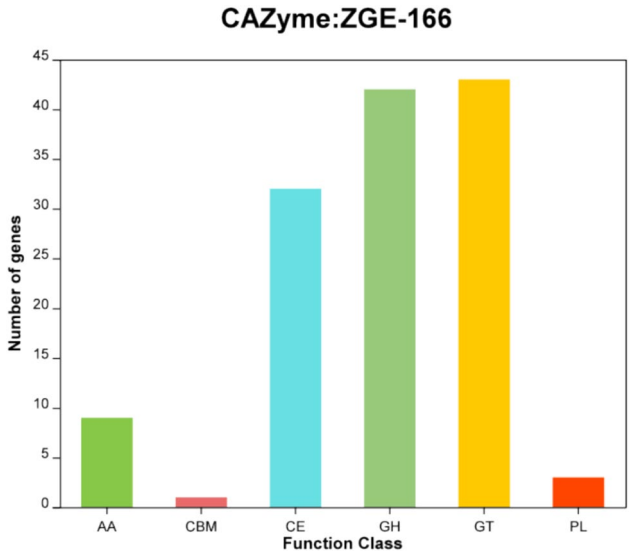

Fig. 9 Gene count distributions of carbohydrate-active enzyme (CAZy) families

degrade lignin and other recalcitrant carbohydrates, suggesting greater adaptability.

A burst of ethylene is necessary for many plants to overcome seed dormancy, but a persistently high ethylene level after germination may prevent root extension. The enzyme ACC deaminase can cleave the plant ethylene precursor ACC, which lowers the amount of ethylene in a growing or stressed plant [61, 62]. IAA has an important role in plant growth and development [63, 64]. Two primary strategies have been reported for the plant synthesis of IAA: the Trp-dependent (TD) pathway and the Trp-independent (TI) pathway [65, 66]. In *B. velezensis*, it may be synthesized de novo using tryptophan (*Trp*) as a precursor(*trpABCDE*). *B. velezensis* has a plant growth-promoting effect. Microorganisms are necessary to act on plants for the stable colonization of plant roots, and biofilms influence the stable colonization and carrying of microorganisms [64]; biofilms are assemblages of cells embedded in a matrix composed of exopolysaccharides (EPSs), proteins, and sometimes DNA [67], in strain ZGE166, *TasA*, *epsG*, *spo0 AD*, and others regulate it. During the nitrogen utilization process of plants, the ammonia production and nitrogen fixation by microorganisms play a crucial role. Ammonia production breaks down organic nitrogen (such as proteins and nucleic acids) in the soil into ammonium (NH_4^+), enabling plants to directly absorb and utilize it. Nitrogen fixation converts nitrogen gas (N_2) in the atmosphere into ammonia (NH_3), which is further converted into ammonium or nitrate (through nitrification) and significantly increases the available nitrogen content in the soil [68]. The nitrogenase (nif) genes required for nitrogen fixation include structural genes, genes involved in Fe protein activation, genes for the biosynthesis of iron-molybdenum cofactors, genes for electron donation, and regulatory genes necessary for enzyme synthesis and function [69]. The key genes for ammonia production mainly encode proteases (*apr*, *npr*, *sub*), urease (*ure*), and deaminases (*gdh*, *dadA*, *aspA*) [45, 70, 71]. In *B. velezensis*, genes such as *gln*, *groE*, *apr*, *npr*, and *ure* are included.

A preliminary demonstration of the growth-promoting and Chongming major pathogenic fungal antagonistic effects of the rhizosphere bacterium ZGE166 on saffron was presented. The functions of growth-promoting and antagonism-related genes have not yet been verified. Future

Table 3 Identification of secondary metabolite-related gene clusters in the genome of ZGE166

Type	Similar cluster	From–to (location, bp)	Similarity(%)	MIBiG accession
transAT-PKS	Macrolactin H	1,362,039–1,448,403	100	BGC0000181
transAT-PKS	Bacillaene	1,712,675–1,813,159	100	BGC0001089
NRPS	Fengycin	1,877,866–2,014,251	100	BGC0001095
transAT-PKS	Difficidin	2,298,201–2,391,999	100	BGC0000176
NRPS	Bacillibactin	3,010,956–3,062,749	100	BGC0000309
Other	Bacilysin	3,573,741–3,615,160	100	BGC0001184
NRPS	Surfactin	302,909–367,720	82	BGC0000433
PKS-like	Butirosin A/butirosin B	897,946–939,191	7	BGC0000693
Terpene	-	1,024,063–1,041,345	-	-
Terpene	-	2,037,830–2,059,714	-	-
T3PKS	-	2,128,378–2,169,479	-	-
Lanthipeptide-class-ii	-	1,162,802–1,191,692	-	-

investigations of the roles of these genes will be taken into account. The results of this study are of great significance for the study of the mechanism of action and antagonism, which will lay the foundation for future applications in biological control of plant growth and diseases.

Conclusions

In this study, we discovered that *Bacillus velezensis* exhibited significant antibacterial activity against the pathogenic fungi responsible for crocus corm decay through in vitro screening. Additionally, it was observed to enhance plant growth. Through the analysis whole-genome sequencing, strain-specific genes were identified, revealing crucial antagonistic traits and mechanisms that promote plant growth. This research provides a scientific foundation for the biological control of plant rot caused by pathogenic fungi.

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Data Availability No datasets were generated or analysed during the current study.

Declarations

Ethics Approval No approval of research ethics committees was required to accomplish the goals of this study because experimental work was conducted with an unregulated invertebrate species.

Consent for Publication The authors confirm that all authors are aware of and consent to the submission and publication of this manuscript.

Competing interests The authors declare no competing interests.

References

1. Cardone L, Castronuovo D, Perniola M, Cicco N, Candido V (2020) Saffron (Crocus sativus L.) the king of spices: an overview. Scientia Horticulturae 272:109560. https://doi.org/10.1016/j.scienta.2020.109560
2. Christodoulou E, Kadoglou NP, Kostomitsopoulos N, Valsami G (2015) Saffron: a natural product with potential pharmaceutical applications. J Pharm Pharmacol 67:1634–1649. https://doi.org/10.1111/jphp.12456
3. Alonso G, Zalacain A, Carmona M (2012) Saffron handbook of herbs and spices. Elsevier, pp. 469–498. https://doi.org/10.1533/9780857095671.469
4. Gupta V, Sharma A, Rai PK, Gupta SK, Singh B, Sharma SK, Singh SK, Hussain R, Razdan VK, Kumar D (2021) Corm rot of saffron: epidemiology and management. Agronomy 11:339. https://doi.org/10.3390/agronomy11020339
5. Hu S, Wang X, Sun W, Wamg L, Li W (2021) Investigation on main disease types of Chongming saffron. Chinese Society of Plant Pathology 2021 Annual Conference, GuiyangGuizhouChina, pp. 1. https://doi.org/10.26914/c.cnkihy.2021.063781
6. Hussain S, Siddique T, Saleem M, Arshad M, Khalid A (2009) Impact of pesticides on soil microbial diversity, enzymes, and biochemical reactions. Adv Agron 102:159–200. https://doi.org/10.1016/S0065-2113(09)01005-0
7. Streletskii R, Astaykina A, Krasnov G, Gorbatov V (2022) Changes in bacterial and fungal community of soil under treatment of pesticides. Agronomy 12:124. https://doi.org/10.3390/agronomy12010124
8. Bonaterra A, Badosa E, Daranas N, Francés J, Roselló G, Montesinos E (2022) Bacteria as biological control agents of plant diseases. Microorganisms 10(9):759. https://doi.org/10.3390/microorganisms10091759
9. Fang J (2014) The characteristics and methods of agricultural biological control. Modern agricultural technology: 170+172. https://link.cnki.net/urlid/34.1278.s.20140423.1148.112. Accessed 23 Feb 2025.
10. Anusha B, Naik M, Gopalakrishnan S, Amaresh Y, Gururaj S (2019) A review on the biological control of plant diseases using various microorganisms. J Pharmacogn Phytochem 8:506–510. https://www.jrmds.in/articles/a-review-on-the-biological-control-of-plant-diseases-using-various-microorganisms.pdf. Accessed 18 Mar 2024.
11. Ghorbanpour M, Omidvari M, Abbaszadeh-Dahaji P, Omidvar R, Kariman K (2018) Mechanisms underlying the protective effects of beneficial fungi against plant diseases. Biol Control 117:147–157. https://doi.org/10.1016/j.biocontrol.2017.11.006
12. Wei X, cao C, Huang D, Yao J, yuan Q (2024) Research progress on the mechanism of Trichoderma mycoprotection and its synergistic disease control effects. China Agricultural Science and Technology Herald 26: 126–135. https://link.cnki.net/doi/10.13304/j.nykjdb.2023.0524. Accessed 18 Apr 2025.
13. Castro-Sowinski S, Herschkovitz Y, Okon Y, Jurkevitch E (2007) Effects of inoculation with plant growth-promoting rhizobacteria on resident rhizosphere microorganisms. FEMS Microbiol Lett 276:1–11. https://doi.org/10.1111/j.1574-6968.2007.00878.x
14. Van Der Heijden MG, Klironomos JN, Ursic M, Moutoglis P, Streitwolf-Engel R, Boller T, Wiemken A, Sanders IR (1998) Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. Nature 396:69–72. https://doi.org/10.1038/23932
15. Hrynkiewicz K, Baum C (2012) The potential of rhizosphere microorganisms to promote the plant growth in disturbed soils.

Environmental protection strategies for sustainable development: 35–64. https://doi.org/10.1007/978-94-007-1591-2_2
16. Yang S, Liu H, Xie P, Wen T, Shen Q, Yuan J (2023) Emerging pathways for engineering the rhizosphere microbiome for optimal plant health. J Agric Food Chem 71:4441–4449. https://doi.org/10.1021/acs.jafc.2c08758
17. Bapat S, Shah A (2000) Biological control of fusarial wilt of pigeon pea by *Bacillus brevis*. Can J Microbiol 46:125–132. https://doi.org/10.1139/w99-109
18. Baard V, Bakare OO, Daniel AI, Nkomo M, Gokul A, Keyster M, Klein A (2023) Biocontrol potential of Bacillus subtilis and *Bacillus* tequilensis against four *Fusarium* species. Pathogens 12:254. https://doi.org/10.3390/pathogens12020254
19. Gomaa EZ (2012) Chitinase production by *Bacillus thuringiensis* and *Bacillus licheniformis*: their potential in antifungal biocontrol. The journal of Microbiology 50:103–111. https://doi.org/10.1007/s12275-012-1343-y
20. Dobrzyński J, Jakubowska Z, Kulkova I, Kowalczyk P, Kramkowski K (2023) Biocontrol of fungal phytopathogens by *Bacillus pumilus*. Front Microbiol 14:1194606. https://doi.org/10.3389/fmicb.2023.1194606
21. Wang L-T, Lee F-L, Tai C-J, Kuo H-P (2008) *Bacillus velezensis* is a later heterotypic synonym of *Bacillus amyloliquefaciens*. Int J Syst Evol Microbiol 58:671–675. https://doi.org/10.1099/ijs.0.65191-0
22. Ruiz-Garcia C, Bejar V, Martinez-Checa F, Llamas I, Quesada E (2005) *Bacillus velezensis* sp. nov., a surfactant-producing bacterium isolated from the river *Vélez* in Málaga, southern Spain. Int J Syst Evol Microbiol 55:191–195. https://doi.org/10.1099/ijs.0.63310-0
23. Chen L, Heng J, Qin S, Bian K (2018) A comprehensive understanding of the biocontrol potential of *Bacillus velezensis* LM2303 against Fusarium head blight. Plos One 13:e0198560. https://www.ncbi.nlm.nih.gov/pubmed/29856856. Accessed 20 Feb 2025.
24. Han X, Shen D, Xiong Q, Bao B, Zhang W, Dai T, Zhao Y, Borriss R, Fan B (2021) The plant-beneficial rhizobacterium *Bacillus velezensis* FZB42 controls the soybean pathogen Phytophthora sojae due to bacilysin production. Appl Environ Microbiol 87:e01601–01621. https://doi.org/10.1128/AEM.01601-21
25. Torres M, Llamas I, Torres B, Toral L, Sampedro I, Bejar V (2020) Growth promotion on horticultural crops and antifungal activity of *Bacillus velezensis* XT1. Appl Soil Ecol 150. https://doi.org/10.1016/j.apsoil.2019.103453
26. Wang J, Qu F, Liang J, Yang M, Hu X (2022) Bacillus velezensis SX13 promoted cucumber growth and production by accelerating the absorption of nutrients and increasing plant photosynthetic metabolism. Sci Hortic 301. https://doi.org/10.1016/j.scienta.2022.111151
27. Tian L, Hu S, Wang X, Guo Y, Huang L, Wang L, Li W (2022) Antagonism of rhizosphere Streptomyces yangpuensis CM253 against the pathogenic fungi causing corm rot in saffron (Crocus sativus L.). Pathogens 11:1195. https://doi.org/10.3390/pathogens11101195
28. Zou L, Wang Q, Wu R, Zhang Y, Wu Q, Li M, Ye K, Dai W, Huang J (2023) Biocontrol and plant growth promotion potential of endophytic *Bacillus subtilis* JY-7-2L on Aconitum carmichaelii Debx. Front Microbiol 13:1059549. https://doi.org/10.3389/fmicb.2022.1059549
29. Gao Z, Zhang B, Liu H, Han J, Zhang Y (2017) Identification of endophytic *Bacillus velezensis* ZSY-1 strain and antifungal activity of its volatile compounds against Alternaria solani and Botrytis cinerea. Biol Control 105:27–39. https://doi.org/10.1016/j.biocontrol.2016.11.007
30. Kaláb M, Yang A-F, Chabot D (2008) Conventional scanning electron microscopy of bacteria. Infocus magazine 10:42–61
31. Zhang X-y, Li C, Hao J-j, Li Y-c, Li D-z, Zhang D-m, Xing X, Liang Y (2020) A novel *Streptomyces* sp. strain PBSH9 for controlling potato common scab caused by *Streptomyces galilaeus*. Plant Dis 104:1986–1993. https://doi.org/10.1094/PDIS-07-19-1469-RE
32. Frank JA, Reich CI, Sharma S, Weisbaum JS, Wilson BA, Olsen GJ (2008) Critical evaluation of two primers commonly used for amplification of bacterial 16S rRNA genes. Appl Environ Microbiol 74:2461–2470. https://doi.org/10.1128/AEM.02272-07
33. Kim O-S, Cho Y-J, Lee K, Yoon S-H, Kim M, Na H, Park S-C, Jeon YS, Lee J-H, Yi H (2012) Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. Int J Syst Evol Microbiol 62:716–721. https://doi.org/10.1099/ijs.0.038075-0
34. Wang Y, Sun Z, Zhao Q, Yang X, Li Y, Zhou H, Zhao M, Zheng H (2024) Whole-genome analysis revealed the growth-promoting and biological control mechanism of the endophytic bacterial strain *Bacillus halotolerans* Q2H2, with strong antagonistic activity in potato plants. Front Microbiol 14:1287921. https://doi.org/10.3389/fmicb.2023.1287921
35. Nielsen P, Sørensen J (1997) Multi-target and medium-independent fungal antagonism by hydrolytic enzymes in Paenibacillus polymyxa and Bacillus pumilus strains from barley rhizosphere. FEMS Microbiol Ecol 22:183–192. https://doi.org/10.1111/j.1574-6941.1997.tb00370.x
36. Suarez-Moreno ZR, Vinchira-Villarraga DM, Vergara-Morales DI, Castellanos L, Ramos FA, Guarnaccia C, Degrassi G, Venturi V, Moreno-Sarmiento N (2019) Plant-growth promotion and biocontrol properties of three *Streptomyces* spp. isolates to control bacterial rice pathogens. Front Microbiol 10: 290. https://www.ncbi.nlm.nih.gov/pubmed/30858835. Accessed 18 Mar 2025.
37. Kapadia C, Patel N, Rana A, Vaidya H, Alfarraj S, Ansari MJ, Gafur A, Poczai P, Sayyed R (2022) Evaluation of plant growth-promoting and salinity ameliorating potential of halophilic bacteria isolated from saline soil. Front Plant Sci 13. https://doi.org/10.3389/fpls.2022.946217
38. Nautiyal CS (1999) An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. FEMS Microbiol Lett 170:265–270. https://doi.org/10.1111/j.1574-6968.1999.tb13383.x
39. Zhang L, Zhang M, Huang S, Li L, Gao Q, Wang Y, Zhang S, Huang S, Yuan L, Wen Y (2022) A highly conserved core bacterial microbiota with nitrogen-fixation capacity inhabits the xylem sap in maize plants. Nat Commun 13:3361. https://doi.org/10.1038/s41467-022-31113-w
40. Dubey A, Saiyam D, Kumar A, Hashem A, Abd_Allah EF, Khan ML (2021) Bacterial root endophytes: characterization of their competence and plant growth promotion in soybean (Glycine max (L.) Merr.) under drought stress. International Journal of Environmental Research and Public Health 18. https://doi.org/10.3390/ijerph18030931
41. Sheng MM, Jia HK, Zhang GY, Zeng LN, Zhang TT, Long YH, Lan J, Hu ZQ, Zeng Z, Wang B, Liu HM (2020) Siderophore production by rhizosphere biological control bacteria *Brevibacillus brevis* GZDF3 of Pinellia ternata and its antifungal effects on Candida albicans. J Microbiol Biotechnol 30:689–699. https://www.ncbi.nlm.nih.gov/pubmed/32482934. Accessed 20 Feb 2025.
42. Chen S, Zhou Y, Chen Y, Gu J (2018) fastp: an ultra-fast all-in-one FASTQ preprocessor. Bioinformatics 34:i884–i890. https://doi.org/10.1093/bioinformatics/bty560
43. Chan PP, Lowe TM (2019) tRNAscan-SE: searching for tRNA genes in genomic sequences. Springer. https://doi.org/10.1007/978-1-4939-9173-0_1

44. Kumar G, Engle K (2023) Natural products acting against S. aureus through membrane and cell wall disruption. Nat Prod Rep 40:1608–1646. https://doi.org/10.1039/D2NP00084A
45. Erega A, Stefanic P, Dogsa I, Danevčič T, Simunovic K, Klančnik A, Smole Možina S, Mandic Mulec I (2021) Bacillaene mediates the inhibitory effect of Bacillus subtilis on Campylobacter jejuni biofilms. Appl Environ Microbiol 87:e02955–e12920. https://doi.org/10.1128/AEM.02955-20
46. Tao Y, Bie X-m, Lv F-x, Zhao H-z, Lu Z-x (2011) Antifungal activity and mechanism of fengycin in the presence and absence of commercial surfactin against Rhizopus stolonifer. J Microbiol 49:146–150. https://link.springer.com/article/10.1007/s12275-011-0171-9. Accessed 17 Apr 2025.
47. Qin Y, He Y, She Q, Larese-Casanova P, Li P, Chai Y (2019) Heterogeneity in respiratory electron transfer and adaptive iron utilization in a bacterial biofilm. Nat Commun 10:3702. https://doi.org/10.1038/s41467-019-11681-0
48. Islam T, Rabbee MF, Choi J, Baek K-H (2022) Biosynthesis, molecular regulation, and application of bacilysin produced by *Bacillus* species. Metabolites 12:397. https://doi.org/10.3390/metabo12050397
49. Mzabri I, Addi M, Berrichi A (2019) Traditional and modern uses of saffron (*Crocus sativus*). Cosmetics 6:63. https://doi.org/10.3390/cosmetics6040063
50. Caligiore-Gei PF, Moratalla-López N, Poggi LM, Alonso GL (2023) Isolation, identification, and determination of the virulence of the causal agents of corm rot of saffron (*Crocus sativus* L.) in Valle de Uco, Argentina. Plants 12: 2717. https://doi.org/10.3390/plants12142717
51. Ren T, Dai D, Yu M, Li T, Zhang C (2023) Identification and characterization of pathogens causing saffron corm rot in China. Front Microbiol 14:1188376. https://doi.org/10.3389/fmicb.2023.1188376
52. Khaledi N, Rezaei M, Alizadeh Farddarabad M (2023) Investigation into the prevalence of Fusarium corm rot disease in saffron fields of Iran. Saffron Agronomy and Technology 11: 393–412. https://doi.org/10.22048/jsat.2024.410910.1501
53. Muñoz R, Lerma M, Castillo P, Armengol J, Somoza E, Woodhall J (2020) First report of Stromatinia gladioli causing neck and corm rot of Crocus sativus in Spain. Plant Dis 104:282. https://doi.org/10.1055/s-0034-1375681
54. Meng Q, Jiang H, Hao JJ (2016) Effects of *Bacillus velezensis* strain BAC03 in promoting plant growth. Biol Control 98:18–26. https://doi.org/10.1016/j.biocontrol.2016.03.010
55. Ongena M, Jourdan E, Adam A, Paquot M, Brans A, Joris B, Arpigny JL, Thonart P (2007) Surfactin and fengycin lipopeptides of *Bacillus subtilis* as elicitors of induced systemic resistance in plants. Environ Microbiol 9:1084–1090. https://doi.org/10.1111/j.1462-2920.2006.01202.x
56. Xu Y, Wu J-Y, Liu Q-J, Xue J-Y (2023) Genome-wide identification and evolutionary analyses of SrfA operon genes in Bacillus. Genes 14:422. https://doi.org/10.3390/genes14020422
57. Nithyapriya S, Lalitha S, Sayyed R, Reddy M, Dailin DJ, El Enshasy HA, Luh Suriani N, Herlambang S (2021) Production, purification, and characterization of bacillibactin siderophore of *Bacillus subtilis* and its application for improvement in plant growth and oil content in sesame. Sustainability 13:5394. https://doi.org/10.3390/su13105394
58. Liu G, Kong Y, Fan Y, Geng C, Peng D, Sun M (2017) Whole-genome sequencing of *Bacillus velezensis* LS69, a strain with a broad inhibitory spectrum against pathogenic bacteria. J Biotechnol 249:20–24. https://doi.org/10.1016/j.jbiotec.2017.03.018
59. Ercole TG, Kava VM, Petters-Vandresen DAL, Gomes MEN, Aluizio R, Ribeiro RA, Hungria M, Galli LV (2024) Unlocking the growth-promoting and antagonistic power: a comprehensive whole genome study on *Bacillus velezensis* strains. Gene 927. https://doi.org/10.1016/j.gene.2024.148669
60. La Rosa SL, Lindstad LJ, Westereng B (2023) Carbohydrate esterases involved in deacetylation of food components by the human gut microbiota. Essays Biochem 67:443–454. https://doi.org/10.1042/EBC20220161
61. Glick BR (2005) Modulation of plant ethylene levels by the bacterial enzyme ACC deaminase. FEMS Microbiol Lett 251:1–7. https://doi.org/10.1016/j.femsle.2005.07.030
62. Glick BR, Todorovic B, Czarny J, Cheng Z, Duan J, McConkey B (2007) Promotion of plant growth by bacterial ACC deaminase. Crit Rev Plant Sci 26:227–242. https://doi.org/10.1080/07352680701572966
63. Bialek K, Michalczuk L, Cohen JD (1992) Auxin biosynthesis during seed germination in Phaseolus vulgaris. Plant Physiol 100:509–517. https://doi.org/10.1104/pp.100.1.509
64. Spaepen S, Vanderleyden J, Remans R (2007) Indole-3-acetic acid in microbial and microorganism-plant signaling. FEMS Microbiol Rev 31:425–448. https://doi.org/10.1111/j.1574-6976.2007.00072.x
65. Di D-W, Zhang C, Luo P, An C-W, Guo G-Q (2016) The biosynthesis of auxin: how many paths truly lead to IAA? Plant Growth Regul 78:275–285. https://doi.org/10.1007/s10725-015-0103-5
66. Chlebek D, Płociniczak T, Gobetti S, Kumor A, Hupert-Kocurek K, Pacwa-Płociniczak M (2021) Analysis of the genome of the heavy metal resistant and hydrocarbon-degrading rhizospheric *Pseudomonas qingdaonensis* ZCR6 strain and assessment of its plant-growth-promoting traits. Int J Mol Sci 23:214. https://doi.org/10.3390/ijms23010214
67. Beauregard PB, Chai Y, Vlamakis H, Losick R, Kolter R (2013) *Bacillus subtilis* biofilm induction by plant polysaccharides. Proc Natl Acad Sci 110:E1621–E1630. https://doi.org/10.1073/pnas.1218984110
68. Shang Y, Shi H, Liu M, Lan P, Li D, Liu X, Wang M, Zhang Z, Chen S (2025) Using synthetic biology to express nitrogenase biosynthesis pathway in rice and to overcome barriers of nitrogenase instability in plant cytosol. Trends in Biotechnology. https://www.cell.com/trends/biotechnology/fulltext/S0167-7799(24)00362-7?rss=yes. Accessed 18 Apr 2025
69. Glick BR (2012) Plant growth-promoting bacteria: mechanisms and applications. Scientifica 2012. https://doi.org/10.1073/pnas.1218984110
70. Mobley H, Island MD, Hausinger RP (1995) Molecular biology of microbial ureases. Microbiol Rev 59:451–480. https://doi.org/10.1128/mr.59.3.451-480.1995
71. Schulz-Mirbach H, Müller A, Wu T, Pfister P, Aslan S, von Borzyskowski LS, Erb TJ, Bar-Even A, Lindner SN (2022) On the flexibility of the cellular amination network in E coli. Elife 11. https://doi.org/10.7554/eLife.77492