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Bacillus velezensis YC89-mediated recruitment of rhizosphere bacteria improves resistance against sugarcane red rot

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Abstract

Background Sugarcane red rot is a soil-borne disease caused by *Colletotrichum falcatum*. It can reduce the yield of sugarcane and the purity of sugarcane juice, which seriously restricts the development of sucrose industry. Biocontrol bacteria can control diseases by regulating rhizosphere microecology. In this study, the effects of biocontrol bacteria on sugarcane rhizosphere microecology were studied by metagenomics and metabolomics, and the control effects of biocontrol bacteria and rhizosphere dominant bacteria on sugarcane red rot were further explored by pot experiment.

Results The results of metagenomic sequencing showed that inoculation with *B. velezensis* YC89 and pathogens could significantly change the microbial diversity of the sugarcane rhizosphere. The relative abundance of beneficial strains such as *Streptomyces*, *Burkholderia*, *Sphingomonas*, and *Rhizobium* increased significantly in the rhizosphere of sugarcane in the YC treatment group. *Pseudomonas* was significantly enriched in the rhizosphere of sugarcane in the C treatment group. The results of metabolome sequencing showed that the content of amino acids in sugarcane root exudates increased after inoculation with *B. velezensis* YC89, and the contents of phenolic acids and flavonoids decreased. Spearman correlation analysis showed that there was a significant correlation between differential metabolites and rhizosphere microorganisms. The results of pot experiment showed that YC89 strain and three rhizosphere microorganisms could significantly reduce the disease index of red rot and promote the growth of sugarcane plants. In addition, these strains can also significantly increase the JA and SA content of sugarcane leaves and induce plant system resistance-related enzyme activities. Among them, the synthetic community treatment group had the best biocontrol effect on red rot, and its relative control effect was 67.50%.

Conclusions Therefore, we conclude that *B. velezensis* YC89 could recruit beneficial rhizosphere microorganisms to enrich the rhizosphere and change the content of some phenolic acids and flavonoids in the root exudates. In addition, the isolated rhizosphere dominant bacteria and YC89 strain can resist red rot by inducing plant systemic resistance and promote the growth of sugarcane plants. This study provides a theoretical basis for the use of biocontrol bacteria to regulate rhizosphere bacteria to jointly control plant soil-borne diseases.

Keywords Sugarcane red rot, Root exudates, Rhizosphere microbiome, *Colletotrichum falcatum*, *Bacillus velezensis*

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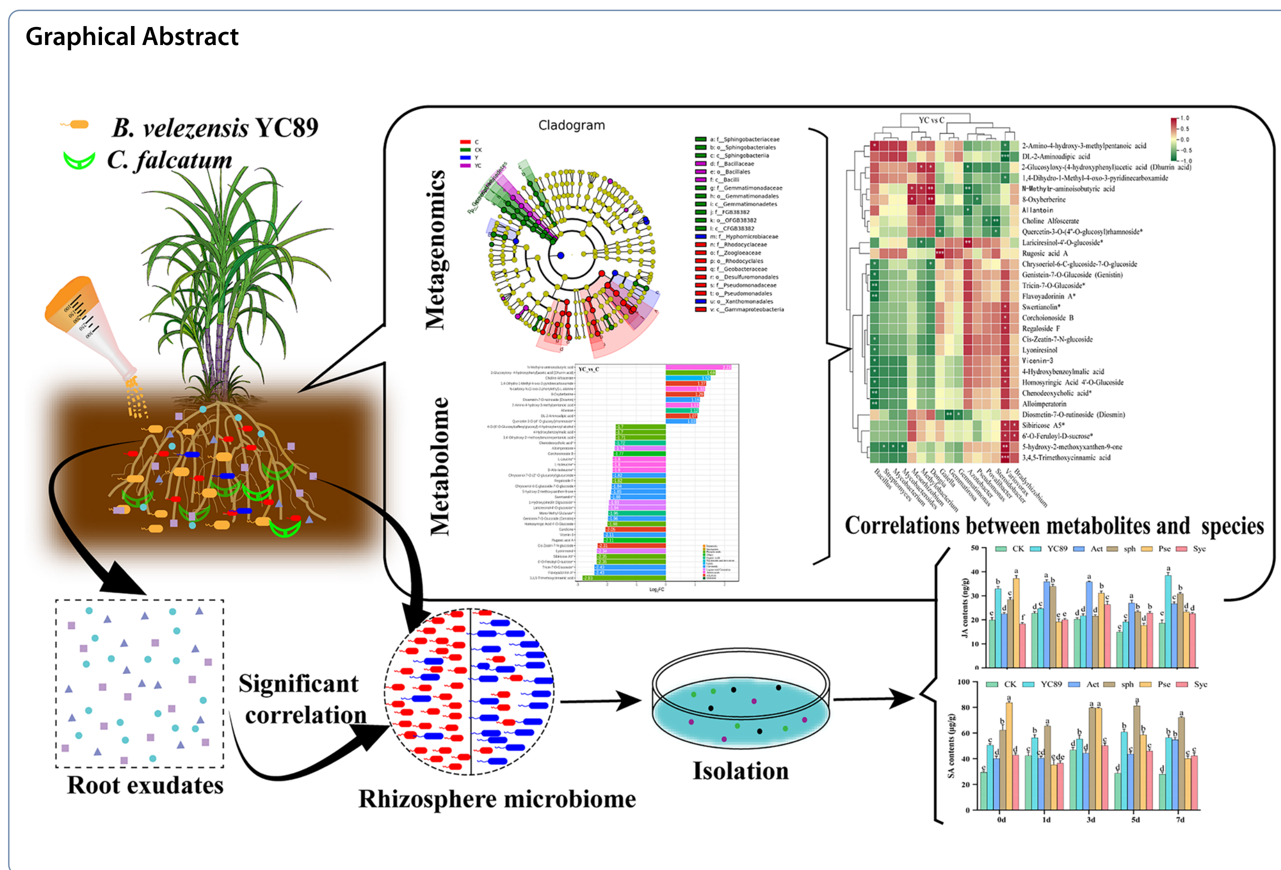
Graphical Abstract



Introduction

Sugarcane is one of the most important sugar crops worldwide, but its production is severely restricted by disease. Sugarcane red rot is a soil-borne disease caused by *Colletotrichum falcatum* [1]. This disease restricts sugarcane production and is often referred to as the "cancer" of sugarcane [2]. Serious damage can lead to a 29.1% reduction in sugarcane production and > 30.8% loss of sucrose, seriously affecting the yield and quality of sugarcane [3]. Sugarcane red rot pathogens exhibit variations, and the emergence of new pathogenic varieties can lead to a "loss" of resistance in sugarcane. Consequently, despite the initial planting of resistant sugarcane strains, susceptibility can ensue within 8–10 years, with the emergence of increasingly virulent varieties [4]. Currently, chemical fungicides are the most effective in controlling sugarcane red rot. However, long-term dependence on chemical fungicides can easily lead to pathogen resistance and reduce their effect [5]. In addition, chemical fungicides lead to the accumulation of pesticide residues, endangering the ecological environment and threatening human health, meanwhile fungicides can affect the composition and function of the soil microbiome [6, 7]. Consequently, the development of green biological defense and control strategies is crucial. Domestic and foreign studies have shown that application of biocontrol bacteria can increase the number of beneficial bacteria genera in the crop rhizosphere of indigenous microorganisms and reduce harmful fungi [8, 9]. However, studies on how biocontrol bacteria affect the distribution of specific dominant microorganisms in the rhizosphere and whether dominant strains can directly participate in plant disease prevention and growth promotion are limited.

Rhizosphere microorganisms have the second most common plant genome and have formed functional holobionts with plants during evolution. Furthermore, they play an important role in the nutrition and health of host plants [10, 11]. Biocontrol bacteria can be used to plant diseases by remodeling the structure and function of rhizosphere microbial communities. The biological agent *Bacillus amyloliquefaciens* W19 reduces the density of banana *Fusarium* wilt pathogens and activates specific plant-beneficial bacteria (such as *Pseudomonas*), directly or indirectly limiting the ability of fungal pathogens to infect plant roots [12]. *B. velezensis* B63 or *Pseudomonas fluorescens* P142 in tomato rhizosphere soil resulted in significant changes in the composition of rhizosphere

bacterial communities, and the corresponding microbial changes may trigger plant defense against *Ralstonia solanacearum* B3B [13]. Similarly, pathogens, when they attack plants, can change the composition of the rhizosphere microbial community, whereas plants can recruit specific microbial groups to fight soil-borne pathogens as a defense strategy during pathogen infection [14]. When *Arabidopsis thaliana* leaves infected with downy mildew enriched the beneficial rhizospheric bacteria *Xanthomonas*, *Stenotrophomonas,* and *Microbacterium*, indicating that *A. thaliana* plants infected with pathogens can promote the enrichment of specific microbial groups in the rhizosphere, helping plants to resist adversity [15].

Root exudates refer to a wide variety of substances secreted or released from different parts of the root system to the growth medium during plant growth [16]. Root exudates are important for plants to adapt to stressful environments. Plants continuously respond to and change their surrounding environment by secreting bioactive compounds, preventing pathogenic microorganisms or invertebrates from negatively affecting the rhizosphere, and promoting positive interactions, such as beneficial microorganisms and rhizosphere symbiosis [17]. Root exudates also contain a variety of plant secondary metabolites (such as flavonoids, benzoxazines, and coumarins), which participate in the signal transduction process of microbial communities by promoting or inhibiting the growth of pathogenic bacteria, thereby affecting the assembly and function of microbial communities [18, 19]. Root exudates shape root microbiota and play an important role in recruiting beneficial soil bacteria to the rhizosphere. Wen et al. showed that the invasion of aboveground pathogens in plants can increase the secretion of long-chain fatty acids and amino acids in root exudates and recruit more *Pseudomonas*, thereby improving plant resistance [20]. Therefore, studying the root exudates that mediate active plant adaptation and resistance to various adverse environments is vital.

Biocontrol bacteria can change the diversity of microbial communities in the rhizosphere of plants. It can recruit beneficial microorganisms to enrich or inhibit the abundance of harmful bacteria in the rhizosphere, thereby inhibiting the occurrence of soil-borne diseases. Therefore, the analysis of the complex interaction between biocontrol bacteria, pathogens and rhizosphere microorganisms is the key to understanding how biocontrol bacteria enhance plant disease resistance by reshaping the rhizosphere soil microbial community. The relative control effect of Bacillus velezensis YC89 on sugarcane red rot was 61.91%, but the effect of the strain on sugarcane rhizosphere microecology remains to be further studied. This study aimed to: (i) determine the effects of the biocontrol bacterium YC89 and red rot disease on the sugarcane rhizosphere bacterial community and root exudate metabolome, (ii) understand the relationship between dominant rhizosphere bacteria and root exudate metabolites using correlation analysis, and (iii) isolate and identify PGPR capable of inducing disease resistance in sugarcane seedlings. Furthermore, we examined the metagenome and metabolome of red rot sugarcane rhizospheres inoculated with *Bacillus velezensis* YC89. The results of this study may provide a theoretical basis for studying biocontrol bacteria and serve as a reference for the biological control of sugarcane red rot.

Materials and methods

Strains and growth conditions

Colletotrichum falcatum was isolated from susceptible sugarcane leaves, sugarcane disease samples were collected from Longchuan County, Yunnan Province, China (24° 08′–24° 39′ N, 97° 39′–98° 17′ E). *B. velezensis* YC89, an endophytic bacterial strain isolated from sugarcane leaves, showed 78% inhibition of *C. falcatum*. *B. velezensis* YC89 (strain N°60, 902) was preserved in China Microbiological Culture Collection Center (Guangdong, China).

Preparation of the biocontrol bacteria suspension: a single colony of biocontrol bacteria was inoculated into LB liquid medium and cultured at 35 °C, 180 rpm for 24 h. Then the fermentation broth was centrifuged at 10,000 rpm for 10 min, the supernatant was discarded and resuspended in sterile water, and the concentration was adjusted to 1×10^8 CFU/mL for further experiments.

Preparation of the pathogen spore suspension: *Colletotrichum falcatum* Went. was inoculated onto solid PDA medium and incubated at 28 °C for 7 days. A sterile punch ($d=5$ mm) was used to remove a plug from the edge of the pathogen colony and transfer it to the potato liquid culture solution. The culture was then incubated for 7 days at 28 °C with shaking at 180 rpm, and filtered through a double layer of sterile gauze to obtain a suspension of pathogen spores. The spores were enumerated under a microscope with a hemocytometer plate, and diluted with sterile water to a concentration of 1×10^7 conidia/mL.

Experimental design and sampling

Experimental design

The experiment was conducted in a greenhouse at the Sugarcane Research Institute of the Yunnan Agricultural University (102° 45′ E, 25° 8′ N). Aseptically propagated tissue culture seedlings (ROC22) were transplanted into a 23×20 cm plastic flowerpot. Experiments were conducted with four treatments: (1) blank control with only

watering (CK), (2) inoculation with only the biocontrol bacterium YC89 (Y), (3) inoculation with the biocontrol bacterial solution YC89 inoculated with *C. falcatum* (YC), and (4) inoculation with *C. falcatum* alone (C). Under the condition of continuous growth of sugarcane, the treatment group inoculated with biocontrol bacteria was inoculated with *B. velezensis* YC89 suspension (1×10^8 CFU/mL) by root-irrigation method, and the biocontrol bacteria were inoculated three times during the whole test stage. For the pathogen infection group, *C. falcatum* suspension (1×10^7 conidia/mL) was inoculated using the root-irrigation method 3 days after inoculation with biocontrol bacteria. Each treatment consisted of 10 pots with three replications, and one seedling was planted in each pot and placed in a greenhouse for unified management and watering at an appropriate time.

Rhizosphere soil collection

Samples were collected 30 days after inoculation with *C. falcatum.* The potted sugarcane was uprooted, large pieces of soil were shaken off, and the soil adhering to the rhizosphere of the sugarcane was considered rhizosphere soil. Three replicates were performed for each treatment. Each soil sample was sieved through a 2 mm sieve and set aside. A total of 36 rhizosphere soil samples (4 treatments × 3 pots × 3 replicates) were collected, and three rhizosphere samples were randomly combined into one sample. Each sample was divided into three parts: one part was used to determine soil enzyme activity, the second part was used for metagenomic sequencing, and the remaining part was used for rhizosphere microbial isolation.

Root exudate collection

After collecting the rhizosphere soil, the roots of sugarcane plants were rinsed with deionized water three times, wrapped with filter paper, and added into 50 mL centrifuge tubes. The tubes were sealed with sealing film, and the sugarcane was buried back in the original pot. After 48 h of treatment, the 50 mL tube was taken out to the laboratory, and then the root covered with filter paper was taken out and put into a conical flask containing 100 mL sterile deionized water, and shaken for 30 min, filtered with a vacuum pump, and preserved at − 80 °C for further analyses [21].

Determination of rhizosphere soil enzyme activity

Activities of soil catalase (S-CAT), solid cellulase (S-CL), soil acid phosphatase (S-ACP), and soil urease (S-UE) in the rhizosphere were determined using soil enzyme activity quantification detection kits (Suzhou Grius Biotechnology Co., Ltd., Suzhou, China).

Rhizosphere soil DNA extraction and high-throughput sequencing

Rhizosphere soil was collected, and DNA was extracted using a Power Soil DNA kit (MO BIO Laboratories) following the manufacturer's instructions. DNA concentration was measured using a Qubit® 2.0 fluorometer (Life Technologies, CA, USA). For each sample, 1 μg DNA was taken for metagenomic library preparation. The NEBNext® UltraTM DNA Library Preparation Kit for Illumina (NEB, USA) was used as sequencing library generation according to the manufacturer's instructions. Sequencing was performed using a PacBio RS II platform at Metware Biotechnology Co. (Wuhan, China).

Default software parameters were set and raw data quality control was performed using Fastp software to preprocess raw data obtained from the Illumina Hi Seq sequencing platform to obtain clean data for subsequent analysis. Following quality control, metagenomic assembly was performed using the clean data, and the unused reads of each sample were mixed together for assembly. To better detect the low abundance species information in the sample, Meta Gene Mark was used for gene prediction starting from the continuous cloning and mixed assembly of a single sample. Gene catalogs were constructed by putting together the predicted genes and hybrid assemblies for each sample, removing redundancies [22]. The metagenomic analysis are described in the Supplementary Information.

Root exudates metabolome assay

The collected sugarcane root exudates were removed from the refrigerator at − 80 °C and then vacuum freeze-dried. After freeze-drying, 70% methanol containing internal standard extract was added according to the ratio of 30 times of concentration (for example, 300 μL extractant was added to 9 mL sample after freeze-drying, and 200 μL extractant was added to 6 mL sample after freeze-drying). After vortexing for 15 min, sonicate for 10 min in an ice-water bath and centrifuge for 3 min at 4 °C at 12,000 r/min. The supernatant was filtered with 0.22 μm microporous membrane and stored in the injection bottle for LC–MS / MS detection.

The sample extracts were analyzed using a system (UPLC, ExionLC™ AD, https://sciex.com.cn/) and a tandem mass spectrometry system (https://sciex.com.cn/). The UPLC conditions were as follows: the column as Agilent SB-C18 (1.8 µm, 2.1 mm × 100 mm),

column temperature 40 °C, injection volume 2 μL, flow rate was 0.35 mL/min. The separation of sugarcane root secretion fractions was carried out using gradient elution with the following elution procedure: at 0.00 min the solution B was 5%, the solution B increased linearly to 95% within 9.00 min and was maintained at 95% for 1 min, and from 10.00 to 11.10 min, the solution B was reduced to 5% and equilibrated at 5% for up to 14 min. Comparison of mass spectrometry databases to determine composition [23]. Metabolome sequencing was performed by Metware Biotechnology (Wuhan, China). The metabolomic analysis are described in the Supplementary Information.

Isolation of culturable rhizosphere microorganisms and screening of antagonistic strains

Next, 100 μL of the suspension was diluted to the appropriate concentration and applied to different bacterial media (LB and NB) using a sterile applicator. The petri dishes were incubated at 35 °C for 2–3 days. Single colonies were picked for purification. The antagonistic strains against sugarcane red rot were screened using the plate confrontation culture method. A pathogen was inoculated at the center of the PDA medium plate with a diameter of 5 mm, and purified bacteria were inoculated at a distance of 30 mm from the center. The bacteria were cultured at 28 °C for 5 days, and each treatment was repeated three times. When the control mycelia grew over the plate, the diameters of the control and treatment groups were measured using the cross method, and the bacteriostatic rate was calculated according to the following formula:

$$\text{Inhibition rate} = \left[\left(\text{diameter of control colony} - \text{diameter of treated colony}\right) / \text{diameter of control colony}\right] \times 100\%.$$

Biocontrol effect of dominant rhizospheric strains on sugarcane red rot in the greenhouse

Four- and one-heart-large plants were inoculated with *B. velezensis* YC89, *Streptomyces* sp., *Sphingomonas* sp., and *Pseudomonas* sp., or a synthetic community composed of these four bacteria (Syc), all strains at a concentration of 1×10^8 CFU/mL, and the control group was water only. Subsequently, 1 week after inoculation with bacteria, the *C. falcatum* (1×10^7 conidia/mL) was inoculated using the root irrigation method. Samples were collected at 0, 1, 3, 5, and 7 days after inoculation to determine the levels of phenylalanine ammonia-lyase (PAL), polyphenol oxidase (PPO), superoxide dismutase (SOD), chitinase activity, jasmonic acid (JA), and salicylic acid (SA). The average disease index and control efficacy were determined 7 days after inoculation with the pathogen according to the morbidity class grading criteria [24]:

$$\text{Disease index} = \left[\sum \left(\text{number of diseased plants in this grade} \times \text{disease grade}\right) / \left(\text{total number of plants investigated} \times \text{the highest disease grade}\right)\right] \times 100.$$

$$\text{Control efficacy (\%)} = \left[\left(\text{disease index of control} - \text{disease index of treated group}\right) / \text{disease index of control}\right] \times 100.$$

Statistical analysis

Statistical analyses were performed using SPSS 20.0 (IBM Corporation, New York, USA). $P < 0.05$ was considered statistically significant for all tests.

Results

Determination of soil enzyme activity

As shown in Fig. S1, the catalase activity with YC89 treatment was 24.5% and 26.66% higher than that of CK and *C. falcatum*, respectively. However, no significant difference was observed in rhizosphere acid phosphatase activity after inoculation with YC89 alone compared with the CK, but it was significantly higher than that of YC treatment group and C treatment group, indicating that inoculation with pathogens significantly reduced sugarcane rhizosphere acid phosphatase activity. Furthermore, no significant difference was observed in urease activity between the Y treatments and YC treatments; however, it was significantly higher than that in the CK and inoculated pathogen groups. The cellulase activity of sugarcane rhizosphere soil with YC treatments was significantly higher than that of the other three treatments; in particular, it was 83.34% higher than that of *C. falcatum* alone.

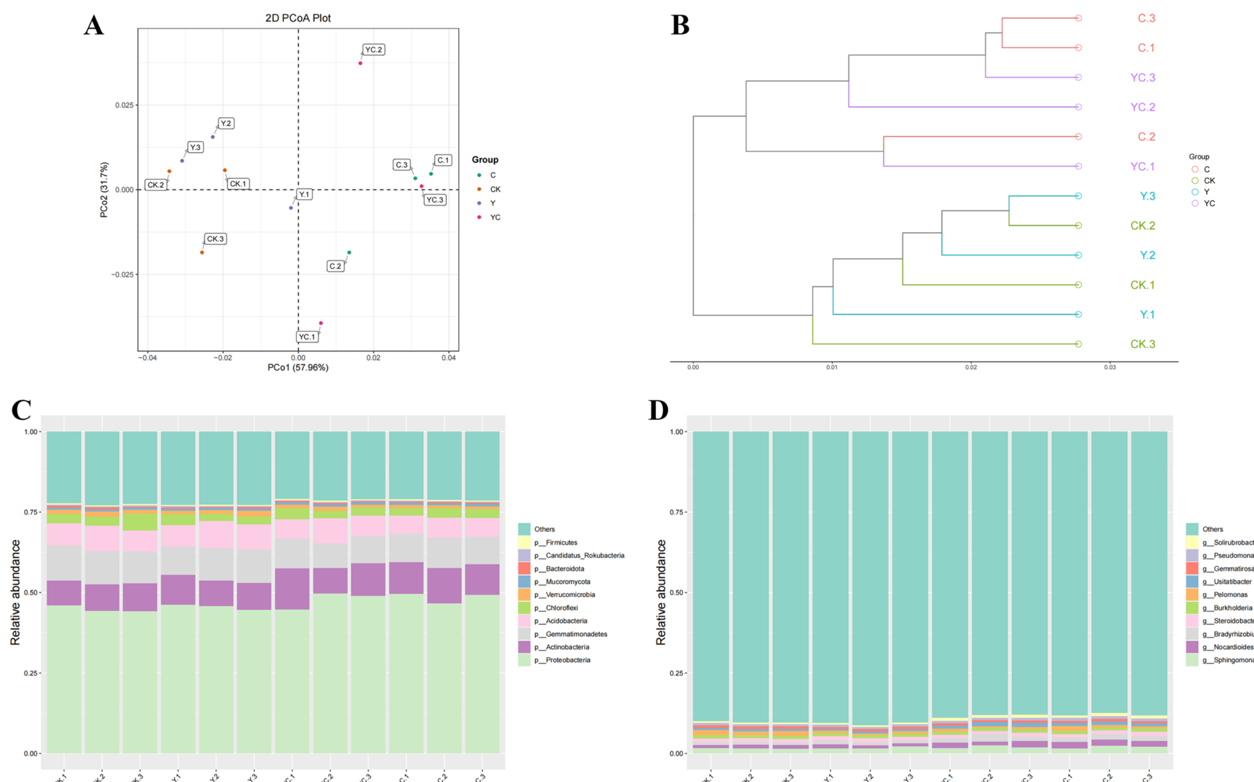


Fig. 1 Compositions of rhizosphere bacterial communities under different treatments. **A** PCoA dimensionality reduction analysis based on the Bray–Curtis distance of species abundance. **B** Cluster analysis between samples based on the Bray–Curtis distance of species abundance. **C** Relative abundances of major phyla present in the bacterial communities under different treatments. **D** Relative abundances of major genera present in the bacterial communities under different treatments

Rhizosphere microbial community compositions

Bray–Curtis-based PCoA showed significant differences in the composition of rhizosphere bacterial communities among the treatments, PCoA1contributes 57.96% and PCoA2 contributes 31.7% (Fig. 1A). The results of sample hierarchical clustering indicated that the sugarcane rhizosphere community could be divided into two significantly different taxa, where groups YC and C and groups CK and Y clustered together (Fig. 1B). Inoculation with *B. velezensis* YC89 and pathogens increased the α-diversity of the rhizosphere bacterial community (Table S1), and the Shannon index following the YC treatment was the highest.

The relative abundance levels of species-annotated phyla showed that Proteobacteria, Actinobacteria, Gemmatimonadetes, Acidobacteria, and Chloroflexi were the dominant phyla in each treatment group (Fig. 1C). Proteobacteria and Actinobacteria were more abundant in the YC and C groups than in the CK and Y groups. Acidobacteria and Gemmatimonadetes showed higher relative abundances in the CK and Y groups than in the YC and C groups. The relative abundance of Firmicutes was higher in the YC group than in the other three groups, and the relative abundance of Chloroflexi was higher in the CK group than in the other three treatment groups (Fig. S2). At the genus level, *Sphingomonas*, *Nocardioides*, *Bradyrhizobium*, *Steroidobacter*, and *Burkholderia* were the dominant genera in the rhizosphere following each treatment (Fig. 1D). The abundances of *Burkholderia*, *Sphingomonas*, and *Pseudomonas* were higher in the YC and C groups than in the CK group. The abundances of *Streptomyces* and *Bacillus* were higher in the YC group than in the other treatment groups. The relative abundance of *Colletotrichum* in the Y and YC groups was lower than that in the CK and C groups, with the Y groups exhibiting the lowest relative abundance, suggesting that inoculation with *B. velezensis* YC89 reduced the abundance of *Colletotrichum* (Fig. S3).

To further analyze the bacterial composition in the different treatments, heat maps of the first 35 genera from the 4 treatments were analyzed (Fig. S4). The bacterial compositions of the four treatments differed significantly in terms of dominant genera and their

abundance. The relative abundance of *Pelomonas*, *Gemmatirosa* spp., *Gemmatimonas* spp. and *Rubrivivax* in CK was significantly higher than the other treatments, it indicated that these strains may be indigenous microorganisms in sugarcane rhizosphere. The relative abundance of *Povalibacter* spp., *Steroidobacter* spp. and *Gaiella* spp. in the rhizosphere of sugarcane inoculated with *B. velezensis* YC89 alone was higher than that of other treatment groups. The relative abundance of *Streptomyces*, *Burkholderia*, *Sphingomonas* and *Rhizobium* increased significantly in YC treatments. *Azotobacter* and *Pseudomonas* were significantly enriched in the treatment group inoculated with pathogens alone. The results showed that the microbial community structure was significantly changed after inoculation with biocontrol bacteria and pathogens.

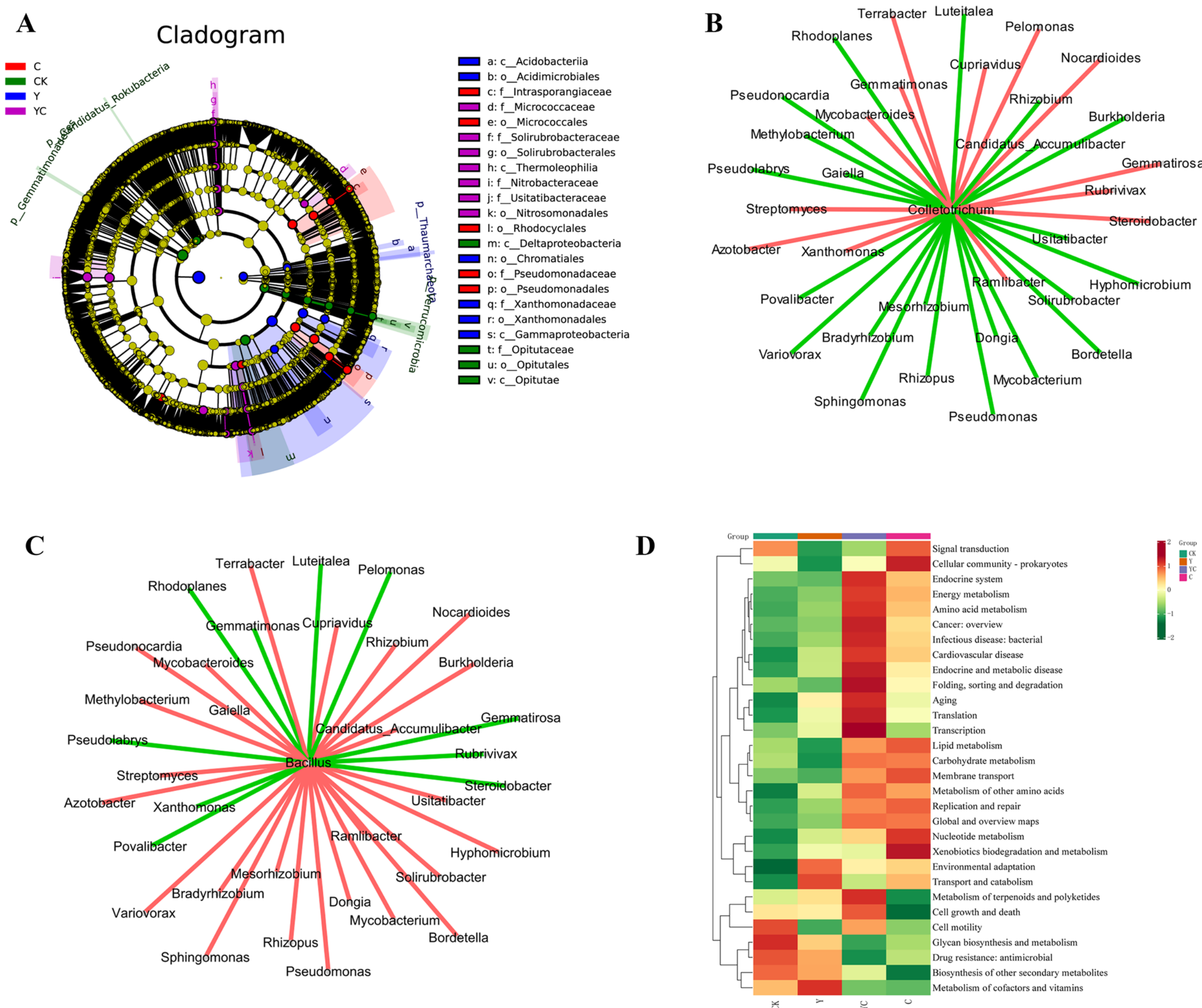


Fig. 2 Taxonomic and functional characteristics of the rhizosphere bacterial community after inoculation with *B. velezensis* YC89 and *C. falcatum*. **A** Least discriminant analysis (LDA) effect size classification spectrogram comparing bacteria under different treatments. Different taxonomic classes are mentioned from inside to outside. Red nodes indicate abundant species in rhizosphere inoculated with *C. falcatum*, blue nodes indicate abundant species in rhizosphere inoculated with YC89, green nodes indicate abundant species in the rhizosphere of CK, purple nodes indicate abundant species in the rhizosphere inoculated with YC89 and *C. falcatum,* and yellow nodes indicate no difference. **B** Relationship between root dominant bacterial genera and *Colletotrichum* calculated using the Spearman correlation method. Red indicates a positive correlation, and green indicates a negative correlation. **C** Relationships between root dominant bacterial genera and *Bacillus* calculated using Spearman's correlation method. Red indicates a positive correlation, and green indicates a negative correlation. **D** Rhizosphere functional profiles. The color scale represents enrichment or reduction of the predicted function

Functional characteristics of differential species and bacterial communities

Opitutaceae and Deltaproteobacteria were significantly enriched in the CK group, indicating that Opitutaceae and Deltaproteobacteria were the inherent flora of the sugarcane rhizosphere in this study. Pseudomonadaceae, Micrococcales, Intrasporangiaceae, and Rhodocyclales were significantly enriched in the rhizosphere after inoculation with *C. falcatum* alone. Acidimicrobiales, Chromatiales, Xanthomonadaceae, and Gammaproteobacteria were significantly enriched in the sugarcane rhizosphere after inoculation with YC89 alone. Micrococcaceae, Solirubrobacteraceae, Thermoleophilia, and Nitrobacteraceae were significantly enriched in the rhizosphere of sugarcane after inoculation with YC89 and *C. falcatum* (Fig. 2A). Notably, the abundance of strains of Burkholderiales, Micrococcales, Nitrosomonadales, Pseudomonadales, and Solirubrobacterales was significantly increased under YC treatment than under YC89 inoculation alone. Furthermore, the abundance of strains of Rhodanobacteraceae, Caulobacteraceae, Hyphomonadaceae, Kaistiaceae, and Rhodospirillaceae was significantly increased under YC treatment compared with that under *C. falcatum* treatment (Fig. S5). To further analyze the relationship between rhizosphere microorganisms and pathogenic and biocontrol bacteria, we analyzed the relationships between the top 35 dominant genera and *Colletotrichum* and *Bacillus*. The abundances of *Sphingomonas*, *Pseudomonas*, *Burkholderia,* and *Xanthomonas* were negatively correlated with *Colletotrichum* but positively correlated with *Bacillus* (Fig. 2B, C).

To investigate whether the bacteria recruited in each treatment altered the functional profile of the bacterial community, unigenes were compared to each functional database using the DIAMOND software. The KEGG sub-functional clustering heatmap (Fig. 2D) showed that microbial functional structures were similar in the CK and Y groups and YC and C groups. The functions of rhizosphere microbial biomarkers of pathogenic inoculation with *B. velezensis* YC89 were related to "Endocrine system," "Energy metabolism," "Transcription," "Folding, sorting and degradation," and "Amino acid metabolism." The functions of rhizosphere microbial biomarkers inoculated with YC89 alone were related to the "Metabolism of cofactors and vitamins," "Biosynthesis of other secondary metabolites," "Transport and catabolism," and "Environmental adaptation." Inoculation of *C. falcatum* with rhizosphere microbial biomarkers was associated with "Signal transduction," "Cellular community-prokaryotes," "Lipid metabolism," "Nucleotide metabolism," and "Xenobiotis biodegradation and metabolism."

Metabolomic analysis of root exudates

Principal component and component analysis of metabolites in root exudates

LC–MS/MS was used to detect 1382 metabolites from 12 sugarcane root exudates. Metabolomic data were evaluated using PCA of the predicted compounds. The first two principal components explained 32.41% and 14.11% of the variance (Fig. 3A), it can be seen from the figure that the different treatment groups are distributed in different quadrants, indicating that there are significant differences between the treatments, but the differences between the YC and C groups are small. The 1382 metabolites mainly belonged to 12 classes, of which flavonoids, phenolic acids, lipids, and alkaloids accounted for 17.08%, 16.06%, 12.23%, and 9.26% of the total metabolites, respectively (Fig. 3B). OPLS-DA showed a clear separation of the treatment groups from the CK group (Fig. S6). Based on the OPLS-DA results (VIP > 1.0, FC ≥ 2,

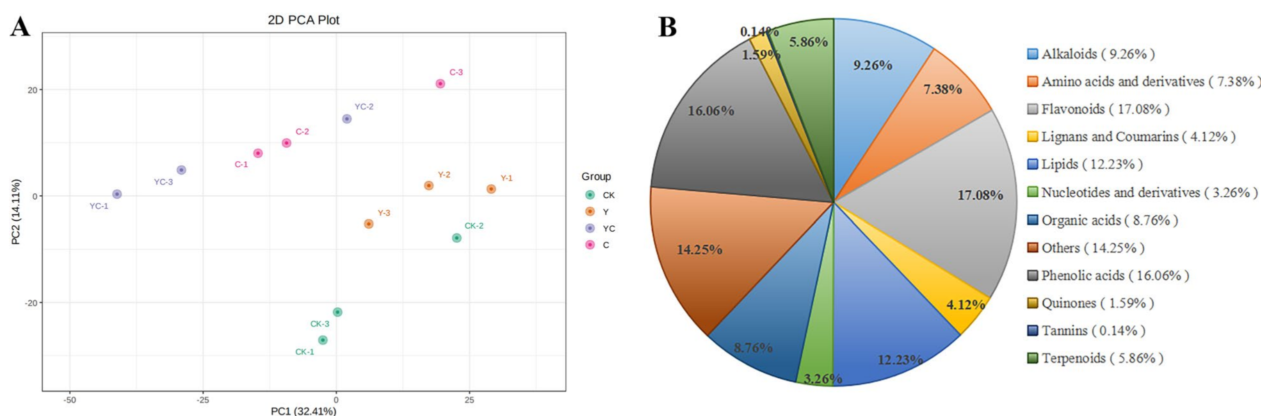


Fig. 3 Effects of the inoculation with *B. velezensis* YC89 and *C. falcatum* on the metabolism of sugarcane root exudates. **A** Principal component analysis. **B** Chemical classification of all identified metabolites

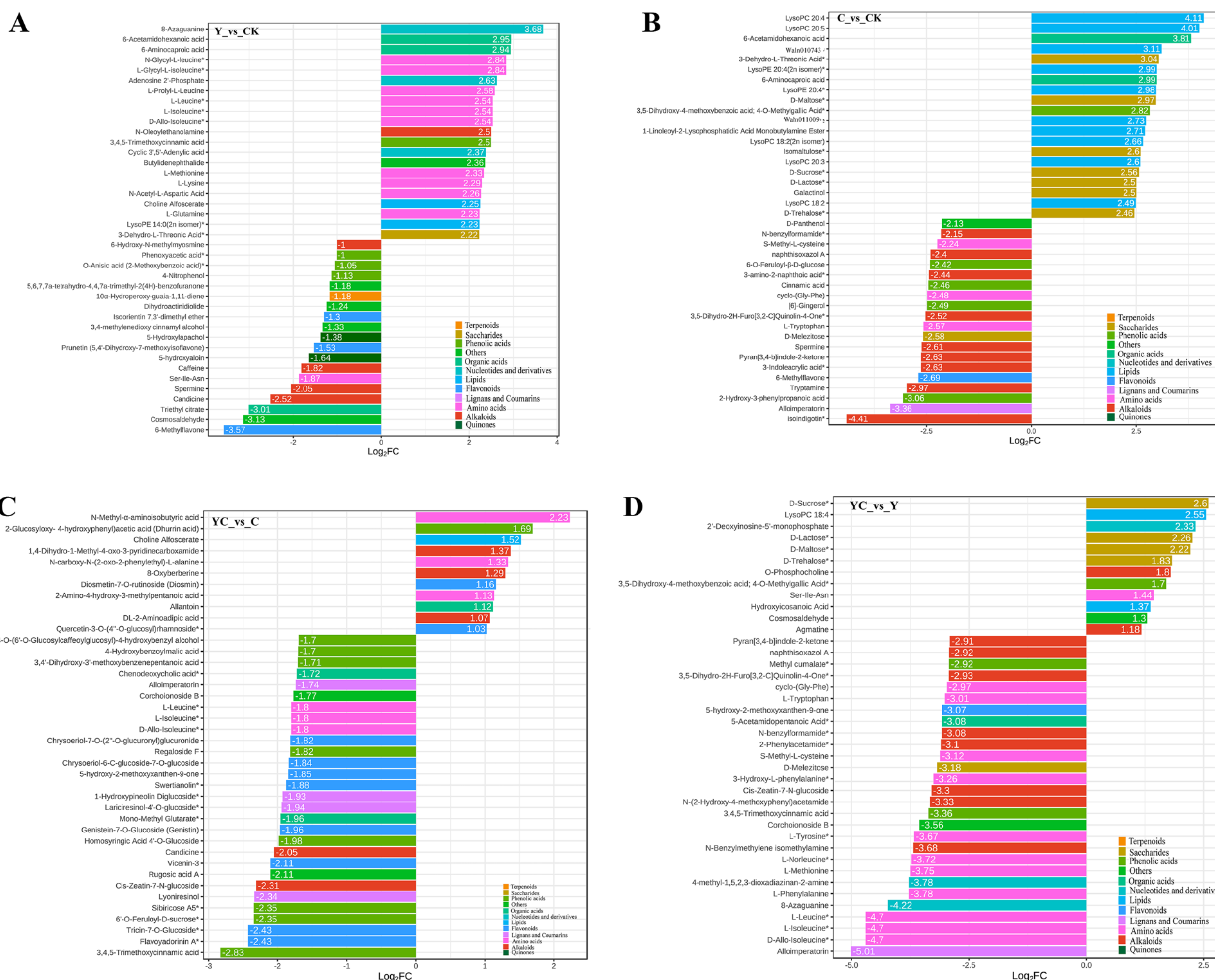


Fig. 4 Difference fold analysis of differential metabolites in different comparison groups. **A** Difference multiple analysis of differential metabolites in the comparison group of Y and CK; **B** Difference multiple analysis of differential metabolites in the comparison group of C and CK; **C** Difference multiple analysis of differential metabolites in the comparison group of YC and C; **D** Difference multiple analysis of differential metabolites in the comparison group of C and CK. Different colours indicate different classes of compounds

and FC ≤ 0.5), a total of 694 differentially expressed root exudates were identified among the different groups of metabolites (Supplementary Data 1).

Response of sugarcane root exudates to B. velezensis YC89 and C. falcatum

Compared with that in the CK, the content of amino acids such as L-leucine, L-isoleucine, L-methionine, and L-lysine was significantly increased following inoculation with *B. velezensis* YC89, whereas the contents of phenolic acids such as phenoxyacetic acid, O-anisic acid (2-methoxybenzoic acid), and 4-nitrophenol significantly decreased (Fig. 4A). Inoculation with *C. falcatum* resulted in a significant increase in the contents of lipids, such as LysoPC20:4, LysoPC20:5, and LysoPE20:4 (2nisomer), and a decrease in the contents of alkaloids, such as isoindigotin, tryptamine, and 3-indoleacrylic acid (Fig. 4B). Compared with the inoculation with *C. falcatum* alone, that with *B. velezensis* YC89 followed by *C. falcatum* resulted in significantly increased contents of root exudates such as *N*-methyl-a-aminoisobutyric acid, 2-glucosyloxy-4-hydroxyphenyl acetic acid (Dhurrin acid), and choline alfoscerate, whereas the contents of phenolic acids such as 3,4,5-trimethoxycinnamic acid, 6’-O-feruloyl-D-sucrose, and sibiricose A5, and flavonoids such as flavoyadorinin A, tricin-7-O-glucoside, and vicenin-3 were decreased (Fig. 4C). Compared with the inoculation with *B. velezensis* YC89 alone, that with *B. velezensis* YC89 followed by *C. falcatum* resulted in an increase in the contents of saccharides, such as

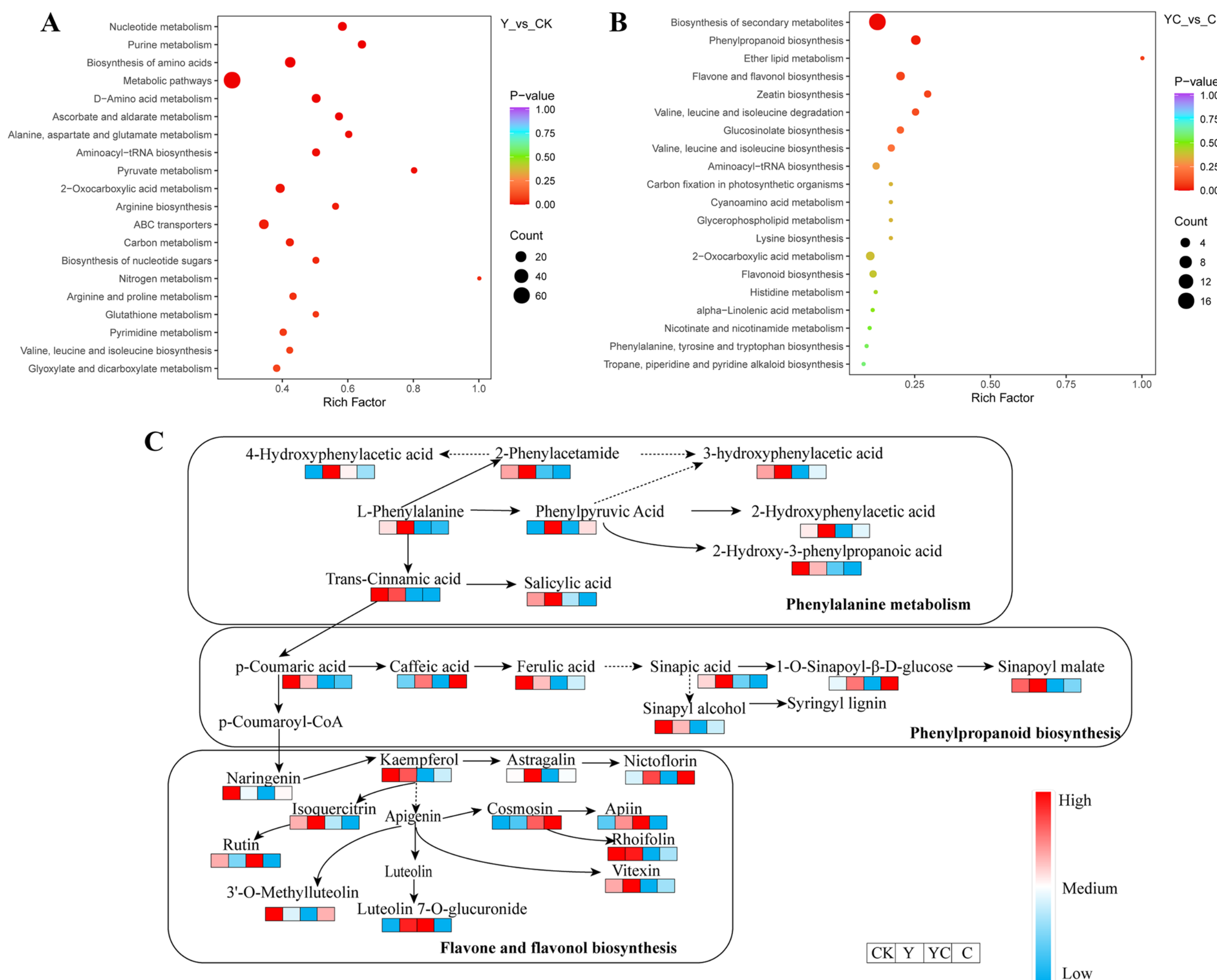


Fig. 5 Differential metabolite KEGG pathway enrichment and key metabolic pathways. **A** KEGG functional enrichment of differential metabolites between the Y and CK groups. **B** KEGG functional enrichment of differential metabolites between the YC and C groups. **C** Analysis of key metabolic pathways

D-sucrose, D-lactose, and D-maltose, and a decrease in the contents of amino acids, such as L-leucine, L-isoleucine, and D-allo-isoleucine (Fig. 4D).

KEGG enrichment of differential metabolites and analysis of key metabolic pathways

KEGG enrichment analysis was performed to explore the potential effects of differential metabolites. The differential metabolites between the Y and CK groups showed relatively high enrichment in metabolic pathways, amino acid biosynthesis, and ABC transporters (Fig. 5A). The differential metabolites between YC and C showed relatively high enrichment in the biosynthesis of secondary metabolites, phenylpropanoids, flavone, and flavonol (Fig. 5B). Notably, phenylpropanoids, flavonoids, plant hormones, flavones, and flavonols are associated with enhanced plant resistance.

Based on the results of the KEGG analysis, three key metabolic pathways related to disease resistance (phenylalanine metabolism, phenylpropanoid biosynthesis, and flavonoid and flavonol biosynthesis) were further analyzed (Fig. 5C). Treatment with YC89 alone significantly increased the expression of l-phenylalanine, phenylpyruvic acid, trans-cinnamic acid, 2-phenylacetamide, sinapic acid, sinapoyl malate, astragalin, rhoifolin, vitexin, and other metabolites in these three metabolic pathways. However, the levels of these metabolites were significantly reduced in the YC and C groups. The contents of caffeic acid, 1-O-sinapoyl-β-D-glucose, nicotiflorin, and cosmosin in the treatment group inoculated with the

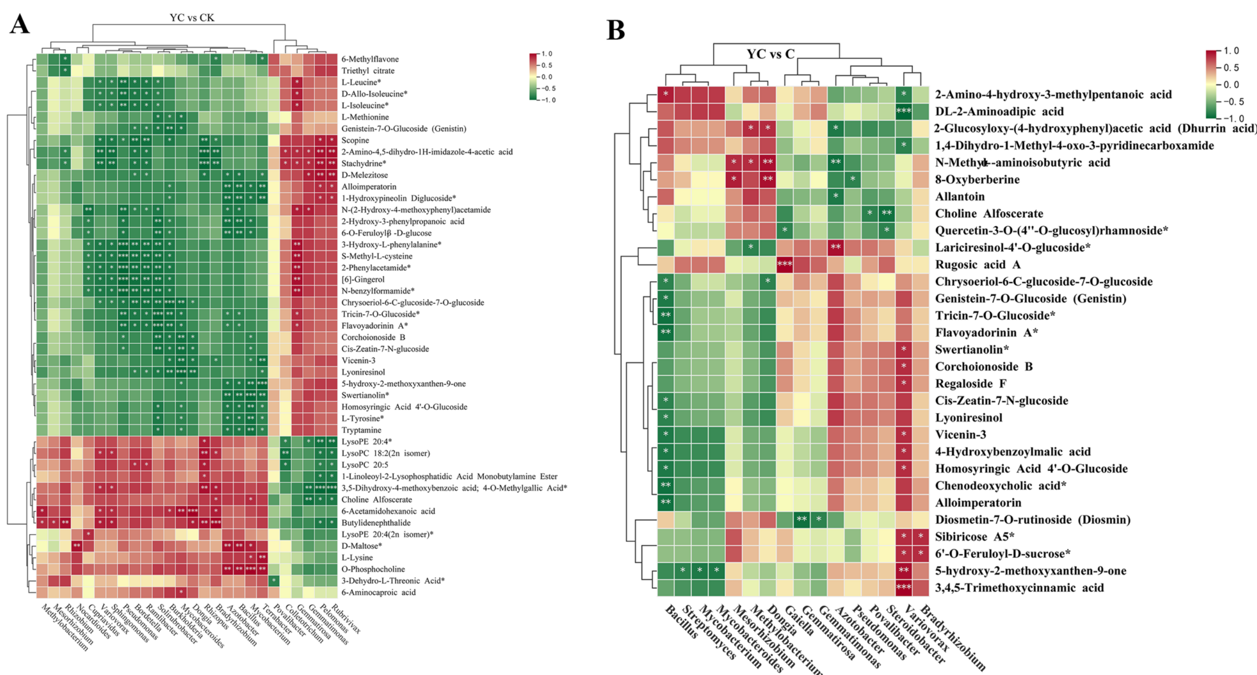


Fig. 6 Correlation between root exudates and several root microorganisms. **A** Heat map analysis of the correlation between differential metabolites and the relative abundance of rhizosphere-dominant microorganisms between the YC and CK groups. **B** Heat map analysis of the correlation between differential metabolites and the relative abundance of rhizosphere-dominant microorganisms between the YC and C groups. The horizontal axis represents the name of the genus of microorganisms in the soil, the vertical axis represents different metabolites, and color depth represents the correlation strength. Significance of each metabolite: *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$

pathogen alone were significantly higher than those in other treatment groups, indicating that these metabolites may be related to plant susceptibility. The apiin, rutin, salicylic acid, and luteolin 7-O-glucuronide contents in the YC group were higher than those in the C group, indicating that these substances play an important role in biocontrol and disease resistance.

Root exudates and microbial association analysis

Figure 6 shows the relationship between inoculated *B. velezensis* YC89 and *C. falcatum* rhizosphere microorganisms and differential metabolites. The abundance of *Bacillus* was positively correlated with the upregulated metabolites and negatively correlated with the downregulated metabolites. The abundances of *Sphingomonas*, *Bordetella*, *Pseudomonas*, and *Burkholderia* were significantly correlated with root exudates. The abundance of *Bacillus* was significantly positively correlated with saccharides (D-maltose) and alkaloids (O-phosphocholine) (Fig. 6A), and significantly negatively correlated with phenolic acids (4-hydroxybenzoylmalic acid, homosyringic acid, 4′-O-glucoside, chenodeoxycholic acid) (Fig. 6B).

Effects of rhizosphere dominant strains on sugarcane red rot

Screening and identification of rhizosphere beneficial antagonistic strains

A total of 174 bacterial strains were isolated from the rhizosphere soil of the YC and C groups using traditional isolation and culture techniques. Among them, 24 strains with antagonistic effects against *C. falcatum* were screened using the plate standoff method (Table S2). Based on morphological characteristics and molecular identification, three potential rhizosphere strains (YC37, C62, and P6) were selected for subsequent experiments. Notably, YC37 and P6 exerted antagonistic effects on *C. falcatum*, and C62 had no antagonistic effect on *C. falcatum* mycelia. The three YC37, C62, and P6 strains were identified as *Streptomyces*, *Sphingomonas*, and *Pseudomonas*, respectively (Fig. 7).

Effects of rhizosphere dominant strains on sugarcane induced systemic resistance (ISR)

To determine the effect of different isolates on sugarcane ISR, JA and SA contents and disease-related enzyme activity were measured at different time points after inoculation with *C. falcatum*. We found that all strains

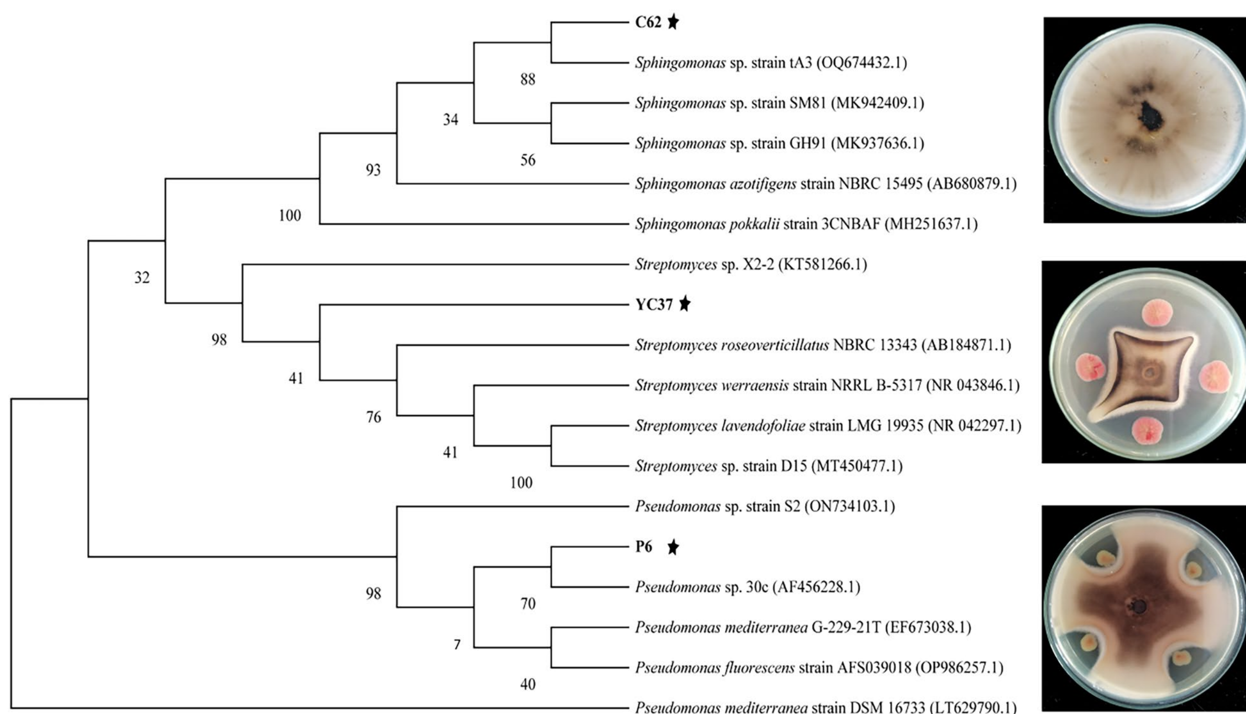


Fig. 7 Effects of potential dominant strains in rhizosphere on *C. falcatum* and their identification

significantly increased JA and SA content in plants. Although the JA and SA contents fluctuated with different treatment times, the JA content of plants inoculated with *Sphingomonas* sp. and YC89 was significantly higher than that of *Streptomyces* sp., Scy, and CK, and the SA content of plants inoculated with *Streptomyces* sp., *Sphingomonas* sp., and YC89 was significantly higher than that of CK throughout the study period. The contents of JA and SA in the Scy treatment were significantly higher than those in CK on days 3 to 7 after inoculation (Fig. 8A, B).

All strains showed significantly increased PAL, PPO, SOD, and chitinase activities. The PAL enzyme activity of plants treated with *Streptomyces* sp., *Sphingomonas* sp., and Scy peaked on day 1 after inoculation and was significantly higher than that of the other treatment groups. PAL enzyme activity was lower on day 5 after inoculation with the pathogen, and the enzyme activity following the *Streptomyces* sp., *Pseudomonas* sp., and Syc treatments was not significantly different from that of the CK group (Fig. 8C). The PPO activities of *Streptomyces* sp., *Sphingomonas* sp., and YC89 were significantly higher than those of the CK group during the entire evaluation period. The PPO activity following the Scy treatment peaked at day 7 after inoculation and was significantly higher than that of other treatment groups and 58.77% higher than that of the CK group (Fig. 8D). SOD activity was lower on day 3 after inoculation and higher on days 5 and 7. The SOD activity following Syc treatment peaked at day 5, which was significantly higher than that of other treatment groups and 57.51% higher than that of the CK group. The SOD activity following *Sphingomonas* sp. treatment peaked at day 7 and was 52.61% higher than that of the CK group (Fig. 8E). The chitinase activity of the single inoculated strain treatment group was lower than that of the CK group on day 3 after inoculation, but the chitinase activity following the Scy treatment was significantly higher than that of the CK group throughout the measurement period and peaked at day 7, which was 60.75% higher than that of the CK group (Fig. 8F).

Control and growth-promotion effects of dominant rhizosphere strains on sugarcane red rot disease

In the potting efficacy test, all strains and their synthetic communities significantly reduced the disease index of sugarcane red rot. Notably, the synthetic community was the most effective in controlling red rot, with a relative efficacy of 67.50%, followed by YC89 and *Streptomyces* sp. No significant difference was observed between the YC89 and *Streptomyces* sp. and the synthetic community treatments (Table 1). In addition, all inoculation treatments promoted plant growth. Particularly, *Streptomyces*

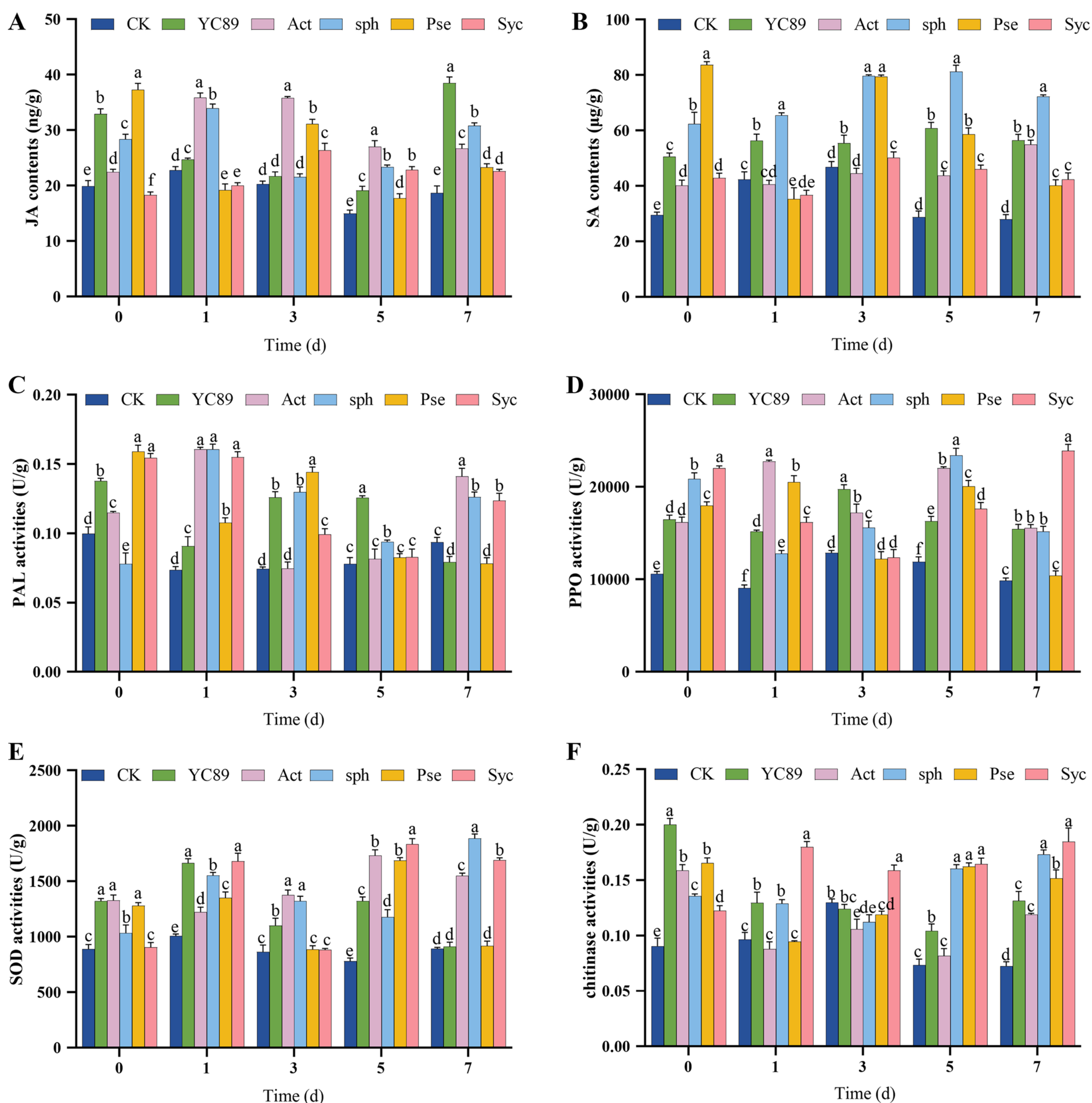


Fig. 8 Effects of different strains on sugarcane ISR. **A** JA content; **B** SA content; **C** PAL enzyme activity assay; **D** PPO activity; **E** SOD enzyme activity; **F** chitinase activity. Note: the bars indicate the mean ± SE (standard error) of three replicates, and different letters indicate significant differences from the other treatments ($p < 0.05$)

sp. and *Sphingomonas* sp. exerted the best growth-promoting effect on sugarcane and significantly increased plant height and stem thickness. Collectively, the potted results indicate that these strains can control the red rot disease of sugarcane and promote the growth of sugarcane plants (Fig. S7).

Discussion

Pseudomonas spp. [25], *Trichoderma* spp. [26] and *Bacillus* spp. [27] are all biocontrol strains with good control effect on sugarcane red rot. At present, most of the studies on biocontrol bacteria against sugarcane red rot focus on the screening of antagonistic strains, but the

Table 1 Efficacy of different treatments against sugarcane red rot and growth promotion evaluation

	Plant height (cm)	Stem diameter (mm)	Disease index	Biocontrol effect
CK	52.80 ± 1.12d	8.82 ± 0.15c	81.33 ± 1.54a	
Syc	67.40 ± 1.00bc	11.22 ± 0.34ab	26.43 ± 0.45c	67.50%
Pse	68.47 ± 1.01bc	10.66 ± 0.36ab	35.72 ± 0.89b	56.08%
YC89	65.33 ± 2.13c	10.69 ± 0.2ab	29.87 ± 1.20c	63.28%
Sph	69.97 ± 0.24ab	10.44 ± 0.29b	39.23 ± 1.82b	51.76%
Act	72.40 ± 0.56a	11.72 ± 0.5a	29.24 ± 1.37c	64.05%

Different letters after the values indicate significant at 0.05 level

research on biocontrol bacteria against sugarcane red rot by changing the rhizosphere microecology of sugarcane has not been reported. Microbial inoculants have been shown to recruit combinations of beneficial taxa such as *Flavobacterium*, *Pseudomonas*, *Agrobacterium*, and *Lysobacter* and suppress soil-borne diseases [28, 29]. Yang et al. showed that treatment with the biocontrol bacteria *Bacillus cereus* AR156 significantly increased the relative abundance of 35 genera, including *Bacillus* and *Pseudomonas,* in rhizosphere soil [30]. In this study, the relative abundance of *Streptomyces*, *Burkholderia*, *Sphingomonas* and *Rhizobium* increased significantly in YC treatments, suggesting that the YC89 strain recruited them for enrichment in the rhizosphere of sugarcane (Fig. S4). The interaction between biocontrol bacteria, soil microecology, and plant roots is complex, and the mechanism regulating soil microbial community changes and resistance to soil-borne diseases remains unclear. Therefore, studying the effects of biocontrol strains on plant rhizosphere microbial communities is crucial.

Under various stress conditions, plants regulate the composition of their root exudates to alleviate adverse conditions [31]. In the present study, the differential metabolites in the roots after inoculation with biocontrol bacteria and pathogens were mainly lipids, flavonoids, phenolic acids, and alkaloids, indicating that these metabolites play an important role in plant resistance to disease pathways (Fig. 3). Phenolic acids are important allelochemicals that are often reported to mediate plant fungal diseases. Clocchiatti et al. showed that although phenolic acids have a slight effect on fungal mycelial biomass, they can promote the relative abundance of soil-borne fungi (*Fusarium*, *Trichoderma*, and *Clostridium*) with the ability to invade plant roots [32]. In the present study, phenolic acids such as 4-hydroxybenzoylmalic acid, homosyringic acid 4'-O-glucoside, and 6-O-feruloyl-β-D-glucose were significantly reduced in the root exudates of plants infected with red rot after treatment with *B. velezensis* YC89. Lipids are an important component of the plant plasma membrane and rhizosphere metabolites and help plants adapt to biotic and abiotic stresses [33]. In this study, the content of lipids, such as LysoPC20:4 and LysoPC20:5, increased after inoculation with *C. falcatum* alone, indicating that lipids play an important role in the adaptation of sugarcane plants to biotic stress. Moreover, amino acid contents in sugarcane root exudates increased significantly after inoculation with *B. velezensis* YC89 alone, indicating that *B. velezensis* YC89 enhances the ability of plants to synthesize basic life macromolecules and metabolism.

Root exudates can shape the rhizosphere microbial community, and a specific change in root exudates triggered by plant pathogens is a mechanism of plant defense [34]. Some root exudates, including amino acids, organic acids, flavonoids, alkaloids, and terpenoids, play important roles in shaping rhizosphere microbial communities [35, 36]. Yuan et al. reported that *A. thaliana* leaves secreted more amino acids, nucleic acids, and long-chain organic acids after pathogen (*Pseudomonas syringae* pv tomato) infection, but that the contents of carbohydrates and short-chain organic acids decreased. Furthermore, soil culture experiments showed that adding a mixture of amino acids and long-chain organic acids improved plant disease resistance [37]. Under ginsenoside stress, *Panax notoginseng* activated phenylpropanoid biosynthesis and α-linolenic acid metabolic pathways and increased the content of cinnamic acid in root exudates, which could promote the chemotaxis and growth of *Burkholderia* B36 and enhance its ability to colonize the rhizosphere, resulting in high-density accumulation of B36 in the inter-radial soil, protecting the plant from root rot [38]. In our study showed that there was a significant correlation between differential metabolites and rhizosphere microorganisms. Phenolic acids such as 4-hydroxybenzoyl malic acid, homosyringic acid 4′-O-glucoside and 6-O-feruloyl-D-glucose were negatively correlated with *Bacillus.* This study provides a theoretical basis for the interaction between plants and microbiomes driven by root exudates. However, the roles of specific compounds and strains remain to be further investigated.

Microorganisms, plants, and the environment are complex, multidimensional, and spatiotemporal systems, and whether all bacteria recruited to the rhizosphere can participate in plant disease resistance and synthetic microbial communities (SynComs) serve as an effective approach to better understand the interactions between multiple organisms remains unclear [39, 40]. Ma et al. found that the bacterial community in the roots changed significantly after inoculation with *P. liquidambaris*; for example, the abundance of *Bradyrhizobium* sp. and *Streptomyces* sp. increased significantly, and then the synthetic community (synII) was constructed based on key taxa, the synII can significantly inhibit *F. oxysporum* and increase disease resistance-related enzyme activity [41]. Inoculation with two or more beneficial microbial strains is more beneficial to plants than single-strain inoculation [42]. *Streptomyces* spp. [43], *Sphingomonas* spp. [44] and *Pseudomonas* spp. [45] are potential biocontrol agents. *Streptomyces* spp., *Sphingomonas* spp. and *Pseudomonas* spp. significantly enriched in the rhizosphere of sugarcane after inoculation with biocontrol strain YC89. To explore whether they are involved in plant disease resistance, they were mixed with YC89 strain to construct a synthetic community. The results showed that the synthetic community treatment group significantly improved the relative control effect on sugarcane red rot. This study provides a new control strategy for the prevention and control of sugarcane red rot. Two basic principles are usually adopted for designing SynComs-function-based and interaction-based approaches—where interactions between bacteria play a key role in stability and robustness [46]. In this study, we inoculated only a mixture of the four strains, and further research is needed regarding the interactions among the strains, as well as their stability.

In addition to their role in inhibiting fungal pathogens and promoting plant growth, bacterial communities can activate plant immunity against pathogen infections [47]. Some beneficial rhizosphere bacteria can activate the JA and SA synthesis pathways when interacting with plants. Plants deficient in the JA and SA anabolic pathways are more susceptible to pathogen infestation, whereas increased accumulation of JA and SA significantly reduces morbidity [48, 49]. PAL, PPO, and SOD are key enzymes in the synthesis and oxidation of plant secondary metabolites and are commonly used as defense-related enzymes for disease resistance evaluation [50]. *B. velezensis* F21 mediated the role of ISR in watermelon *Fusarium* wilt and significantly increased defense-related enzyme activities (CAT, POD, and SOD) compared with a control treatment [51]. In this study, inoculation with *B. velezensis* YC89 and the three rhizosphere-dominant strains significantly increased the contents of SA and JA and the activities of SOD, PPO, PAL, and chitinase, indicating that the strains could induce systemic resistance to plant diseases. *Sphingomonas*, as a resident genus of soil bacteria, exhibits a close relationship with plants and helps them to cope with various stressors [52]. *Sphingomonas* strain HJY enhances plant resistance to pesticides

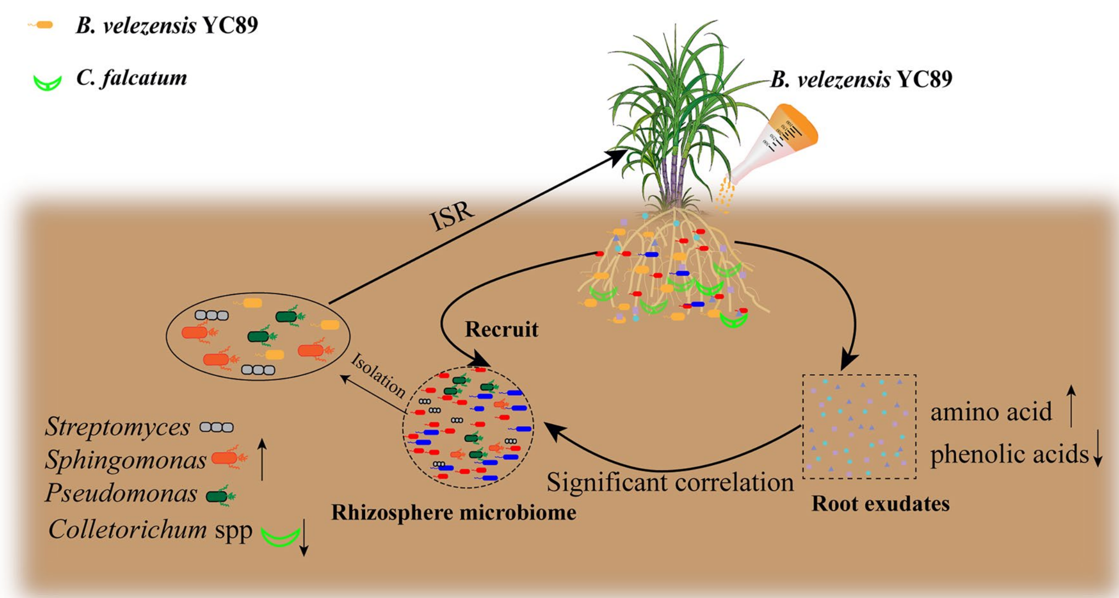


Fig. 9 Mechanistic model of *B. velezensis* YC89 mediating sugarcane rhizosphere microecology against red rot disease

by activating plant defense enzymes [53]. The endophytic bacterium *Sphingomonas melonis* can accumulate in resistant rice seeds, pass from generation to generation, and produce anthranilic acid, making highly susceptible varieties more resistant [44]. In this study, although *Sphingomonas* sp. did not have a direct inhibitory effect on *C. falcatum*, it significantly increased the SA and JA contents and activated ISR resistance-related enzyme activities, indicating that *Sphingomonas* sp. can resist disease through ISR. These three rhizosphere microorganisms have a certain control effect on sugarcane red rot in the pot control effect test, but the biocontrol mechanism of the strains and the field test need to be further studied.

Conclusion

Inoculation with the *B. velezensis* YC89 and pathogens, can significantly change the diversity of rhizosphere bacteria and the composition of the root exudate metabolome. *B. velezensis* YC89 can recruit beneficial rhizosphere microorganisms to enrich the rhizosphere and can also change the contents of some phenolic acids and flavonoids in root exudates. Spearman correlation analysis showed that there was a significant correlation between differential metabolites and rhizosphere microorganisms. To further explore the effects of *B. velezensis* YC89 and rhizosphere dominant strains on sugarcane red rot, rhizosphere microorganisms were isolated and synthetic colonies were constructed. The results showed that both single inoculation and synthetic community could significantly reduce the disease index of red rot and promote the growth of sugarcane plants. These findings provide new insights into the mechanisms underlying plant–microbe interactions that contribute to plant disease resistance (Fig. 9).

Abbreviations

PCoA	Principal coordinate analysis
PCA	Principal component analysis
LEfSe	Linear discriminant analysis effect size
LC–MS/MS	Liquid chromatography–tandem mass spectrometry
OPLS-DA	Orthogonal partial least squares discriminant analysis
VIP	Variable importance in projection
UPLC	Ultra-performance liquid chromatography
PAL	Phenylalanine ammonia-lyase
PPO	Polyphenol oxidase
SOD	Superoxide dismutase
JA	Jasmonic acid
SA	Salicylic acid
KEGG	Kyoto Encyclopedia of Genes and Genomes

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40538-024-00627-4.

Supplementary Material 1. Table S1 Rhizosphere bacterial community α-diversity index. Table S2 The inhibition rate of rhizosphere strains to *C. falcatum* mycelium. Fig. S1 Determination of rhizosphere soil enzyme activity under different treatments. A: Catalase, B: Urease, C: Acid phosphatase, D: Cellulase. Bars and lines represent mean values of three replicates ± SE. A different letter at the head of a column indicates a significant differencefrom other treatments. Fig. S2 Relative abundance of bacterial phylum in sugarcane rhizosphere soil under different treatments. Fig. S3 Relative abundance of soil microbial genera in sugarcane rhizosphere in different treatments. Fig. S4 Heat map of the bacterial community composition with cluster analysis. Similar samples were clustered horizontally, and vertical patterns illustrate the phylogenetic relationships among the top 35 bacterial genera across samples. Fig. S5 LEfSe analysis of species differing between groups.LEfSe analysis of the differential species between YC and C.LEfSe analysis of the differential species between YC and Y. Fig. S6 OPLS-DA scores plots between the different comparison groups. The horizontal coordinate indicates the predicted principal component, with the horizontal direction showing the gap between groups; the vertical coordinate indicates the orthogonal principal component, with the vertical direction showing the gap within groups; and the percentage indicates the rate at which the component explains the dataset. Fig. S7 Effect of dominant strains in rhizosphere on the control effect and growth promotion of sugarcane red rot.

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Author contributions
Fusheng Li, Lilian He, and Linyan Xie designed the experiments. Linyan Xie conducted most of the experiments. Lufeng Liu, Yanju Luo, Xibing Rao and Shaozhi LV performed a small number of experiments and provided experimental methods. Zhenfeng Qian, Yining Di and Hongbo Lou carried out the statistical analyses and organized the data. Linyan Xie wrote the manuscript. Fusheng Li and Lilian He revised the manuscript. All authors contributed to the article and approved the submitted version.

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Availability of data and materials
The relevant data in this study have been uploaded to the NCBI (National Center for Biotechnology Information) SRA database, and the search number is PRJNA1090281.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
All co-authors have seen and agreed on the contents of the manuscript, and there is no financial interest to report.

Competing interests
The authors declare no competing interests.

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