scientific reports

Check for updates

OPEN

Bacillus velezensis A-27 as a potential biocontrol agent against *Meloidogyne incognita* and effects on rhizosphere communities of celery in field

Yanan Yao[1], Liyi Wang[1], Haozhen Zhai[1], Hailong Dong[1], Jianming Wang[1], Zengqi Zhao[2] & Yumei Xu[1]✉

***Meloidogyne incognita*, a highly destructive plant-parasitic nematode, poses a significant threat to crop production. The reliance on chemical nematicides for nematode control has been crucial; however, the banning of many effective nematicides due to their adverse effects has necessitated the exploration of alternative solutions. Rhizosphere biocontrol bacteria, particularly strains of *Bacillus*, have demonstrated promising results in managing plant-parasitic nematodes. In this study, strain A-27 was identified as *Bacillus velezensis* based on its morphological, physiological, and molecular characteristics, including 16 S rRNA and gyrA sequencing. Strain A-27 exhibited high larvicidal and ovicidal efficacy in vitro, with estimated LC_{50} values of 4.0570×10^8 CFU/mL for larvicidal efficacy and 3.6464×10^8 CFU/mL for ovicidal efficacy. In a pot experiment, *B. velezensis* A-27 significantly reduced the root gall index, achieving a control efficacy of 85.36%. Field experiments further indicated that A-27 reduced the root gall index with a control efficacy of 67.31%, while also decreasing the J2 population density of *M. incognita* and significantly enhancing the growth of celery plants. Additionally, high-throughput sequencing analysis revealed that *B. velezensis* A-27 significantly increased the relative abundances of *Bacillus* and *Sphingomonas*, while markedly reducing the relative abundances of *Fusarium*, *Mortierella*, and *Cephaliophora* in the celery rhizosphere. These findings suggest that *B. velezensis* A-27 has potential as an effective biocontrol agent against *M. incognita*, offering a promising alternative to chemical nematicides in sustainable agriculture.**

Keywords *Meloidogyne incognita*, *Bacillus velezensis*, Rhizosphere, Mortality, Abundance

Root-knot nematodes (RKNs), belonging to the genus *Meloidogyne* (Nematoda: Meloidogynidae), are widely recognized as highly destructive plant-parasitic nematodes. They have the capacity to infect and damage over 5, 500 plant species, including numerous vegetable crops[1,2]. Annually, RKNs are responsible for approximately $100 billion in global crop losses[3], with losses in vegetable crops alone exceeding 3 billion CNY in China[4]. Among the 100 valid species of *Meloidogyne*, *M. incognita* is the most prevalent and extensively studied, particularly affecting greenhouse vegetables in Shanxi province. Other notable species include *M. arenaria*, *M. javanica*, and *M. hapla*[5]. Furthermore, RKNs can exacerbate infections caused by other pathogens, such as fungi and bacteria, leading to complex disease syndromes and complicating control measures for these pathogens[6].

Synthetic chemical nematicides are effective; however, they pose environmental and health risks and high costs. Therefore, it is essential to develop new and safe biocontrol agents to combat RKNs for sustainable agriculture. Various nematophagous fungi, such as *Paecilomyces tenuis*, have demonstrated over 90% mortality in infective second-stage juveniles (J2s) nematodes within 24 h of exposure to the fungal filtrate, along with approximately 87% parasitization rates[7]. *Rhizophagus irregularis* has been shown to enhance plant growth parameters and combat RKNs by improving the activity of phenolics and antioxidant defense enzymes[8]. Additionally, *Fusarium oxysporum* f. sp. *ciceris*[9] and *Myrothecium verrucaria* have been shown to parasitize the eggs, J2s and adult

[1]Laboratory of Nematology, Department of Plant Pathology, College of Plant Protection, Shanxi Agricultural University, Taigu 030801, China. [2]Auckland Mail Centre, Manaaki Whenua-Landcare Research, Private Bag 92170, Auckland 1142, New Zealand. ✉email: ymxu@sxau.edu.cn

females of RKNs, resulting in mortality rates of up to 71%[10]. Arbuscular mycorrhizal fungi have been reported as effective biocontrol agents for RKNs, enhancing plant tolerance through direct competition for nutrients and space, inducing systemic resistance (ISR), and altering interactions in the rhizosphere[11]. Moreover, bacterial pathogens, particularly plant growth-promoting rhizobacteria (PGPR) such as *Pseudomonas stutzeri* PPB1, *Bacillus subtilis* PPB2, and *Stenotrophomonas maltophilia* PPB3[12], as well as *B. cereus* Bc-cm103[13], *B. firmus* YBf-10[14], *B. amyloliquefaciens*[15], *P. fluorescens* CHA0[16], and *Streptomyces antibioticus* M7[17], exhibit systemic nematicidal activity. They achieve this through lethal effects, inhibition of egg hatching and motility, or by activating systemic resistance pathways such as salicylic acid (SA) and jasmonic acid (JA) signaling pathways. Notably, *B. velezensis* has shown promise in controlling harmful phytopathogens, including *Verticillium dahliae*[18], and *Colletotrichum gloeosporioides*[19]. Furthermore, *B. velezensis* has exhibited potential for nematode control beyond its antifungal capabilities. For instance, *B. velezensis* BZR 86 has been found to reduce RKN disease in tomato and cucumber plants while promoting growth and biomass[20]. Additionally, strain Bv-25, isolated from cucumber rhizosphere soil, has demonstrated inhibitory activity against *M. incognita* egg hatching and a high mortality rate in J2s after exposure to the fermented broth of the strain[21].

Plants preferentially recruit microorganisms from the rhizosphere that are essential for their growth and development. A diverse microbiome in this region enhances the metabolic capabilities of plants, thereby, supporting critical processes such as seed germination, seedling establishment, nutrient absorption, water uptake, growth promotion, and defense against pathogens[22]. The application of microbial compost has been shown to increase the diversity of soil bacterial communities and influence their structure[23]. For example, microbial compost derived from *B. velezensis* has been found to increase soil organic matter content, enhance enzyme activity, and reshape the bacterial community structure in the rhizosphere, ultimately alleviating replanting disease and promoting the growth of apple trees[24]. Consequently, the composition of the rhizosphere microbiome can serve as an important indicator of plant health.

This study investigates the efficacy of the isolate A-27 in controlling *M. incognita* and its impact on the composition of the rhizobial microbiota. The objectives of this study are: (*i*) to isolate and identify strain A-27; (*ii*) to evaluate the nematicidal activities of *B. velezensis* A-27 against *M. incognita* through in vitro assays, pot experiments, and field trials; and (*iii*) to further analyze the impact of *B. velezensis* A-27 on the microbial diversity of rhizosphere soils in the field.

Results

Identification of strain A-27

In the primary screening, a total of 51 bacterial strains were isolated. Subsequent nematicidal bioassays revealed that strain A-27 exhibited strong nematicidal activity, resulting in 100% mortality of J2 nematodes (Table S1). Furthermore, strain A-27 was selected by our group as a candidate for potential biocontrol against *Heterodera glycines*[25] and *M. incognita*. It is important to note that the identification of strain A-27 was conducted within the scope of this study.

The morphological and biochemical characterization of strain A-27 revealed that it is Gram-positive and capable of forming endospores (Fig. 1; Table 1). Analysis of the 16 S rRNA and gyrA gene sequences of A-27 using BLAST homology and phylogenetic analysis demonstrated that A-27's 16 S rDNA (OK614085) shared 100% similarity with *B. velezensis* CR-502^T (AY603658), as indicated by GenBank search data[26]. Phylogenetic analysis further revealed that A-27 clusters with *B. velezensis* strain CR-502^T, exhibiting with a Bayesian posterior probability (BPP) of 0.98 in the 16 S rDNA tree, and with *B. velezensis* strain NRRLB-41,580^T (EU138598), showing with a BPP of 1.00 in the gyrA tree. These findings confirm the classification as *B. velezensis* (Fig. 2A,B).

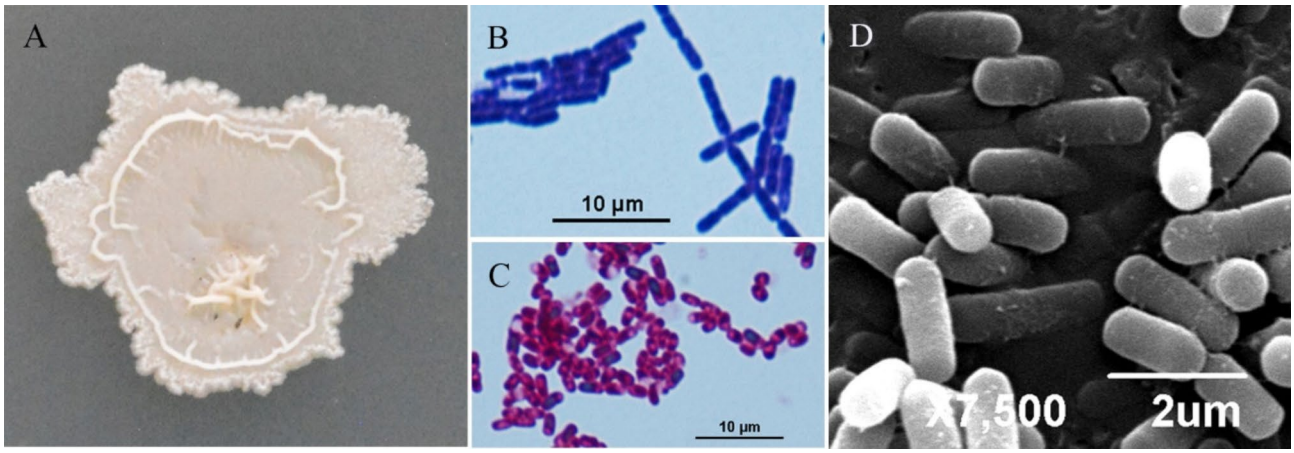

Fig. 1. Culture, Gram straining, spore staining, and characteristics of A-27. (**A**) Culture characteristics on NA plate. (**B**) gram staining results. (**C**) endospore staining results; (**D**) external characteristics observed by SEM.

Characteristics	A-27	Characteristics	A-27
Cell length/μm	1.6–1.8	Sucrose	+
Cell diam./μm	0.6–0.7	Nitrate reduction	−
Gram stain	+	Use of citrate	+
Flagellum	−	Hydrolyze starch	+
Spore	+	Glutamic acid	−
Oxidase	+	Leucine	+
Catalase	+		

Table 1. Physiological and biochemical characteristics of strain A-27. "+" means positive, "−" means negative.

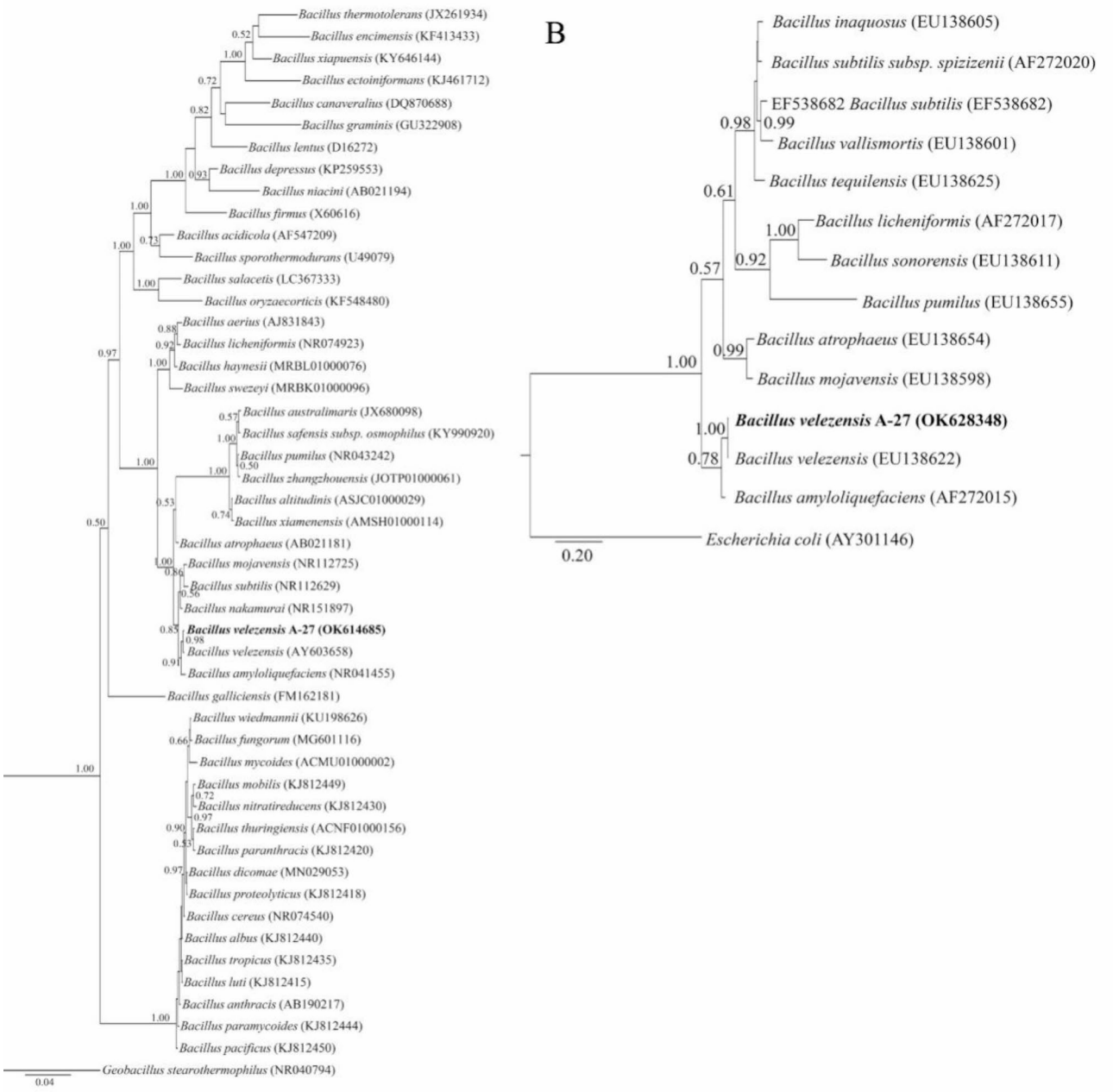

Fig. 2. Bayesian tree inferred from 16 S rRNA gene (**A**) and gyrA gene (**B**) sequences of *Bacillus* spp. (posterior probabilities exceeding 50% are given on appropriate clades).

Treatments	Units	Corrected mortality (%)	
		24 h	48 h
Fermentation broth	1 ×	100.00 ± 0.00 a	100.00 ± 0.00 a
	5 ×	97.36 ± 3.13 a	100.00 ± 0.00 a
	10 ×	41.86 ± 7.31 b	44.85 ± 4.20 b
Fermentation filtrate	1 ×	85.17 ± 7.36 a	86.85 ± 5.65 a
	5 ×	65.82 ± 9.44 b	72.60 ± 5.12 b
	10 ×	54.84 ± 1.08 b	62.65 ± 3.16 c
Bacterial suspension(CFU/mL)	10^6	14.93 ± 5.76 c	18.88 ± 2.33 c
	10^7	21.79 ± 5.21 b	22.31 ± 2.65 b
	10^8	26.70 ± 2.73 a	27.20 ± 2.19 a

Table 2. Larvicidal efficacy of different A-27 treatments on J2 in vitro. *Data are represented as the mean ± standard deviation ($n = 3$). Different letters (a, b, c) within each column indicate significantly as determined by one-way analysis of variance (ANOVA) and Tukey's multiple comparison test ($P < 0.05$).

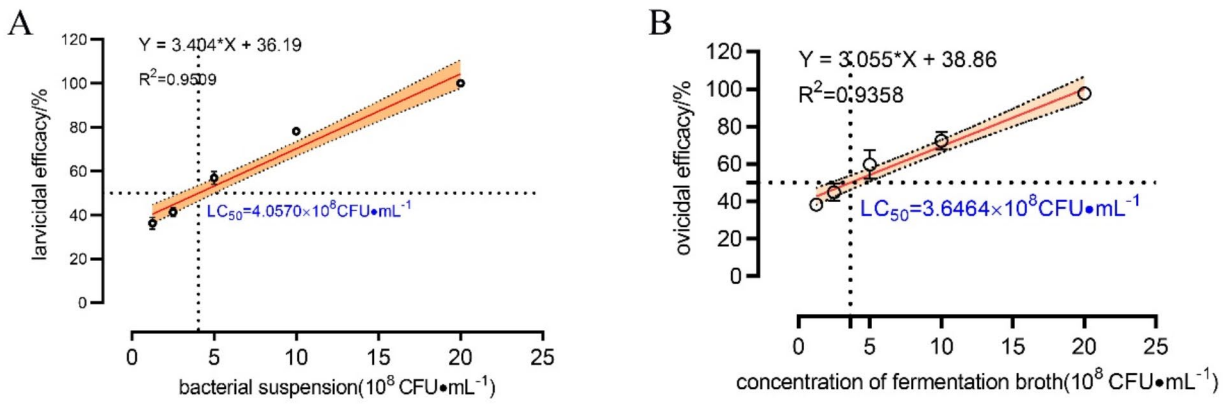

Fig. 3. Larvicidal (**A**) and ovicidal (**B**) efficacy of A-27 on *M. incognita* (the dashed lines mean the bacterial suspension when the larvicidal and ovicidal efficacy is 50%; orange areas mean 95% confidence interval).

Larvicidal and ovicidal efficacy of *B. velezensis* A-27 in vitro

In vitro assessments were conducted to evaluate the larvicidal potential of *B. velezensis* A-27 against J2 nematodes. The results indicated that all treatments effectively reduced the J2 population, with the mortality rate increasing over time, although it decreased with increasing dilution factors (Table 2). At the 24-hour post-inoculation time point, the A-27 fermentation broth demonstrated the highest corrected J2 mortality rate, reaching 100.00%. The fermentation filtrate resulted in an 85.32% mortality rate, while the lowest corrected juvenile mortality was observed with a bacterial suspension of 10^6 CFU/mL. The LC_{50} value for larvicidal efficacy was determined to be 4.0570×10^8 CFU/mL (Fig. 3A).

Furthermore, an in vitro test was conducted to evaluate the ovicidal efficacy of A-27. The egg hatching percentage in the A-27 fermentation broth decreased from 97.62 to 38.26% as the concentration of the bacterial suspension was reduced from 2×10^9 CFU/mL to 1.25×10^8 CFU/mL. The estimated LC_{50} value for ovicidal efficacy was calculated to be 3.6464×10^8 CFU/mL (Fig. 3B).

Control efficacy of *B. velezensis* A-27 in the pot experiment

In the pot experiment, treatment with *B. velezensis* A-27 resulted in a significant reduction in the root gall index compared to the control group ($F = 25.750$, df = 2, $P < 0.001$) (Fig. 4B; Table 3). The control efficacy of A-27 reached 85.36%, which is 16.20% higher than that of fosthiazate. Furthermore, A-27 significantly enhanced various plant growth indices, including plant height (38.03%, $F = 19.144$, df = 2, $P < 0.01$), fresh plant weight (17.20%, $F = 5.745$, df = 2, $P < 0.05$), and dry root weight (32.46%, $F = 7.896$, df = 2, $P < 0.001$) when compared to the control (Fig. 4A).

Control efficacy of *B. velezensis* A-27 in the field experiment

Results from the field experiment indicated that inoculating celery roots with *B. velezensis* A-27 led to a reduction in both the gall index and the density of soil-resident J2 populations (Table 4). The root gall index significantly decreased ($F = 13.255$, df = 2, $P < 0.001$) after A-27 treatments compared to the control and AW

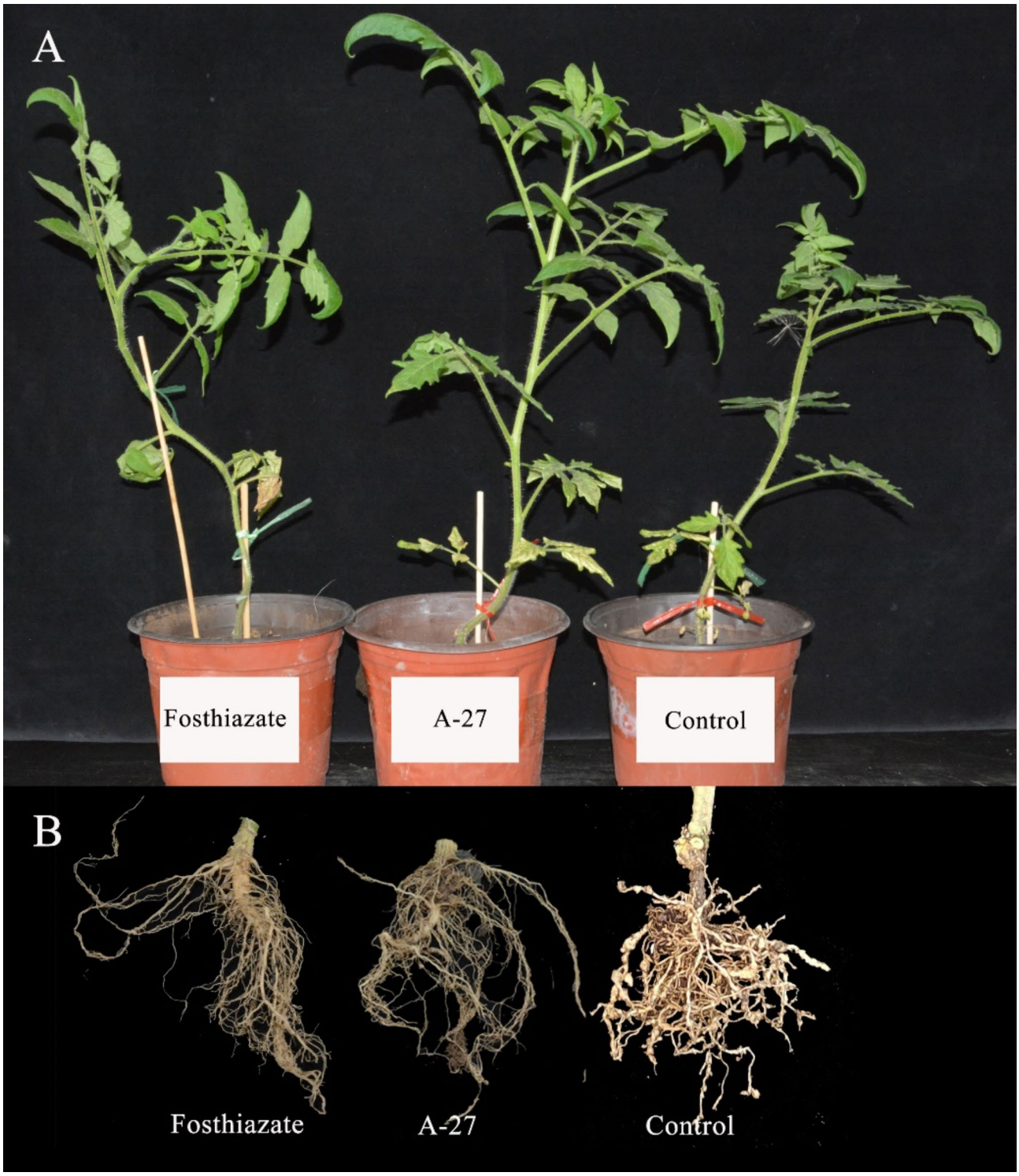

Fig. 4. Aboveground growth and root galls of tomatoes observed in pot experiments at 60 dpi. (**A**) The growth status of potted tomatoes plants under different treatments is shown from left to right: fosthiazate, A-27, and control. (**B**) Root galls observed in tomatoes under different treatments are presented from left to right: fosthiazate, A-27, and control.

treatments. Additionally, the control efficacy of A-27 reached 67.31%, which was higher than that of AW. The growth parameters assessed at 60 days post-inoculation (dpi) showed that inoculation with *B. velezensis* A-27 significantly enhanced celery growth compared to the control ($P < 0.05$) (Table 4). Specifically, the top height increased from 65.20 cm to 83.70 cm ($F = 23.800$, df = 2, $P < 0.001$), and the fresh weight increased from 0.27 kg

Treatments	Plant height (cm)	Plant fresh weight (g)	Root dry weight (g)	Root gall index	Control efficacy (%)
A-27	64.34 ± 5.25 a	39.25 ± 3.09 a	4.67 ± 0.57 a	0.08 ± 0.02 b	85.36 ± 3.97 a
Fosthiazate	60.62 ± 4.01 a	37.06 ± 1.69 a	4.25 ± 0.36 b	0.17 ± 0.03 b	69.16 ± 3.61 b
CK	46.61 ± 2.40 b	33.49 ± 3.11 b	3.52 ± 0.42 b	0.55 ± 0.03 a	-

Table 3. Plant indices and root gall index of tomato infected with *M. incognita* in pot experiment. *Data are represented as the mean ± standard deviation ($n = 15$). Different letters (a, b, c) within each column indicate significantly as determined by one-way analysis of variance (ANOVA) and Tukey's multiple comparison test ($P < 0.05$).

Treatment	Top height (cm)	Fresh weight (kg)	Gall Index	Control efficacy
A-27	83.70 ± 6.55 a	0.45 ± 0.14 a	0.20 ± 0.02 c	67.31 ± 0.08 a
AW	75.90 ± 6.47 b	0.40 ± 0.11 a	0.39 ± 0.03 b	36.62 ± 0.03 b
CK	65.20 ± 4.89 c	0.27 ± 0.07 b	0.61 ± 0.05 a	

Table 4. Control efficacy of *B. velezensis* A-27 in field experiment. *Data are presented as the mean ± standard deviation ($n = 30$). Different letters (a, b, c) within each column indicate significantly as determined by one-way analysis of variance (ANOVA) and Tukey's multiple comparison test ($P < 0.05$).

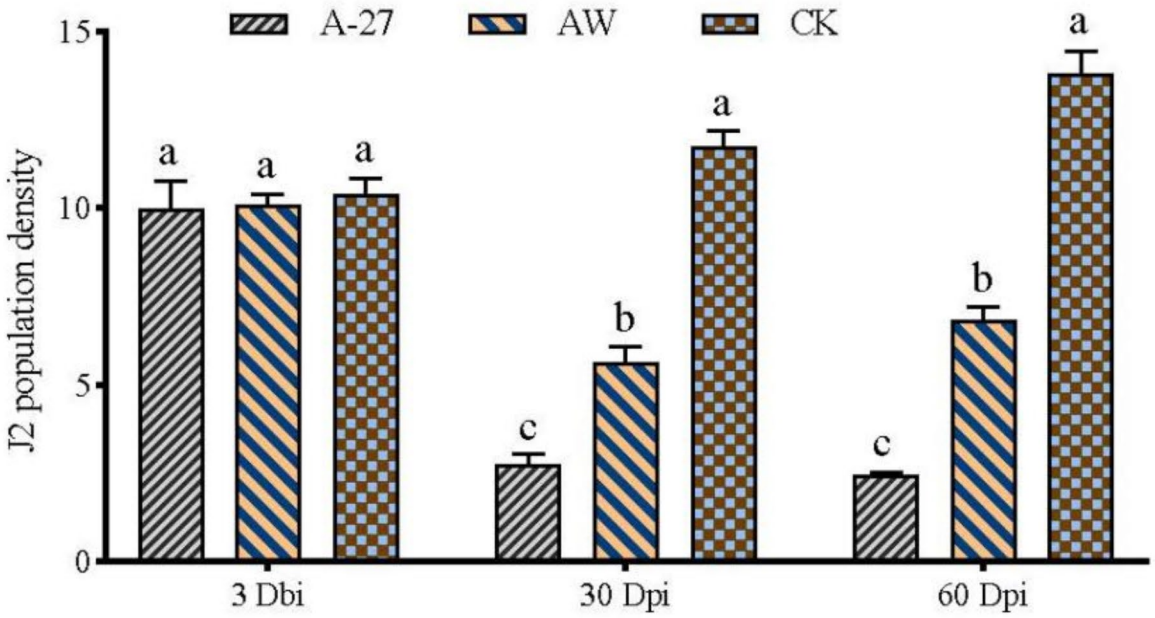

Fig. 5. Effects of A-27 fermentation broth on the population density of *M. incognita*. Different letters (a, b, c) above the bars indicate significant differences as determined by one-way analysis of variance (ANOVA) and Tukey's multiple comparison test ($P < 0.05$) at the corresponding time points, such as 3 dbi, 30 dpi, 60 dpi.

per plant to 0.45 kg per plant ($F = 7.603$, df = 2, $P < 0.01$). Furthermore, inoculation with A-27 resulted in greater top height, fresh weight, and control efficacy compared to AW.

The dynamic changes in the *M. incognita* J2 population density treated with A-27 are shown in Fig. 5. At 3 days before inoculation (dbi), no significant differences were observed between the A-27 treatment, the AW treatment, and the control group. However, at 30 dpi, both the A-27 fermentation broth and AW significantly reduced the population density, whereas the population density in the control group exhibited a gradual increase.

Effects of *B. velezensis* A-27 on soil microbial diversity

Quality assessment of soil microbial sequencing results

The composition of the rhizospheric soil microbiome was analyzed using Illumina MiSeq sequencing of the 16 S and ITS rRNA genes. Each sample was sequenced to a uniform depth, yielding 30,799 bacterial reads and

67,524 fungal reads. This sequencing resulted in the identification of 8,595 operational taxonomic units (OTUs) for bacteria and 5,962 OTUs for fungi at a 97% similarity threshold. Good's coverage for the observed OTUs exceeded 99.71%, and the rarefaction curves demonstrated clear asymptotes (Fig. 6A, B). A comparative analysis of each soil sample using Partial Least Squares Discriminant Analysis (PLS-DA) indicated that samples A-27 (Sxau03, Sxau07, Sxau11), CK (Sxau01, Sxau05, Sxau09), and AW (Sxau02, Sxau06, Sxau10) clustered distinctly, suggesting significant difference among the three treatments (Fig. 6C, D).

Analysis of microbial alpha-diversity index

The microbial alpha-diversity indices, specifically Chao1 and Shannon are summarized in Table 5. No significant changes were observed in the Shannon and Chao1 indices among the three treatments after the application of A-27 and AW.

Analysis of rhizospheric soil species composition

A Venn diagram demonstrated over 97% similarity in the observed overlap and specificity of OTUs in the soil samples across the three treatments. Inoculation with A-27 significantly increased the number of unique microbial OTUs compared to the control. Specifically, the inoculations with A-27, AW, and CK resulted in 209, 222, and 196 unique bacterial OTUs, respectively, with a total of 3, 520 OTUs shared among all treatments (Fig. 6E). For fungal OTUs, A-27, AW, and CK yielded 193, 182, and 134 unique OTUs, respectively, with a total of 468 OTUs common to all treatments (Fig. 6F).

Comparison of rhizospheric soil microbial communities

At the phylum level, the rhizospheric soil microbial communities following inoculation with A-27, AW and CK exhibited the same predominant phyla, although their relative abundances varied. The most abundant bacterial phylum was Proteobacteria, which declined from 30.78% in CK to 26.03% in A-27 (Fig. 7A). The most preval fungal phylum was Ascomycota, which significantly declined from 77.89% in CK to 57.90% in A-27; conversely, Basidiomycota exhibited a significant increase from 3.28% in CK to 18.78% in A-27 (Fig. 7B). Further analysis at the genus level revealed *Bacillus* and *Sphingomonas*, both beneficial bacteria that contribute to plant protection against RKNs. The relative abundances of *Bacillus* and *Sphingomonas* increased significantly by 49.51% and 28.35%, respectively, with A-27 treatment compared to CK (Fig. 7C). In terms of fungi, the relative abundances of *Fusarium*, *Mortierella*, and *Cephaliophora* were significantly reduced by 36.88%, 54.63%, and 84.89%, respectively, with A-27 treatment compared to CK (Fig. 7D). Notably, many species of *Fusarium*, *Mortierella*, and *Cephaliophora* are recognized as plant pathogens.

Predictive functional analysis

The functional changes in the rhizospheric soil microbiome were characterized by predicting the functional composition profiles using PICRUSt and FUNGuild based on 16 S rRNA and ITS sequencing data. Analysis of the bacterial Cluster of Orthologous Groups (COG) functional categories revealed that all three treatments were enriched and exhibited similar functional features. These features included energy production and conversion, amino acid transport and metabolism, carbohydrate transport and metabolism, translation, ribosomal structure and biogenesis, transcription, replication, recombination and repair, cell wall/membrane/envelope biogenesis, inorganic ion transport and metabolism, general function prediction only and signal transduction mechanisms. However, no significant differences were observed among these functional features (Fig. 8A).

The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway abundance composition analysis (level 1) indicated that the A-27 treatment could enhance the abundance of major pathways associated with cellular processes, environmental information processing, genetic information processing, and metabolism. However, no significant differences were observed among these functional features (Fig. 8C). The FUNGuild analysis (Fig. 8B) suggested that the functional composition did not change significantly after inoculation with the various bacterial isolates; however, the relative abundance of functional group such as saprotroph-wood saprotroph, endophyte-litter saprotroph-soil, saprotroph-undefined saprotroph, and undefined saprotroph, decreased following the A-27 treatment compared to the CK. Conversely, the relative abundance of certain functional compositions, including animal pathogen-endophyte-lichen, parasite-plant, pathogen-soil, saprotroph-wood saprotroph, endophyte-litter saprotroph-soil, saprotroph-undefined saprotroph, and undefined saprotroph, also decreased following the A-27 treatment in comparison to the CK.

Discussion

Bacillus velezensis A-27 was initially isolated from the rhizosphere of *Cucurbita pepo* plants. Following its isolation, two separate studies were conducted within our group to evaluate its biocontrol potential. One study focused on its effects against *H. glycines*[25], while the other investigated its impact on *M. incognita.*

The study on *H. glycines* investigated the molecular mechanisms through which the plant growth-promoting rhizobacterium (PGPR) *B. velezensis* A-27 enhances the resistance of red kidney beans. Using TMT proteomics technology, the research highlighted the significant role of the jasmonic acid (JA) biosynthesis pathway in enhancing disease resistance. qRT-PCR analysis confirmed the upregulation of four key genes in the JA biosynthesis pathway, while ELISA analysis demonstrated a significant increase in JA content in the roots. These fundings reveal that *B. velezensis* A-27 induces systemic resistance (ISR) in red kidney beans by modulating the JA biosynthesis pathway, thereby enhancing the plant's defense against SCN.

In the study on *M. incognita*, strain A-27 was identified as *B. velezensis* based on its morphological, physiological, and molecular characteristics, including 16 S rRNA and gyrA sequencing. *B. velezensis* A-27 demonstrated significant larvicidal and ovicidal activities against *M. incognita* in vitro. Both pot and field experiments further confirmed its biocontrol efficacy, demonstrating that A-27 effectively reduced the root

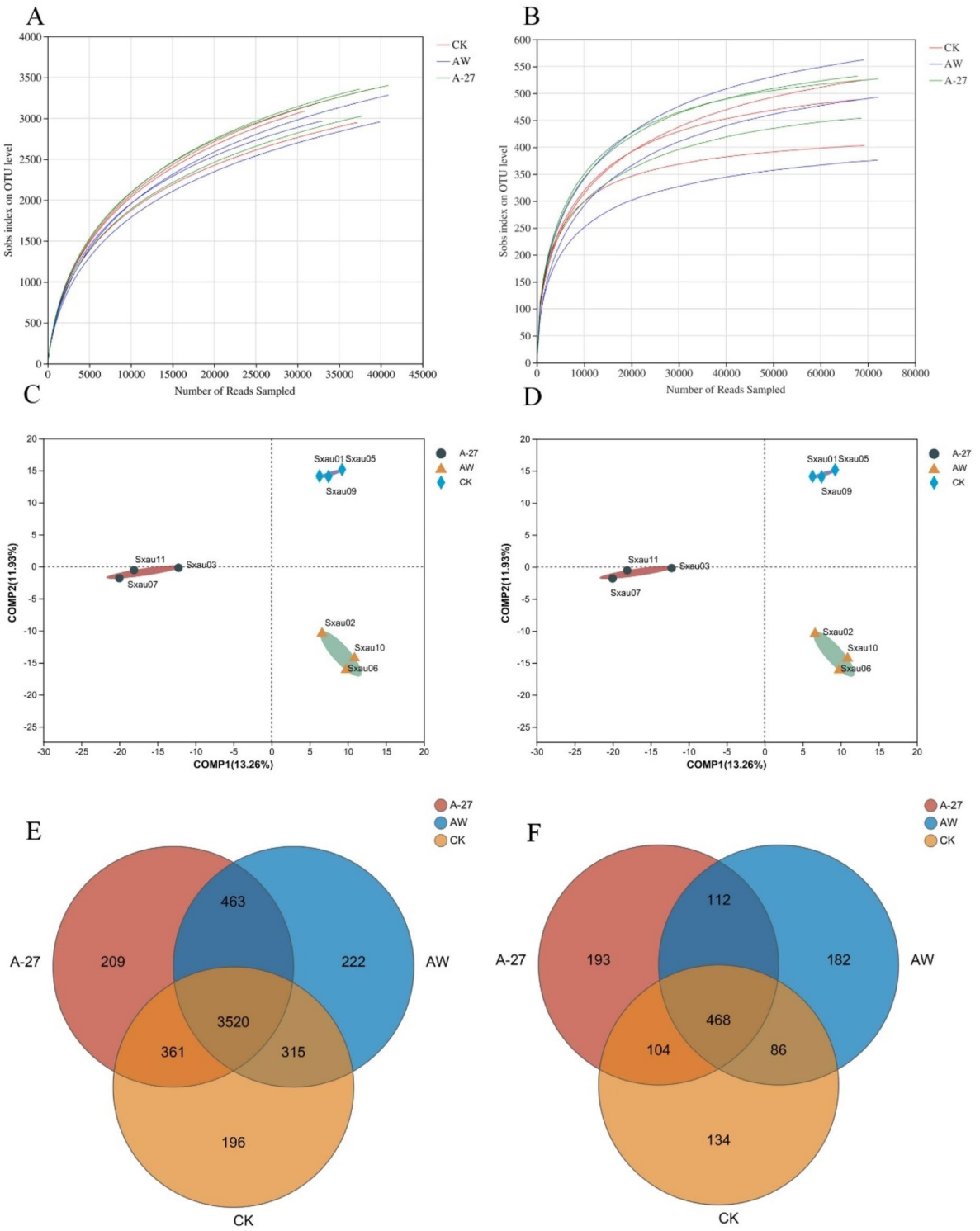

Fig. 6. Rarefaction curves, PLS-DA analysis, and Venn diagrams for bacteria and fungi. (**A**) Rarefaction curves for bacteria. (**B**) Rarefaction curves for fungi. (**C**) PLS-DA analysis of bacteria. (**D**) PLS-DA analysis of fungi. (**E**) Venn diagram for bacteria. (**F**) Venn diagram for fungi. All analysis is based on OTU data from rhizosphere soil samples, which are detailed as follows: CK (Sxau01, Sxau05, Sxau09); AW (Sxau02, Sxau06, Sxau10); and A-27 (Sxau03, Sxau07, Sxau11).

Items	Treatment	Shannon index	Chao1 index
Bacteria	A-27	5.2042 ± 0.1128 a	811.6786 ± 60.4293 a
	AW	5.1767 ± 0.1209 a	767.2485 ± 51.4002 a
	CK	5.1407 ± 0.0819 a	775.1334 ± 4.0362 a
Fungi	A-27	3.6458 ± 0.2397 a	531.6826 ± 47.3151 a
	AW	3.3588 ± 0.4939 a	521.7581 ± 105.1923 a
	CK	3.7224 ± 0.1794 a	509.5726 ± 83.9535 a

Table 5. Rhizospheric soil bacterial and fungal diversity indices (Shannon and Chao1) influenced by *B. velezensis* A-27. *Data are represented as the mean ± standard error. Different letters (a, b, c) above the bars indicate significant differences as determined by one-way analysis of variance (ANOVA) and Tukey's multiple comparison test ($P < 0.05$).

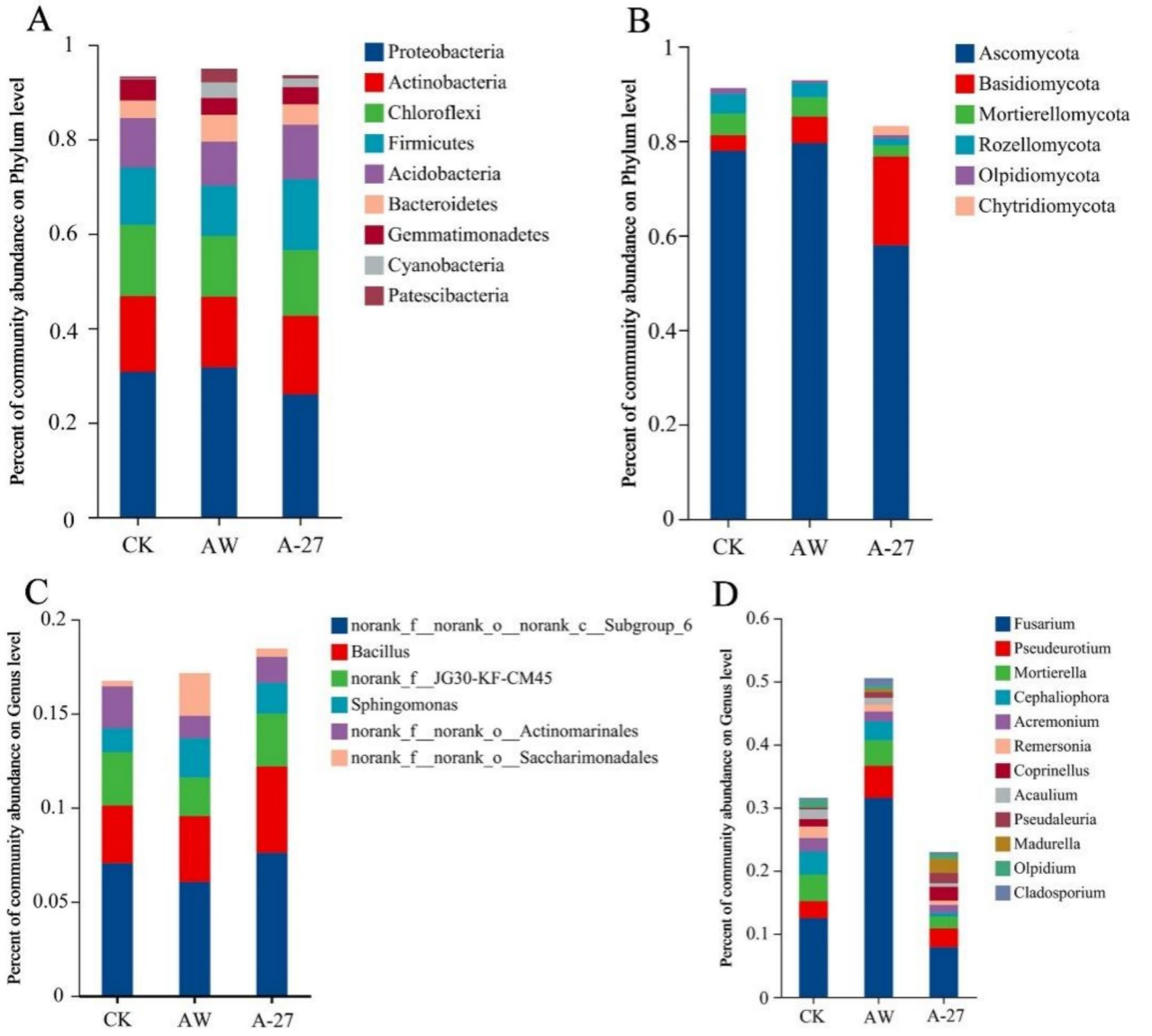

Fig. 7. Taxonomic profiles of sample groups at the phylum and genus level. (**A**) Bacterial phyla. (**B**) Fungal phyla. (**C**) Bacterial genera. (**D**). Fungal genera. Unknown or unclassified clusters are not shown. The height of the bars for each phylum/genus indicates the relative abundance in the sample.

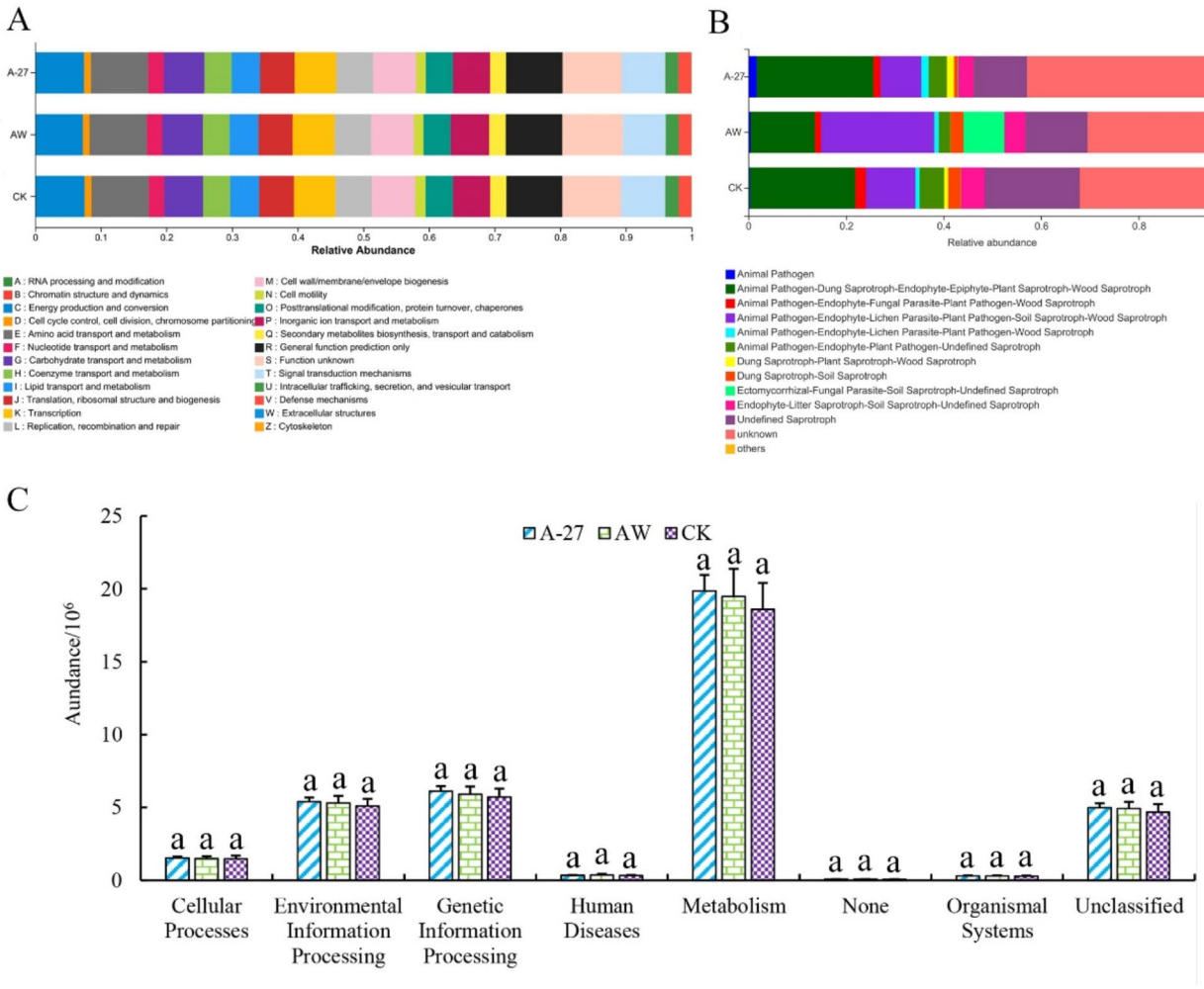

Fig. 8. Predicted functionality of the rhizospheric soil microbiome. (**A**) Relative abundance of bacterial COG functional analysis for treated and untreated samples. (**B**) Relative abundance of assigned fungal functional guilds for treated and untreated samples. (**C**) Relative abundance composition of the bacterial KEGG pathway (level 1) for treated and untreated samples. Different letters (a, b, c) above the bars indicate significant differences based on one-way analysis of variance (ANOVA) and Tukey's multiple comparison test, with a significant level of $P < 0.05$ for the same X-axis options.

gall index and suppressed *M. incognita* populations. Furthermore, field trials revealed that *B. velezensis* A-27 decreased the density of *M. incognita* J2s, influenced microbial diversity and abundance, and promoted celery growth. These findings suggest that *B. velezensis* A-27 may serve as a promising biological agent against *M. incognita*.

Previous studies have utilized *B. velezensis* for the biological control of various phytopathogenic fungi, including *Fusarium graminearum*[27], *Magnaporthe oryzae*[28], *Streptomyces galilaeus*[29], and *Bipolaris sorokiniana*[30]. However, reports on the antagonistic effects of *B. velezensis* against RKNs remain limited. Notably, *B. velezensis* strains Bv-DS1[31], and VB7[32] exhibited significant mortality rates against RKNs. These isolates were obtained from diverse sources, including the rhizosphere soil of cucumber plants, a tidal soil sample, and the rhizosphere of winter wheat, while strain A-27 was specifically isolated from the rhizosphere soil of *Cucurbita pepo*. This indicates that *B. velezensis* has origins in various soil environments and plant root systems, suggesting that its role may vary depending on specific environmental conditions.

This study highlights the considerable potential of *B. velezensis* A-27 in effectively managing RKN disease. Field experiments demonstrated that treatment with *B. velezensis* A-27 significantly reduced both the root gall index and J2 density, while enhancing the height and fresh weight of celery plants. The findings of our study align with previous research on this bacterium; however, most prior studies have primarily focus on host interactions and aimed to induce systemic resistance pathways, including SA and JA signaling pathways, tonoplast intrinsic proteins (TIPs), and microbe-associated molecular pattern (MAMP)-triggered immunity through the expression of transcription factors and defense genes. Furthermore, *B. velezensis* A-27 has been shown to stimulate ISR in red kidney beans and to promote JA biosynthesis by regulating the expression of key enzymes in the α-LeA metabolic pathway against soybean cyst nematodes[25]. Nonetheless, this study emphasizes the significance of the

soil environment and primarily examines the effects of *B. velezensis* A-27 treatment on soil microbial diversity. Our results demonstrate that *B. velezensis* A-27 can recruit beneficial bacteria and mitigate plant pathogens, thereby protecting plants against RKNs. This study is the first to investigate the potential implications of utilizing *B. velezensis* as a drench and biocontrol agent for RKN on the composition of rhizosphere microorganisms in treated soil.

PGPRs colonize the root systems of plants and can modulate growth by enhancing nutrient availability, inducing metabolic activities through phytohormones and their analogs, activating defense mechanisms such as systemic acquired resistance (SAR) and ISR, or by reducing phytotoxic microbial communities[33]. The production of reactive oxygen species (ROS) mediated by FER regulates the relative abundance of beneficial pseudomonads in the rhizosphere microbiome of *Arabidopsis*[34]. Inoculation with *B. amyloliquefaciens* selectively increased the relative abundances of Pseudomonadaceae and Flavobacteriaceae in the rhizosphere of oilseed rape[35]. Our high-throughput sequencing analysis of rhizosphere soil samples indicated that the presence of *B. velezensis* A-27 significantly enhanced the relative abundances of *Bacillus* and *Sphingomonas*, while significantly reducing the relative abundances of *Fusarium*, *Mortierella*, and *Cephaliophora* in the celery rhizosphere. These results demonstrate that inoculation with *B. velezensis* A-27 can recruit beneficial bacteria and decrease the relative abundance of harmful microorganisms. Previous reports have shown that *B. velezensis* exhibits protease, cellulase, and β-1,3-glucanase activities, which are associated with the degradation of phytopathogen cell walls, and produces growth-promoting substances such as indole-3-acetic acid (IAA) and siderophores[30]. It is plausible that *B. velezensis* A-27 may also secrete secondary metabolites that reduce the concentration of soil pathogens while increasing the abundance of beneficial or benign microorganisms, thereby protecting celery against *M. incognita* and enhancing growth through the modulation of the rhizosphere microbial community. Regarding the mechanism, certain *Bacillus* species, including *B. megaterium*[36], *B. subtilis*[37], *B. pumilus*[38], and *B. cereus*[39], produce nematicidal and antimicrobial compounds, such as antibiotics, cyclic lipopeptides, polyketides and bacteriocins. In our study, the fermentation broth and filtrate of *B. velezensis* A-27 exhibited stronger larvicidal activities against *M. incognita* compared to the bacterial suspension. This suggests that A-27 may have released a range of antimicrobial peptides or enzymes that effectively target *M. incognita*, leading to a significantly higher mortality rate of eggs. Further research is necessary to explore its specific components and elucidate the mechanisms of action involved.

Materials and methods

Sources and isolation of rhizospheric bacteria

Root systems along with their associated rhizosphere and soil samples were collected from greenhouses in three locations in Shanxi Province, China, in July 2017. These locations were associated with *Cucurbita pepo* (Jinzhong, 37°29'58.7976" N, 112°46'14.736" E), *Solanum lycopersicum* (Jincheng, 35°27'15.9372" N, 112°54'37.1484" E), and *Cucumis melo* (Jinzhong, 37°23'41.0568" N, 112°36'5.7276" E). The plants were selected based on their healthy appearance and moderate symptoms of nematode infection. These plant roots and soil samples were used to screen for potential biological control agents against RKNs.

Bacterial candidates were isolated using the serial dilution plate method. One gram of each soil sample was dissolved in a conical flask containing 50 mL of sterile water and shaken in a constant temperature shaker at 28℃ and 150 rpm for 15 min. Subsequently, 1 mL of the mixed soil suspension was transferred into a clean test tube, followed by the addition of 9 mL of sterile water and thorough shaking. The suspension concentration of the soil sample was set at 10^{-1} and subsequently diluted to 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5} using the same method. A volume of 20 µL of soil suspension from the 10^{-4} and 10^{-5} concentrations was then applied to nutrient agar (NA) plates. The plates were cultured at 28℃ for 24 h in the dark. Single colonies were subsequently picked, streaked, purified on NA at 28℃, and finally stored in glycerol at -20℃.

Preparation of *M. incognita* inoculum

Meloidogyne incognita nematodes were cultured on tomato plants (*Solanum lycopersicum* cultivar Dahong 368) obtained from Shanxi Agricultural University. The plants were maintained in a growth chamber under a light: dark cycle of 16:8 h, with a temperature of 28℃ during the light phase and 24℃ during the dark phase, and a humidity level of 50%. Eggs were extracted from the roots of the plants, surface-sterilized using 0.5% NaClO for 3 min, and then thoroughly rinsed with sterilized water. Additional egg masses were placed on moist sterile filter paper and incubated in the dark at 28℃ for 5 days to collect the J2s[36].

Subsequently, the eggs and J2s were collected and adjusted to concentrations of 500 eggs/mL and 500 J2/mL, respectively, for the following experiments.

Primary screening of rhizobacteria isolates

The isolated bacterial strains were cultured in 100 mL of Nutrient Broth (NB), which consisted of 3.0 g of beef extract, 10.0 g of peptone, 5.0 g of sodium chloride, and 1000 mL of distilled water, with the pH adjusted to 7.0. The cultures were incubated at 160 rpm and 28℃ for 48 h. Following incubation, the bacterial culture was centrifuged at 8000 rpm for 5 min and subsequently filtered using 0.22 µm Millipore filters.

For the nematicidal assay, 100 µL of *M. incognita* J2 suspensions were mixed with 2 mL of bacterial fermentation filtrate in a 12-well microtitre plate. Wells containing sterile NB liquid medium served as the control (CK). Each bacterial strain was tested in triplicate. The J2 populations were observed under a microscope at 24- and 48-hours post-treatment. The nematicidal efficacy was calculated using the formula: juvenile corrected mortality percentage (%) = (juvenile mortality in treatment - juvenile mortality of CK) / (1 - juvenile mortality of CK) × 100[40].

Identification of strain A-27

Strain A-27 was identified at the genus level based on its morphological characteristics, which included colony morphology, cell length/width, Gram staining results, and the presence of endospores or flagella. Additionally physiological and biochemical traits such as oxidase and catalase activity, acid production from glucose, maltose, and sucrose, nitrate reduction, starch hydrolysis, and the utilization of glutamic acid, citrate, and leucine were assessed, as outlined in Bergey's Manual of Determinative Bacteriology.

For molecular identification, genomic DNA of A-27 was extracted during the logarithmic growth phase using the Ezup Column Bacteria Genomic DNA Purification Kit (Sangon Biotech Co., Shanghai, China). This DNA served as a template for subsequent polymerase chain reaction (PCR) analysis. The 16 S rRNA gene was amplified using primers 27 F and 1492R. Additionally, the gyrA gene of A-27 was amplified using primers p-gyrA-f and p-gyrA-r[41]. The PCR reaction mixture comprised 0.5 µL of template DNA (50 ng/µL), 0.5 µL of each primer (10 µM), 1 µL of 10 × Buffer (with Mg^{2+}), 1 µL of dNTP (2.5 mM), and 22 µL of ddH_2O. The PCR program consisted of an initial denaturation at 94℃ for 5 min, followed by 30 cycles of denaturation at 94℃ for 45 s, annealing at 55℃ for 45 s, and extension at 72℃ for 1 min, concluding with a final extension at 72℃ for 10 min. The PCR products were subsequently separated by electrophoresis on 1% agarose gels, purified, and sent to Sangon Biotech (Shanghai) Co. Ltd. for sequencing.

The obtained DNA sequences were compared to the GenBank database at the National Center for Biotechnology Information (NCBI) using the BLAST program. Sequence alignment was conducted using ClustalX2[42]. The best-fit DNA evolution model was determined using jModelTest 2.1.4 based on the Akaike Information Criterion (AIC)[43]. Finally, a phylogenetic Bayesian tree was constructed using MrBayes V3.1.2[44].

Larvicidal and ovicidal efficacy in vitro

To evaluate the larvicidal efficacy in vitro, the fermentation broth, fermentation filtrate and bacterial suspension of strain A-27 were utilized. The fermentation broth was prepared in NB medium at 28℃ for 48 h with shaking at 160 rpm. Subsequently, the broth was centrifuged at 8000 rpm for 5 min and filtered through 0.22 µm Millipore filters. This A-27 fermentation filtrate eliminated any potential effects from the strain's cells and macromolecular metabolites on the test nematodes. The A-27 fermentation broth and fermentation filtrate were then diluted fivefold and tenfold, respectively. Additionally, a bacterial suspension of A-27 with a concentration of 10^{10} CFU/mL was further diluted to concentrations of 10^6, 10^7, and 10^8 CFU/mL to assess the effects of lower bacteria concentrations.

In the larvicidal assay, a nematicidal J2 assay was conducted in a 12-well microtiter plate, involving three different treatments: (*i*) 100 µL of J2 suspension (approximately 50 J2s) with 2 mL of A-27 fermentation broth; (*ii*) 100 µL of J2 suspension with 2 mL of A-27 fermentation filtrate; and (*iii*) 100 µL of J2 suspension with 2 mL of A-27 bacterial suspension. The J2 populations were monitored microscopically in triplicate at 24- and 48-hours post-treatment.

In the ovicidal assay, 100 µL of egg suspensions were mixed with 2 mL of A-27 fermentation broth, and the egg populations were monitored microscopically 4 days post-treatment. Sterile NB medium served as the control (CK) in both assays. Each assay was conducted in triplicate, with all treatments performed at 28℃ under dark conditions. The larvicidal and ovicidal efficacy were calculated using the formula: juvenile/egg corrected mortality percentage (%) = (mortality of treatment – mortality of CK) / (1 – mortality of CK) × 100[45].

The lethal concentration (LC_{50}) for *M. incognita* J2s and eggs was determined through simple linear regression analysis. This analysis established a correlation between the bacterial concentration of strain A-27 and the corrected mortality rate of *M. incognita* J2s and eggs, utilizing the equation $y = ax + b$, where 'y' denotes the corrected mortality rate among J2s and eggs, and 'x' represents the bacterial concentration of A-27[46].

Efficacy in controlling RKNs in the pot experiment

A pot experiment was conducted at Shanxi Agricultural University, China, within a greenhouse environment. Tomato seeds (*Solanum lycopersicum* cultivar Dahong 368) were surface-sterilized using a 0.5% sodium hypochlorite (NaClO) solution for 4 min, followed by three successive rinses with sterile water. The sterilized seeds were then sown in pots measuring 18 cm in diameter and 15 cm in depth. Each pot was filled with a substrate mixture of silica sand and soil in a 1:1 ratio, totaling 1 kg per pot. Both the silica sand and soil were sterilized using high-pressure steam prior to use.

Tomato seedlings at the four-leaf stage were root-inoculated with three different treatments: *(i)* 15 mL of 2×10^9 CFU/mL A-27 fermentation broth, (*ii*) 15 mL of 10% fosthiazate granules (applied according to the manufacturer's instructions) as a pharmaceutical control, and *(iii)* 15 mL of sterile NB broth as a blank control (CK). Each treatment was replicated five times. The pots were arranged in a randomized block design and maintained in the greenhouse under ambient light at a temperature of 28 ± 3 °C. Tomato plants were watered every 2 days to ensure optimal growth, and the pots were covered with gauze to prevent the introduction of diseases or pests.

After 3 days, a hole was created near each plant, 3 cm from the root and 5 cm deep, for the inoculation of nematodes. Each pot was inoculated with 1000 J2s of *M. incognita.* At 60 dpi, the tomato seedlings were assessed for several growth parameters, including shoot length, root length, fresh root weight, and dry root weight. The root gall index was evaluated according to the specified method[47]. The control efficacy was calculated using the formula: control efficacy (%) = [(root gall index of CK – root gall index of treatment) / root gall index of CK] × 100.

Efficacy of strain A-27 in controlling RKNs in the field experiment

Field experiments were conducted in a greenhouse located in Lixiu Village, Taigu County, Jinzhong City, Shanxi Province, China (coordinates: 37°23′29.54″N, 112°34′20.57″E), from August to November 2019. The greenhouse

had undergone a rotational cultivation of tomato (*Solanum lycopersicum*) and celery (*Apium graveolens*) for three years, resulting in a significant infestation of *M. incognita*. No fumigants or contact nematicides had been previously applied to the soil.

Seven days after transplanting the celery and confirming its stability, the celery plants were root-inoculated at a depth of 5 cm with three different treatments: (i) sterile NB broth as a blank control (CK), (ii) a 5% abamectin oil emulsion (AW, applied according to the manufacturer's instructions) as a pharmaceutical control, and (iii) 10 ml of 2×10^8 CFU/mL A-27 fermentation broth. The field experiment was organized into randomly assigned blocks, divided into nine plots, each measuring 16 m^2 (8 m × 2 m). During the field experiment, the temperature was maintained between 30–35℃, with humidity at approximately 70%.

The population density of *M. incognita* in the soil was measured at 3 dbi, and at 30 dpi and 60 dpi. Soil samples were collected from each plot using a five-point sampling method. Two hundred grams of each soil sample were extracted using the Whitehead and Hemming tray method[48]. Each treatment was replicated three times. The root gall index and control efficacy were calculated as described in the pot experiments.

Effects of strain A-27 on soil microbial diversity

At 30 days post-treatment with strain A-27 in the field experiments, five soil cores were collected from each plot. These soil cores were combined for each plot and subsequently divided into three portions. The portions were then immediately frozen in liquid nitrogen and stored at -80℃ for DNA extraction.

Genomic DNA was extracted from a 0.5 g wet-weight soil sample using the E.Z.N.A ® Soil DNA Kit (OMEGA, USA). The purity and integrity of the extracted DNA were assessed using a NanoDrop2000 (Thermo Scientific, USA) and agarose gel electrophoresis, respectively. The bacterial 16 S rRNA gene V3-V4 region was amplified using primers 338F and 806R[49], while the fungal ITS1 rRNA gene was amplified using primers ITS1F and ITS2R[50]. The PCR products from each sample were mixed, analyzed using 2% agarose gel electrophoresis, purified with an AxyPrep DNA Gel Extraction Kit (Axygen, USA), and quantified using a Quantus™ Fluorometer. After individual quantification, equal amounts of the amplicons from each sample were pooled together for sequencing. Paired-end sequencing was performed using an Illumina MiSeq platform with a NEXTFLEX Rapid DNA-Seq Kit (Bioo Scientific, USA) and MiSeq PE300 (Illumina, USA) at Shanghai Meggie Biomedical Technology Co., Ltd (http://www.majorbio.com/).

Following sequencing, the raw FASTQ files were demultiplexed and quality-filtered using Trimmomatic software[51]. Paired-end reads were assembled using FLASH v1.2.7[52]. OTUs were clustered with a 97% similarity cutoff using UPARSE[53]. Subsequently, a representative sequence for each OTU was selected and classified using the RDP Classifier[54] based on the Silva[55] 16 S and UNITE ITS databases for bacteria and fungi, respectively. Data analysis included generating dilution curves, Venn diagrams, community histograms, and PLS-DA charts using R language v3.2.0. Alpha diversity, Shannon diversity, and Chao1 indices were calculated using Mothur software[56]. Functional prediction analysis of microbial communities was performed using Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt)[57] and FUNGuild[58].

Statistical analysis

Statistical analysis was performed using Microsoft Office Excel 2019 and IBM SPSS Statistics version 25.0 (IBM, Armonk, NY, USA). General linear models were utilized to assess for significant differences. The mortality rates of J2s and eggs, gall index, growth index, and microbial diversity index were analyzed using one-way analysis of variance (ANOVA). Tukey's multiple range tests were conducted to identify significant differences ($P<0.05$). The data is presented as mean values accompanied by standard deviation (SD).

Data availability

The study accession numbers for the sequencing data are as follows: SRR24999916, SRR24999915, SRR24999917, SRR24999919, SRR24999920, SRR24999921, SRR24999923, SRR24999912, SRR249999114, SRR25000167, SRR25000166, SRR25000163, SRR25000161, SRR25000160, SRR25000159, SRR25000157, SRR25000156, and SRR25000165. The bioproject accession number is PRJNA986599, and the biosample accession numbers are SAMN35847138, SAMN35847139, SAMN35847140, SAMN35847142, SAMN35847143, SAMN35847144, SAMN35847146, SAMN35847147, and SAMN35847148. For more information, please visit the online link: https://dataview.ncbi.nlm.nih.gov/object/PRJNA986599?reviewer=9prhnlgk7b50iseo1ovlmvugko.

Received: 9 September 2024; Accepted: 16 December 2024
Published online: 07 January 2025

References

1. Jones, J. T. et al. Top 10 plant-parasitic nematodes in molecular plant pathology. *Mol. Plant Pathol.* **14**, 946–961 (2013). https://www.ncbi.nlm.nih.gov/pubmed/23809086
2. Forghani, F. & Hajihassani, A. Recent advances in the development of environmentally benign treatments to control root-knot nematodes. *Front. Plant Sci.* **11**, 1125 (2020). https://www.ncbi.nlm.nih.gov/pubmed/32793271
3. Oka, Y. et al. New strategies for the control of plant-parasitic nematodes. *Pest Manag. Sci.* **56**, 983–988. https://doi.org/10.1002/1526-4998(200011)56:11 (2000).
4. Peng, D. Plant nematode diseases: serious challenges to China's food security. *Biotechnol. Bull.* **37**, 1–2 (2021).
5. Adam, M., Westphal, A., Hallmann, J. & Heuer, H. Specific microbial attachment to root knot nematodes in suppressive soil. *Appl. Environ. Microbiol.* **80**, 2679–2686. https://doi.org/10.1128/AEM.03905-13 (2014).
6. Hajji-Hedfi, L., Regaieg, H., Larayedh, A., Chihani, N. & Horrigue-Raouani, N. Biological control of wilt disease complex on tomato crop caused by *Meloidogyne Javanica* and *Fusarium oxysporum* f.sp. *Lycopersici* by *Verticillium Leptobactrum*. *Environ. Sci. Pollut Res. Int.* **25**, 18297–18302 (2018).

7. Kassam, R. et al. Evaluation of *Paecilomyces tenuis* producing Huperzine A for the management of root-knot nematode *Meloidogyne incognita* (Nematoda: Meloidogynidae). *J. Pest Sci.* **96**, 723–743. https://doi.org/10.1007/s10340-022-01521-4 (2023).
8. Sharma, I. P. & Sharma, A. K. Physiological and biochemical changes in tomato cultivar PT-3 with dual inoculation of mycorrhiza and PGPR against root-knot nematode. *Symbiosis* **71**, 175–183. https://doi.org/10.1007/s13199-016-0423-x (2017).
9. Kundu, A., Saha, S., Walia, S. & Dutta, T. K. Anti-nemic secondary metabolites produced by Fusarium oxysporum f. sp ciceris. *J. Asia. Pac. Entomol.* **19**, 631–636. https://doi.org/10.1016/j.aspen.2016.06.003 (2016).
10. Dong, H. L., Zhou, X. G., Wang, J. M., Xu, Y. M. & Lu, P. *Myrothecium verrucaria* strain X-16, a novel parasitic fungus to *Meloidogyne hapla*. *Biol. Control.* **83**, 7–12. https://doi.org/10.1016/j.biocontrol.2014.12.016 (2015).
11. Schouteden, N., De Waele, D., Panis, B. & Vos, C. M. Arbuscular mycorrhizal fungi for the biocontrol of plant-parasitic nematodes: a review of the mechanisms involved. *Front. Microbiol.* **6**, 1280. https://doi.org/10.3389/fmicb.2015.01280 (2015).
12. Islam, S., Akanda, A. M., Prova, A., Islam, M. T. & Hossain, M. M. Isolation and identification of plant growth promoting rhizobacteria from cucumber rhizosphere and their effect on plant growth promotion and disease suppression. *Front. Microbiol.* **6**, 1360. https://doi.org/10.3389/fmicb.2015.01360 (2016).
13. Yin, N. et al. Volatile organic compounds of *Bacillus cereus* strain Bc-cm103 exhibit fumigation activity against *Meloidogyne incognita*. *Plant Dis.* **105**, 904–911. https://doi.org/10.1094/PDIS-04-20-0783-RE (2021).
14. Xiong, J. et al. Systemic nematicidal activity and biocontrol efficacy of *Bacillus firmus* against the root-knot nematode *Meloidogyne incognita*. *World J. Microbiol. Biotechnol.* **31**, 661–667 (2015). https://www.ncbi.nlm.nih.gov/pubmed/25672545
15. Messa, V. Nunes, Joselito, Mattei, Danielle. Seed treatment with *Bacillus amyloliquefaciens* for the control of *Meloidogyne Javanica in vivo* bean culture and its direct effect on the motility, mortality and hatching of *M. Javanica in vitro*. *Agron. Sci. Biotechnol.* **5**, 59. https://doi.org/10.33158/ASB.2019v5i2p59 (2019).
16. Sahebani, N. & Gholamrezaee, N. The biocontrol potential of *Pseudomonas fluorescens* CHA0 against root knot nematode (*Meloidogyne Javanica*) is dependent on the plant species. *Biol. Control*. 152. https://doi.org/10.1016/j.biocontrol.2020.104445 (2021).
17. Sharma, M., Jasrotia, S., Ohri, P. & Manhas, R. K. Nematicidal potential of *Streptomyces antibioticus* strain M7 against *Meloidogyne incognita*. *AMB Express*. **9**, 168 (2019).
18. ZHANG, T., Li, X. Y., Yang, H. M., Chu, M. & Ying-Wu, S. H. I. Isolation screening and identification of antagonistic bacteria against *Verticillium Dahliae* Kleb. *Xinjiang Microbiol. China*. **45**, 2418–2428. https://doi.org/10.13344/j.microbiol.china.180003 (2018).
19. Jin, P. et al. Antifungal mechanism of bacillomycin D from *Bacillus velezensis* HN-2 against *Colletotrichum gloeosporioides* Penz. *Pestic. Biochem. Physiol.* **163**, 102–107. https://doi.org/10.1016/j.pestbp.2019.11.004 (2020).
20. Migunova, V. D. et al. Selection of bacterial strains for control of root-knot disease caused by *Meloidogyne incognita*. *Microorganisms* **9**, 1698. https://doi.org/10.3390/microorganisms9081698 (2021).
21. Tian, X. L., Zhao, X. M., Zhao, S. Y., Zhao, J. L. & Mao, Z. C. The biocontrol functions of *Bacillus velezensis* strain Bv-25 against *Meloidogyne incognita*. *Front. Microbiol.* **13**, 843041. https://doi.org/10.3389/fmicb.2022.843041 (2022).
22. Kumar, M. et al. Plant-growth-promoting rhizobacteria emerging as an effective bioinoculant to improve the growth, production, and stress tolerance of vegetable crops. *Int. J. Mol. Sci.* **22**, 12245. https://doi.org/10.3390/ijms222212245 (2021).
23. Jiang, S. Q. et al. High-throughput absolute quantification sequencing reveals the effect of different fertilizer applications on bacterial community in a tomato cultivated coastal saline soil. *Sci. Total Environ.* **687**, 601–609 (2019). https://www.ncbi.nlm.nih.gov/pubmed/31220714
24. Yu, S. et al. The mechanism of compound microbial fertilizer KMM alleviating apple replant disease. *J. Plant. Prot.* **51**, 352–360. https://doi.org/10.13802/j.cnki.zwbhxb.2024.2023005 (2024). https://link.cnki.net/doi/
25. Hu, Y. et al. Research on the mechanism of *Bacillus velezensis* A-27 in enhancing the resistance of red kidney beans to soybean cyst nematode based on TMT proteomics analysis. *Front. Plant Sci.* **15**, 1458330 (2024). https://www.ncbi.nlm.nih.gov/pubmed/39376238
26. Ruiz-Garcia, C., Bejar, V., Martinez-Checa, F., Llamas, I. & Quesada, E. *Bacillus velezensis* sp. nov., a surfactant-producing bacterium isolated from the river Velez in Malaga, southern Spain. *Int. J. Syst. Evol. Microbiol.* **55**, 191–195 (2005). https://www.ncbi.nlm.nih.gov/pubmed/15653875
27. Palazzini, J. M. et al. Spray-drying process as a suitable tool for the formulation of *Bacillus velezensis* RC218, a proved biocontrol agent to reduce Fusarium Head Blight and deoxynivalenol accumulation in wheat. *Biocontrol Sci. Technol.* **30**, 329–338. https://doi.org/10.1080/09583157.2019.1709621 (2020).
28. Ma, Z. W., Zhang, S. Y., Sun, K. & Hu, J. C. Identification and characterization of a cyclic lipopeptide iturin A from a marine-derived *Bacillus velezensis* 11 – 5 as a fungicidal agent to *Magnaporthe oryzae* in rice. *J. Plant Dis. Prot.* **127**, 15–24. https://doi.org/10.1007/s41348-019-00282-0 (2020).
29. Cui, L. X., Yang, C. D., Wei, L. J., Li, T. H. & Chen, X. Y. Isolation and identification of an endophytic bacteria *bacillus velezensis* 8 – 4 exhibiting biocontrol activity against potato scab. *Biol. Control*. **141**, 104156. https://doi.org/10.1016/j.biocontrol.2019.104156 (2020).
30. Huang, T., Zhang, Y., Yu, Z. H., Zhuang, W. Y. & Zeng, Z. Q. *Bacillus velezensis* BV01 has broad-spectrum biocontrol potential and the ability to promote plant growth. *Microorganisms* **11**, 2627. https://doi.org/10.3390/microorganisms11112627 (2023).
31. Hu, Y. F. et al. Biocontrol efficacy of *Bacillus velezensis* strain YS-AT-DS1 against the root-knot nematode *Meloidogyne incognita* in tomato plants. *Front. Microbiol.* **13**, 1035748. https://doi.org/10.3389/fmicb.2022.1035748 (2022).
32. Kamalanathan, V., Sevugapperumal, N. & Nallusamy, S. Antagonistic bacteria *bacillus velezensis* VB7 possess nematicidal action and induce an immune response to suppress the infection of Root-Knot Nematode (RKN) in tomato. *Genes* **14**, 1335. https://doi.org/10.3390/genes14071335 (2023).
33. Ping, L. Signals from the underground: bacterial volatiles promote growth in *Arabidopsis*. *Trends Plant Sci.* **9**, 263–266. https://doi.org/10.1016/j.tplants.2004.04.008 (2004).
34. Song, Y. et al. FERONIA restricts Pseudomonas in the rhizosphere microbiome via regulation of reactive oxygen species. *Nat. Plants*. **7**, 644–654 (2021). https://www.ncbi.nlm.nih.gov/pubmed/33972713
35. Liu, Y. et al. Unraveling mechanisms and impact of microbial recruitment on Oilseed rape (Brassica napus L.) and the rhizosphere mediated by plant growth-promoting rhizobacteria. *Microorganisms* **9**, 161 (2021). https://www.ncbi.nlm.nih.gov/pubmed/33445684
36. Zhou, Y. et al. Efficacy of *Bacillus megaterium* strain Sneb207 against soybean cyst nematode (*Heterodera glycines*) in soybean. *Pest Manag. Sci.* **77**, 568–576 (2021). https://www.ncbi.nlm.nih.gov/pubmed/32815305
37. Cao, H. Y. et al. Analysis of the activity and biological control efficacy of the *Bacillus subtilis* strain Bs-1 against *Meloidogyne incognita*. *Crop Prot.* **122**, 125–135. https://doi.org/10.1016/j.cropro.2019.04.021 (2019).
38. Liu, G. Y. et al. Screening, identification and application of soil bacteria with nematicidal activity against root-knot nematode (*Meloidogyne incognita*) on tomato. *Pest Manag. Sci.* **76**, 2217–2224. https://doi.org/10.1002/ps.5759 (2020).
39. Hu, H. J. et al. Identification and nematicidal characterization of proteases secreted by endophytic bacteria *bacillus cereus* BCM2. *Phytopathology* **110**, 336–344. https://doi.org/10.1094/PHYTO-05-19-0164-R (2020).
40. Zhao, D. et al. Isolation and identification of bacteria from rhizosphere soil and their effect on plant growth promotion and root-knot nematode disease. *Biol. Control*. **119**, 12–19. https://doi.org/10.1016/j.biocontrol.2018.01.004 (2018).
41. Chun, J. & Bae, K. S. Phylogenetic analysis of *Bacillus subtilis* and related taxa based on partial gyrA gene sequences. *Antonie Van Leeuwenhoek*. **78**, 123–127 (2000). https://www.ncbi.nlm.nih.gov/pubmed/11204764

42. Larkin, M. A. et al. Clustal W and Clustal X version 2.0. *Bioinformatics* **23**, 2947–2948 (2007). https://www.ncbi.nlm.nih.gov/pubmed/17846036
43. Darriba, D., Taboada, G. L., Doallo, R. & Posada, D. jModelTest 2: more models, new heuristics and parallel computing. *Nat. Methods*. **9**, 772 (2012). https://www.ncbi.nlm.nih.gov/pubmed/22847109
44. Ronquist, F. et al. MrBayes 3.2: efficient bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol.* **61**, 539–542 (2012). https://www.ncbi.nlm.nih.gov/pubmed/22357727
45. Saikia, S. K., Tiwari, S. & Pandey, R. Rhizospheric biological weapons for growth enhancement and *Meloidogyne incognita* management in *Withania somnifera cv. Poshita Biol. Control*. **65**, 225–234. https://doi.org/10.1016/j.biocontrol.2013.01.014 (2013).
46. Du, B., Xu, Y., Dong, H., Li, Y. & Wang, J. *Phanerochaete chrysosporium* strain B-22, a nematophagous fungus parasitizing *Meloidogyne incognita*. *PLoS One*. **15**, e0216688 (2020). https://www.ncbi.nlm.nih.gov/pubmed/31931510
47. Bridge, J. & Page, S. L. J. Estimation of root-knot nematode infestation levels on roots using a rating chart. *Trop. Pest Manage.* **26**, 296–298. https://doi.org/10.1080/09670878009414416 (2009).
48. Bell, N. L. & Watson, R. N. M. Optimising the Whitehead and Hemming tray method to extract plant parasitic and other nematodes from two soils under pasture. *Nematology* **3**, 179–185. https://doi.org/10.1163/156854101750236312 (2001).
49. Xu, N., Tan, G. C., Wang, H. Y. & Gai, X. P. Effect of biochar additions to soil on nitrogen leaching, microbial biomass and bacterial community structure. *Eur. J. Soil Biol.* **74**, 1–8. https://doi.org/10.1016/j.ejsobi.2016.02.004 (2016).
50. Adams, R. I., Miletto, M., Taylor, J. W. & Bruns, T. D. Dispersal in microbes: fungi in indoor air are dominated by outdoor air and show dispersal limitation at short distances. *ISME J.* **7**, 1262–1273. https://doi.org/10.1038/ismej.2013.28 (2013).
51. Bolger, A. M., Lohse, M. & Usadel, B. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* **30**, 2114–2120. https://doi.org/10.1093/bioinformatics/btu170 (2014).
52. Magoc, T. & Salzberg, S. L. FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics* **27**, 2957–2963. https://doi.org/10.1093/bioinformatics/btr507 (2011).
53. Edgar, R. C. UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nat. Methods*. **10**, 996–998. https://doi.org/10.1038/nmeth.2604 (2013).
54. Wang, Q., Garrity, G. M., Tiedje, J. M. & Cole, J. R. Naïve bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl. Environ. Microbiol.* **73**, 5261–5267. https://doi.org/10.1128/AEM.00062-07 (2007).
55. Quast, C. et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res.* **41**, D590–D596. https://doi.org/10.1093/nar/gks1219 (2013).
56. Pitta, D. W. et al. Bacterial diversity dynamics associated with different diets and different primer pairs in the rumen of kankrej cattle. *Plos One*. **9**, e111710. https://doi.org/10.1371/journal.pone.0111710 (2014).
57. Langille, M. G. et al. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. *Nat. Biotechnol.* **31**, 814–821 (2013). https://www.ncbi.nlm.nih.gov/pubmed/23975157
58. Nguyen, N. H. et al. FUNGuild: an open annotation tool for parsing fungal community datasets by ecological guild. *Fungal Ecol.* **20**, 241–248. https://doi.org/10.1016/j.funeco.2015.06.006 (2016).

Author contributions

YX, JW, and YY conceived and designed the experiment. YY, LW, and HD prepared the experimental materials. YY and HZ conducted the experiment, as well as collected and analyzed the data. YY wrote the initial draft of the manuscript. ZZ and YX revised the manuscript, addressed the authors′ responses to comments, and edited the language. All authors approved the final version of the manuscript for publication.

Funding

This research was funded by the Central Project Guide Local Science and Technology for Development of Shanxi Province (YDZJSX2021A033), the National Natural Science Foundation of China (31801958), Postgraduate Scientific Research Innovation Project of Shanxi Province (2024KY306), and the Cultivate Scientific Research Excellence Programs of Higher Education Institutions in Shanxi Province (2019KJ021).

Declarations

Competing interests

The authors declare no competing interests.

Additional information

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-024-83687-8.

Correspondence and requests for materials should be addressed to Y.X.