



ORIGINAL ARTICLE

Open Access



Preliminary study on the effects of *Bacillus subtilis* GYH20240327-1 on the microbiome and metabolomics of *Platycodon grandiflorus* and *Scutellaria baicalensis* during growth

Jing He^{1†}, Na Sai^{1†}, Yang Cao^{1†}, Ying Zhang¹, Xiao Qi¹, Ruiwen Shi¹, Fengyu Han², Lingjiang Meng², Xiuling Yu², Yue Zhang¹, Jing Zhao¹, Jianxia Gao¹, Jian Shen^{1*}  and Haibin Guan^{1*} 

Abstract

Objective To investigate the effects of *Bacillus subtilis* GYH20240327-1, isolated from traditional fermented yogurt, on the rhizosphere microbiomics and metabolomics of *Platycodon grandiflorus* and *Scutellaria baicalensis* Georgi.

Methods The *Bacillus subtilis* strain was isolated from yogurt, applied to fertilizer, and used in the planting field. By measuring four apparent plant growth indicators, high-throughput sequencing (16S rRNA and ITS), and LC-MS-based metabolomics analysis of related rhizosphere soil samples, we preliminarily explored and compared the effects of *Bacillus subtilis*-containing fertilizer on the growth and development, microbial communities, and metabolite profiles of two medicinal plants.

Results The results show that fertilization treatment has a certain growth-promoting effect on *Platycodon grandiflorus* and *Scutellaria baicalensis* during the critical three-month period from flowering to harvest, and it also reshapes their rhizosphere microbial communities. In *Platycodon grandiflorus* soil, the abundances of *Acidobacteriota* and *Gemmatimonadota* increased, while *Proteobacteria* decreased. In *Scutellaria baicalensis* soil, *Basidiomycota* increased, whereas *Ascomycota* and *Mortierellomycota* decreased. Metabolomics revealed significant enrichment in pathways for plant hormone biosynthesis, linoleic acid metabolism, biosynthesis of plant secondary metabolites, and vitamin B6 metabolism.

Conclusion *Bacillus subtilis* GYH20240327-1 can effectively promote the growth and development of the rhizosphere of medicinal plants, alter the soil microbial community structure, and affect the composition of plant metabolites, showing its potential as a biofertilizer to improve cultivation efficiency.

[†]Jing He, Na Sai and Yang Cao contributed equally to this work and share first authorship.

*Correspondence:

Jian Shen
20120124@immu.edu.cn
Haibin Guan
20070095@immu.edu.cn

Full list of author information is available at the end of the article



© The Author(s) 2026. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

Keywords *Bacillus subtilis*, Plant growth-promoting rhizobacteria, *Platycodon grandiflorus*, *Scutellaria baicalensis* Georgi, Microbiome, Metabolomics

Introduction

Plant growth-promoting rhizobacteria (PGPR) refer to beneficial rhizobacteria within the soil bacterial community that colonize the narrow zone around plant roots, stimulate plant growth, and reduce the incidence of plant diseases (Bhardwaj et al. 2025; Dong et al. 2025). PGPR can promote plant growth and development, improve nutrient acquisition, enhance soil fertility, and consequently alter plant yield by secreting various metabolites and enzymes, dissolving organic matter, facilitating mineral absorption, fixing nitrogen, generating volatile organic compounds, exhibiting antifungal activity, enhancing plant-microbe symbiosis, and secreting a multitude of substances that indirectly affect overall plant morphology. This makes them environmentally friendly and advantageous alternatives to chemical fertilizers and pesticides (Souza et al. 2015; Lopes et al. 2021; Goginyan et al. 2025). Furthermore, PGPR improve tolerance to abiotic stresses such as drought, salt stress, high and low temperatures, and waterlogging by enhancing plant water retention capacity, producing plant hormones, solubilizing nutrients, performing osmoregulation, activating antioxidant enzymes, and regulating stress-resistant genes (Khan et al. 2020; Shahid et al. 2023; Ali et al. 2024). PGPR also demonstrate excellent potential in bioremediation, utilizing mechanisms such as siderophore production and heavy metal removal, acting as biosurfactants, performing biosorption, exhibiting ACC deaminase activity to reduce ethylene levels, and producing exopolysaccharides and polymeric substances to remediate heavy metal-contaminated environments (Saeed et al. 2021). *Bacillus* and *Pseudomonas* are among the most abundant and phylogenetically diverse genera within PGPR. They are effective rhizosphere colonizers with a broad soil distribution, capable of producing a wide range of metabolites that suppress various pathogens in plants and improve crop yield (Sunita et al. 2003; Gupta et al. 2022). *Bacillus subtilis* (*B. subtilis*) is a Gram-positive bacterium widely found in various environments such as soil, plants, and the digestive tracts of animals. It can synthesize lipopeptides, a class of compounds that exhibit potent antimicrobial activity against various pathogens. As it does not produce endotoxins and is “Generally Recognized As Safe” (GRAS), *B. subtilis* is a probiotic widely used in agriculture, biomedicine, and industrial production (Akinsemolu et al. 2024; Gao et al. 2025a; Gharsallah et al. 2025). A report entitled “Biofertilizers Market By Type, By Microorganism, By Application, By Crop Type, By Form, Global Market Size, Segmental analysis, Regional Overview, Company share

analysis, Leading Company Profiles And Market Forecast, 2025–2035” (<https://www.researchandmarkets.com/>) shows that due to the increasing global demand for sustainable agriculture, the biofertilizer market is growing rapidly. In 2024, the global biofertilizer market size is 2.64 billion USD, and it is expected to reach nearly 9 billion USD by 2035, with the Asia-Pacific region, including India and China, being high-growth areas for the biofertilizer market. According to the product registration data from the Ministry of Agriculture and Rural Affairs of China, there have been 1,646 types of microbial fertilizers registered from 2025 to the present. Among the effective strains, 1,259 types contain *B. subtilis*, accounting for 76.488%. However, most of these microbial fertilizers are suitable for vegetables and grains, followed by fruits, with very few suitable for medicinal plants. For example, the microbial fertilizer (2025) approval number (15773) contains *B. subtilis* and can be applied to *Codonopsis pilosula*, while the microbial fertilizer (2025) approval number (15427) also contains *B. subtilis* and can be used for *Panax ginseng* cultivation (https://iarrp.caas.cn/fljzzjzx/ww_djcp/index.htm#). It can be seen that this study on using *B. subtilis* as a biofertilizer for medicinal plants has practical value and economic significance.

Platycodon grandiflorus (Jacq.) A. DC. (*P. grandiflorus*) is a plant within the genus *Platycodon* of the Campanulaceae family, with a long history of both medicinal and edible use (Ma et al. 2025). *P. grandiflorus* is rich in various bioactive components, mainly including saponins, polysaccharides, flavonoids, phenols, polyacetylenes, and various trace elements (Fan et al. 2025). It possesses multiple pharmacological functions such as anti-tumor (Sun et al. 2025), antimicrobial (Kwon et al. 2025), anti-obesity (Zhi et al. 2025), hepatoprotective (Dang et al. 2025), anti-inflammatory and anti-oxidant (Liu et al. 2023). Additionally, in North Korea, South Korea, Japan, and China, *P. grandiflorus* is a popular traditional pickle. In the modern food industry, *P. grandiflorus* has been developed into more edible forms, such as salads, noodles, preserved fruits, and health beverages (Ji et al. 2020). *Scutellaria baicalensis* Georgi (*S. baicalensis*) is a perennial herbaceous plant and a widely used traditional medicine in China. The root of *S. baicalensis* is its medicinal part, and it is included in the Chinese Pharmacopoeia (CP 2020), British Pharmacopoeia (BP 2018), and European Pharmacopoeia (EP 9.0) (Wu et al. 2025a; Zhang et al. 2025b). *S. baicalensis* mainly contains flavonoid compounds and has various pharmacological effects such as treating respiratory diseases (Shen et al. 2025), anti-tumor (Xiang et al. 2025), antimicrobial (Zhang et

al. 2025c), anti-inflammatory (Kuwar And Kalia 2025), antioxidant (Johnson et al. 2025), nervous protective (Song et al. 2025), preventing abortion (Gao et al. 2025b), treating pulmonary hypertension (Xie et al. 2025), and diabetic nephropathy (An et al. 2025).

In recent years, the rapid development of multi-omics technologies, such as the combination of microbiome and metabolome analysis, has provided new perspectives for the in-depth analysis of plant-microbe interactions. The rhizosphere microbiome can influence the chemical composition and exudation of root systems through systematic signaling mechanisms, thereby affecting the metabolic profile of the plant rhizosphere soil (Korenblum et al. 2020). High-throughput sequencing studies can reveal the composition and structure of the soil microbial community, while metabolomics provides direct evidence of changes in metabolites within the plant rhizosphere soil (Lu et al. 2025; Zhang et al. 2025a). Unraveling the changes in rhizosphere soil microorganisms and metabolites during plant growth and development is crucial for guiding plant production.

Throughout their life cycle, plants are constantly subjected to biotic and abiotic stresses. The technology of using biofertilizers formulated with PGPR contributes to the development of more tolerant and productive plants. This study investigated the effects of applying *B. subtilis* GYH20240327-1, isolated from traditional fermented yogurt of herders, on the metabolic and microbial composition during the growth of *P. grandiflorus* and *S. baicalensis* plants, using high-throughput sequencing and UPLC-MS/MS. The objective of this study was to detect whether there are significant differences in the metabolite profiles and microbial community composition of *P. grandiflorus* and *S. baicalensis* upon application of *B. subtilis* GYH20240327-1. This research will deepen our understanding of the physiological mechanisms arising from bacteria/host associations and provide a theoretical basis for further enhancing the impact of PGPR on plant growth studies.

Materials and methods

Microbial isolation, purification, and identification

Traditional fermented yogurt was purchased from herders' households. According to the "National Standard of the People's Republic of China - Food Microbiological Inspection - Lactic Acid Bacteria Inspection" (GB 4789.35-2023), the strains in the yogurt were cultured using the specified detection and culture methods. Bacterial isolation was then performed using the quadrant streak plate method. Gram staining was conducted to explore the morphological characteristics of the root bacterial isolates under a microscope. After triple purification, biochemical tests including casein hydrolysis, starch hydrolysis, Voges-Proskauer (VP) test, oxidase

test, catalase test, citrate utilization test, gelatin liquefaction test, and sugar fermentation tests were performed. Molecular biological identification was carried out by extracting bacterial genomic DNA using a Bacterial Genomic DNA Extraction Kit (DP302-021, Tiangen Biochemical Technology (Beijing) Co., Ltd.) according to the manufacturer's instructions. The 16S rRNA gene was amplified using self-designed forward primer (5'-AGAG TTTGATCCTGGCTCAG-3') and reverse primer (5'-TACCGTTACCTTGTTACGACTT-3'). The amplification system consisted of: 6 µL bacterial DNA template, 1 µL forward primer, 1 µL reverse primer, 12.5 µL of 2×Taq PCR Master Mix (KT201-01, Tiangen Biochemical Technology (Beijing) Co., Ltd.), and 4.5 µL dd H₂O. Amplification of the 16 S rRNA gene was performed using a PCR amplifier (Model WD-9402D, Beijing Liuyi Biotechnology Co., Ltd.) with an initial denaturation at 94°C for 3 min, followed by 30 cycles of denaturation (94°C, 30 s), annealing (55°C, 30 s), and extension (72°C, 1 min). The PCR amplification products of the 16 S rDNA from strains showing a single band on 1.2% agarose gel electrophoresis were sent to Sangon Biotech (Shanghai) Co., Ltd. for bidirectional sequencing. The returned 16 S rDNA upstream and downstream sequences were assembled using DNAMAN 9 software. The assembled sequences were compared using the Basic Local Alignment Search Tool (BLAST) in the GeneBank database of the National Center for Biotechnology Information (NCBI) to determine the species and name of the strain.

Research location and field experiments

The study was conducted from June 2022 to October 2024 at a *Platycodon grandiflorus* and *Scutellaria baicalensis* cultivation base in Qianmeilihe Village, Meilihe Town, Yuanbaoshan District, Chifeng City, Inner Mongolia Autonomous Region, China (East Longitude 119°09', North Latitude 42°12'). The area is located in a temperate continental monsoon climate zone, with an average annual surface temperature of 6.2°C, an average of -12.5°C in January, and an average of 23.2°C in July. The average annual precipitation is 355 mm, and the annual evaporation ranges between 2000 and 2300 mm. The main soil types include gravel soil, cinnamon soil, and black soil (Liu et al. 2025). Each plot contained 130 *P. grandiflorus* plants or 160 *S. baicalensis* plants. The size of the experimental plots was 4 m², with a spacing of approximately 15 × 20 cm for *P. grandiflorus* and 12 × 20 cm for *S. baicalensis*. Each treatment had three replicates, and the plots were separated and surrounded by at least two rows of buffer zones in each direction.

Production and application of probiotic compound fertilizer

Organic fertilizer refers to commonly used chemical fertilizers, such as compound fertilizer 14–16–15 (New Yangfeng Agricultural Technology Co., Ltd.) and diammonium phosphate 18–46–0 (Yunnan Sanhuan Zhonghua Chemical Fertilizer Co., Ltd.) in a 1:1 ratio. Organic fertilizer was applied once per month from June to September 2023, at a rate of 0.06 kg per m². The composition and proportion of the probiotic compound fertilizer, by parts by weight, are as follows: Probacterial agent (*B. subtilis*): Chitosan: Humic acid: Calcium chloride: Cellulase = 10: 3: 14: 2: 1. The biofertilizer was applied once per month from June to September 2023, at a rate of 0.6 kg per m².

Soil sampling

Soil samples were collected in mid-October locally using the diagonal method, with random sampling from five points. Soil from the rhizosphere (1–2 mm around the roots), root surfaces, and adhering soil was obtained using the soil drilling method. Debris such as foreign matter and plant root residues was removed, and the samples were mixed thoroughly, placed into sample bags, and stored for subsequent high-throughput sequencing of 16S rDNA and ITS amplicons and metabolomics analysis.

Measurement of plant growth indicators

Each type of plant was set up with a control group (no fertilizer applied), an organic fertilizer group, and an organic fertilizer+composite biofertilizer group. During the harvesting of medicinal plants, the height of the aboveground parts and the root length of *P. grandiflorus* and *S. baicalensis* were measured. The roots of *P. grandiflorus* and *S. baicalensis* were cleaned according to the 2020 edition of the Chinese Pharmacopoeia. The fresh weight of the roots was measured, and the medicinal materials were dried according to the drying procedures described in the Pharmacopoeia to determine the dry weight of the medicinal materials.

Microbial diversity analysis

Soil microbial DNA extraction and sequencing

Total genomic DNA was extracted from soil samples using the cetyltrimethylammonium bromide (CTAB) method. The V4 region of the bacterial 16S rRNA gene was amplified using the forward primer (5'-ACTCC TACGGGAGGCAGCA-3') and the reverse primer (5'-GGACTACHVGGGTWTCTAAT-3'). The fungal internal transcribed spacer (ITS) region was amplified using the universal primers ITS1F (5'-CTTGGTC ATTTAGAGGAAGTAA-3') and ITS2R (5'-GCTGCG TTCTTCATCGATGC-3'). Soil microbial analysis was

performed with the assistance of Panomix (Suzhou) Biomedical Technology Co., Ltd. Library quality control was performed using the Agilent High Sensitivity DNA Kit on an Agilent Bioanalyzer, followed by library quantification using the Quant-iT PicoGreen dsDNA Assay Kit on a Promega QuantiFluor fluorescence quantification system. The amplification products first underwent sequence end repair: the 5' overhanging bases of the DNA sequences were excised using the End Repair Mix 2 from the kit, while a phosphate group was added and the missing bases at the 3' end were filled in. An 'A' base was added to the 3' end of the DNA sequences to prevent self-ligation of DNA fragments and to ensure the target sequences could ligate to the sequencing adapters (which have a single 'T' base overhang at the 3' end). Sequencing adapters containing library-specific indexes (i.e., Index sequences) were ligated to the 5' ends of the sequences, enabling the DNA molecules to be immobilized on the Flow Cell. Adapter self-ligation fragments were removed, and the post-ligation library system was purified using BECKMAN AMPure XP Beads for size selection. The adapter-ligated DNA fragments were then PCR-amplified to enrich the sequencing library templates, and the enriched library products were purified again using BECKMAN AMPure XP Beads. Final fragment selection and purification of the library were performed via 2% agarose gel electrophoresis. Sequencing libraries were prepared using Illumina's TruSeq Nano DNA LT Library Prep Kit, and high-throughput sequencing analysis of the bacterial rRNA genes was performed.

Statistical and bioinformatics analysis

DADA2 denoising was performed using QIIME2 (version 2019.4). Primer fragments were first removed from the sequences using qiime cutadapt trim-paired, and sequences where primers were not matched were discarded. Subsequently, qiime dada2 denoise-paired was called to execute DADA2 for quality control, denoising, merging, and chimera removal. After denoising all libraries, the ASV feature sequences and ASV table were merged, and singleton ASVs were removed. For both bacterial 16S rRNA gene and fungal ITS sequences, the Silva database (Release 132, <http://www.arb-silva.de>) was used. Taxonomic annotation was performed for each ASV feature sequence or each OTU representative sequence using the pre-trained Naïve Bayes classifier within QIIME2 software with default parameters, employing the classify-sklearn algorithm (Bokulich et al., 2018) (<https://github.com/QIIME2/q2-feature-classifier>). For functional genes or other requirements, the localized nt database (downloaded in 2019.8, <ftp://ftp.ncbi.nih.gov/blast/db/>) was used for annotation. For the nt database, the BROCC algorithm (Nilsson et al., 2006) (<https://github.com/kylebittinger/q2-brocc#the-brocc-algorithm>)

was employed: first, blastn was used to align sequences against the nt database (or a specific subset thereof); then, the brocc.py script was called with recommended parameters to obtain annotation information. Global Untargeted Metabolomics.

Extraction of metabolites from soil samples

In collaboration with Suzhou Panomix Biomedical Technology Co., Ltd., ultra-performance liquid chromatography–tandem mass spectrometry (UPLC-ESI-MS/MS) was employed to profile metabolite composition in soil samples. Specifically, 0.10 g of each soil sample was accurately weighed into a 2 mL centrifuge tube, and 600 μ L of an internal standard solution—2-chloro-L-phenylalanine (4 ppm, Aladdin) prepared in methanol—was precisely added. The mixture was vortex-mixed for 30 s, after which a steel bead was introduced and homogenization performed using a tissue grinder at 55 Hz for 90 s. Subsequently, the sample was subjected to ultrasonication at room temperature for 30 min, followed by incubation on ice for 30 min. Centrifugation was carried out at 12,000 rpm and 4°C for 10 min. The resulting supernatant was filtered through a 0.22 μ m membrane, and the filtrate was transferred into an injection vial for UPLC-MS/MS analysis.

UPLC-ESI-MS/MS analysis

Metabolite profiling in soil samples was performed using a UPLC-ESI-MS/MS system, consisting of a Thermo Vanquish UPLC (Thermo Fisher Scientific, USA) coupled with a Q Exactive mass spectrometer (Thermo Fisher Scientific, USA) via an LC-ESI ion source. Chromatographic separation was achieved on an ACQUITY UPLC® HSS T3 column (2.1 $\times$ 100 mm, 1.8 μ m; Waters, Milford, MA, USA) maintained at 40 °C, with a flow rate of 0.3 mL/min and an injection volume of 2 μ L. For positive ion mode [LC-ESI (+)-MS], the mobile phase consisted of 0.1% formic acid in acetonitrile (B2) and 0.1% formic acid in water (A2). The gradient elution program was as follows: 0–1 min, 8% B2; 1–8 min, 8% to 98% B2; 8–10 min, 98% B2; 10–10.1 min, 98% to 8% B2; 10.1–12 min, 8% B2. For negative ion mode, the mobile phase included acetonitrile (B3) and 5 mM ammonium formate in water (A3), with the following gradient: 0–1 min, 8% B3; 1–8 min, 8% to 98% B3; 8–10 min, 98% B3; 10–10.1 min, 98% to 8% B3; 10.1–12 min, 8% B3 (Zelena et al. 2009). The column effluent was directed into the mass spectrometer for analysis. Data were acquired in both positive and negative ionization modes. The spray voltage was set to 3.50 kV for positive mode and –2.50 kV for negative mode. Sheath gas and auxiliary gas flows were maintained at 40 arb and 10 arb, respectively. The capillary temperature was 325°C. Full-scan MS¹ spectra were acquired at a resolution of 70,000 over the m/z range 100–1000. Data-dependent

MS² acquisition was performed using higher-energy collisional dissociation (HCD) with a collision energy of 30 eV and a resolution of 17,500. The top 10 most intense ions were selected for fragmentation, and dynamic exclusion was applied to avoid redundant MS/MS spectra (Want et al. 2013).

Data processing and statistical analysis

Raw mass spectrometry data were converted to mzXML format using the MSConvert tool within the ProteoWizard software package (v3.0.8789) (Rasmussen et al. 2022). Peak detection, filtering, and alignment were performed using the R package XCMS with the following parameters: bw=2, ppm=15, peakwidth=c(5, 30), mzwid=0.015, mzdiff=0.01, method = “centWave”. A feature table was generated and normalized by total peak area to correct for systematic bias (Navarro-Reig et al. 2015). The preprocessed results were exported as an.xlsx file containing retention time (RT), m/z values, and peak intensities. Base peak chromatograms (BPC) were obtained from continuous MS scanning. Metabolite identification was conducted by querying spectral databases including HMDB (Wishart et al. 2007), MassBank (Horai et al. 2010), LMSD (Sud et al. 2007), mzcloud (Abdelrazig et al. 2020), KEGG (Ogata et al. 1999), and Nuomi Metabolome’s in-house library, with a mass tolerance of <30 ppm. Molecular formulas were inferred based on precursor ion m/z, mass error (ppm), and adduct information for level-1 identification. Level-2 identification was achieved by comparing experimental MS/MS spectra with database records. Metabolites with MS² annotations were further screened using P value and VIP thresholds from univariate analysis to identify differentially expressed metabolites (Kieffer et al. 2016). Multivariate statistical analyses—PCA, PLS-DA, and OPLS-DA—were performed using the R package ropls (Thévenot et al. 2015). Model overfitting was assessed by permutation testing, with R²X, R²Y, and Q² values indicating model fit and predictive ability. Potential biomarkers were selected based on P value < 0.05, VIP > 1, and fold change. Differentially expressed metabolites were subsequently subjected to KEGG pathway enrichment analysis via MetaboAnalyst.

Statistical analysis

The software GraphPad Prism 10.1.2 was used to organize, analyze, and graph data related to plant growth. Analysis was conducted using one-way ANOVA, followed by Tukey’s-b post hoc test, with a confidence interval of 95%. Results are presented graphically and expressed as mean $\pm$ standard deviation (SD).

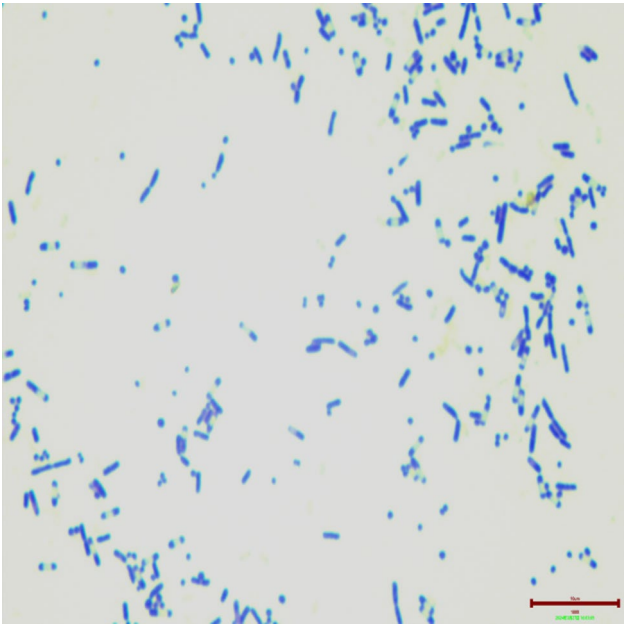


Fig. 1 Microscopic observation results of Gram staining for GYH20240327-1

Table 1 Biochemical characterization of *B. subtilis* GYH20240327-1 isolated from traditional yogurt

Biochemical experiment	Result
Oxygen demand	+
Litmus milk reduction	+
V-P Measurement	+
MP Test	–
Casein hydrolysis	+
Starch hydrolysis	+
Citrate utilization	+
Catalase test	+
Gelatin liquefaction	+
Nitrate reduction	+
Urea utilization	–
Glucose fermentation	+
Fructose fermentation	+
Lactose fermentation	+
Maltose fermentation	+
Sucrose fermentation	+
Xylitol fermentation	+
Mannitol fermentation	+

“+” indicates a positive test result, and “–” indicates a negative test result

Results

Strain identification results

The Gram staining microscopic identification results of the strain are shown in Fig. 1, and the biochemical test results are shown in Table 1. The Strain amplification product sequence is shown in SI. 1, and the System phylogenetic tree are shown in SI. 2. Through sequence comparison, the strain isolated from traditional yogurt was identified as *B. subtilis* and was named GYH20240327-1.

Effects of applying biofertilizer on plant growth

The above-ground height, root length, root fresh weight, and root dry weight of *P. grandiflorus* and *S. baicalensis* samples were measured to evaluate the effects of a bio-composite fertilizer containing strain GYH20240327-1 on the growth of *P. grandiflorus* and *S. baicalensis*. The results are shown in Fig. 2. The results indicate that fertilization treatment had a certain growth-promoting effect on the plants during the critical three-month period from flowering to harvest: overall, plants treated with an organic fertilizer composite biofertilizer showed better promotion on above-ground height, root length, root fresh weight, and root dry weight compared to those treated with only organic fertilizer, and compared with the unfertilized control, all four growth indicators were significantly improved ($p < 0.05$). For *P. grandiflorus*, applying a composite biofertilizer containing GYH20240327-1 has a more significant growth-promoting effect on biennial *P. grandiflorus* (JG2) than on annual *P. grandiflorus* (JG1), especially in promoting root development, with roots being the main site of accumulation of the pharmacologically active components of *P. grandiflorus*. For annual *S. baicalensis*(HQ), after applying the composite biofertilizer, the effect on root length and weight is extremely significant, indicating that a composite biofertilizer containing GYH20240327-1 may play an important role in the accumulation of pharmacologically active components in *S. baicalensis*.

Effects of *B. subtilis* inoculation on the soil microbial community

Diversities of soil bacterial and fungal microbiota

To assess differences in rhizosphere microbial composition between *P. grandiflorus* and *S. baicalensis* following the application of GYH20240327-1 (only applying organic fertilizer/applying both organic fertilizer + compound microbial fertilizer), statistical analyses were conducted at multiple taxonomic levels. Rarefaction was performed at 95% of the minimum sample sequence depth. A total of 253,440 high-quality bacterial sequences were clustered into 38 phyla, 66 classes, 173 orders, 342 families, 1,625 genera, and 46 species. For fungi, 663,972 high-quality sequences were obtained, representing 7 phyla, 4 classes, 13 orders, 15 families, 18 genera, and 121 species. Rarefaction curves nearly paralleled the x-axis, indicating sufficient sequencing depth to capture overall bacterial and fungal diversity (SI. 3). Alpha diversity indices, including Chao1, Simpson, Shannon, Pielou’s evenness, Observed species, Faith’s PD, and Good’s coverage, showed no significant differences between the rhizosphere soils of *P. grandiflorus* and *S. baicalensis* (Fig. 3), suggesting similar microbial richness, diversity, evenness, and coverage. Beta diversity was evaluated using Bray–Curtis-based PCoA and NMDS, along with UPGMA

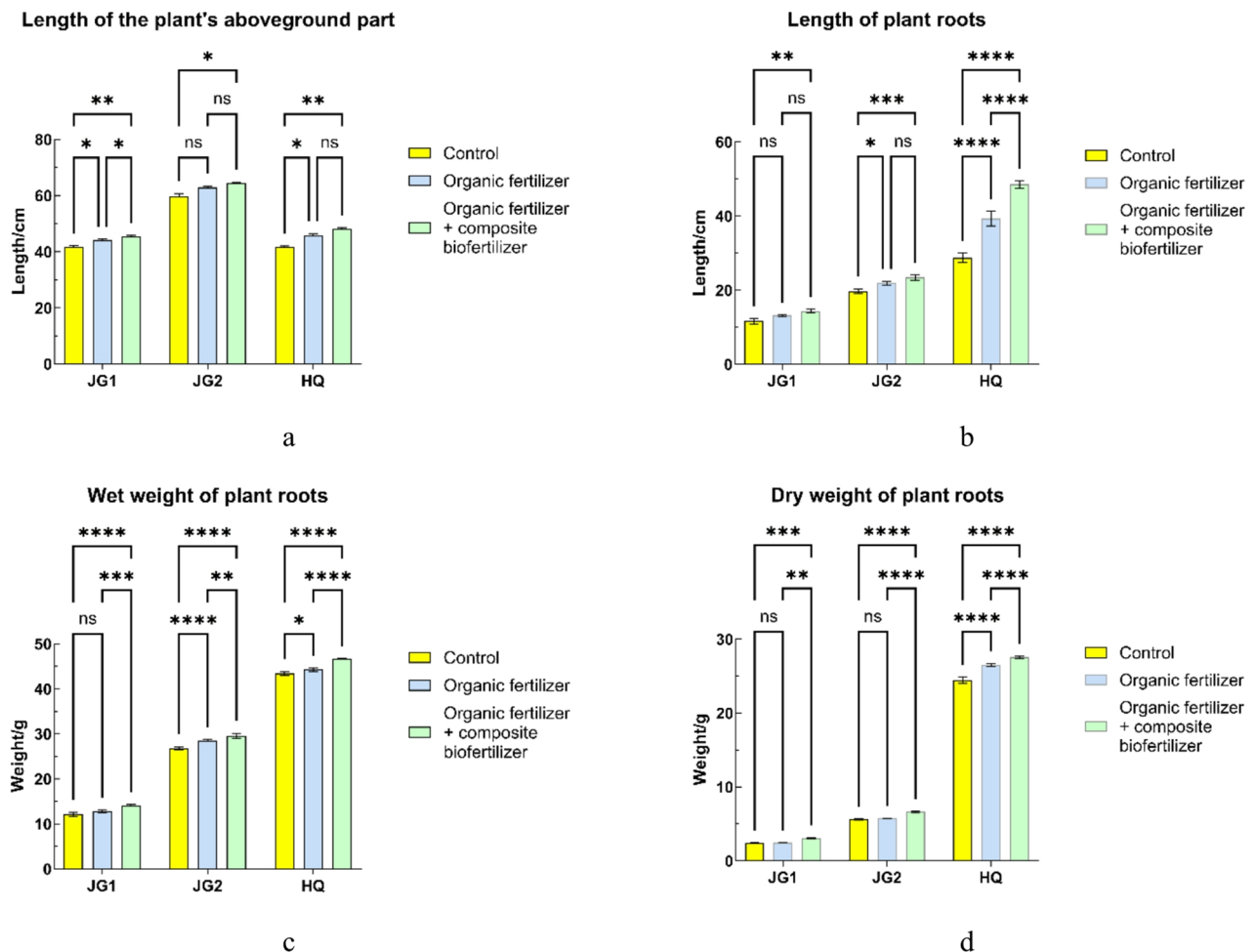


Fig. 2 Effects of Fertilization on Plant Growth. **a** Aboveground height of plants; **b** Root length of plants; **c** Fresh weight of plant roots; **d** Dry weight of plant roots. JG1 represents 1-year-old *P. grandiflorus*, JG2 represents 2-year-old *P. grandiflorus*, HQ represents 1-year-old *S. baicalensis*. * indicates $p < 0.05$, ** indicates $p < 0.01$, *** indicates $p < 0.001$, **** indicates $p < 0.0001$. Values are mean $\pm$ SD of three replicates ($n = 3$)

clustering (Fig. 4). PCoA revealed that the first two principal components collectively explained 38.7% and 41.7% of the variance in bacterial and fungal communities, respectively. While bacterial composition appeared similar between the two species, fungal communities showed clear separation. NMDS (Stress < 0.2) indicated distinct bacterial and fungal profiles between species. UPGMA analysis identified JG1-3 (annual *P. grandiflorus* blank control) and HQ1 (annual *S. baicalensis* blank control) as the most dissimilar. Bacterial communities clustered closely within JG2 (biennial *P. grandiflorus* with GYH20240327-1), JG1-1 and JG1-2 (annual *P. grandiflorus* with GYH20240327-1), and among HQ2, HQ3, and HQ4 (annual *S. baicalensis* with GYH20240327-1). Fungal communities exhibited high similarity within all *P. grandiflorus* samples and within all *S. baicalensis* samples. Composition of the soil bacterial and fungal community.

Taxonomic composition of microbial communities at the phylum and genus levels was determined by comparing representative OTU sequences against reference databases (Fig. 5). The ten most abundant bacterial phyla were largely consistent between *P. grandiflorus* and *S. baicalensis*, including *Acidobacteriota*, *Proteobacteria*, *Actinobacteriota*, *Gemmatimonadota*, *Chloroflexi*, *Myxococcota*, *Bacteroidota*, *Planctomycetota*, *Methylo-**mirabilota*, and *Verrucomicrobiota*. In the control sample JG1-3, *Acidobacteriota* and *Gemmatimonadota* were less abundant, while *Proteobacteria* were more abundant compared to the five treated samples, suggesting that the application of GYH20240327-1 increased the abundance of beneficial bacterial groups and reduced *Proteobacteria*, potentially optimizing the soil micro-environment for *P. grandiflorus* growth. In *S. baicalensis*, the control sample HQ1 showed lower relative abundance of *Acidobacteriota* and variable *Actinobacteriota* levels across samples, indicating a modulatory effect of GYH20240327-1 on

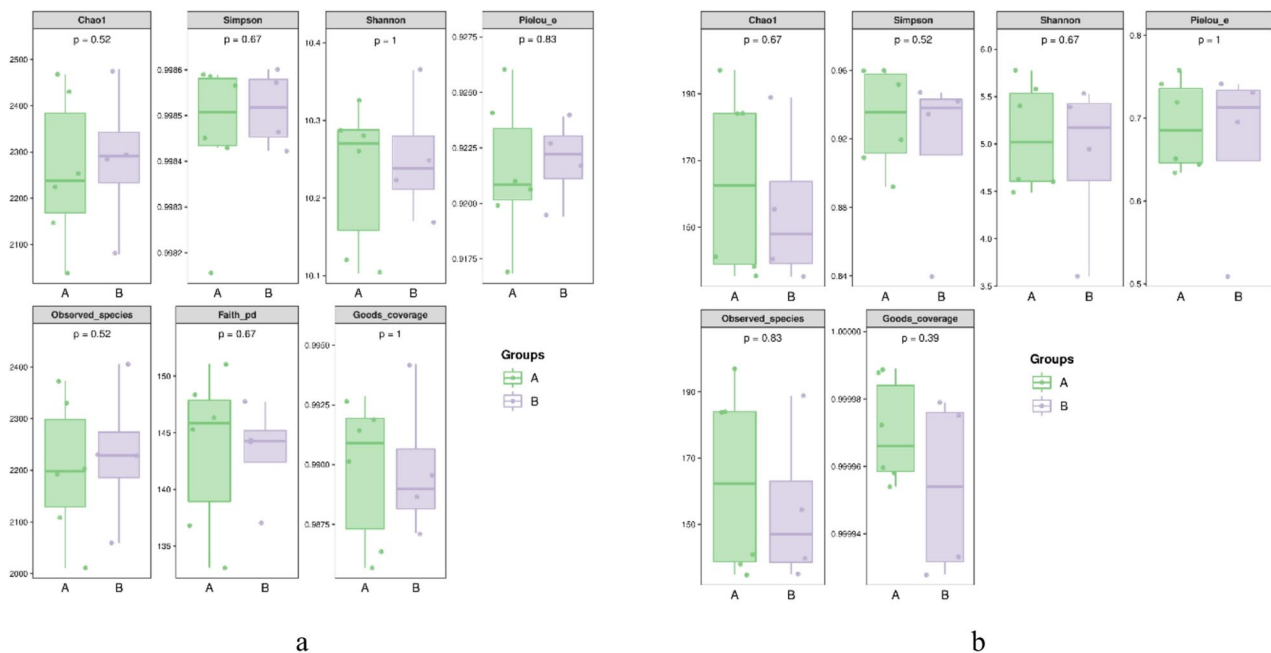


Fig. 3 Boxplot of Alpha Diversity Index by Group. **a** Bacteria. **b** Fung

dominant soil bacteria. For fungi, the top ten phyla included *Ascomycota*, *Basidiomycota*, *Mortierellomycota*, *Chytridiomycota*, *Apheleliomycota*, *Glomeromycota*, *Rozellomycota*, *Olpidiomycota*, *Mucoromycota*, and *Calcarisporiellomycota*. In *P. grandiflorus*, *Mortierellomycota* abundance in JG1-3 resembled that in biennial samples, possibly reflecting an effect of GYH20240327-1. In *S. baicalensis*, HQ1 exhibited higher *Ascomycota* ($\geq 18.56\%$) and lower *Basidiomycota* ($\geq 6.19\%$) and *Mortierellomycota* abundances compared to treated samples. At the genus level, the dominant bacterial genera were similar between species, including *Vicinamibacteraceae*, MND1, RB41, MB-A2-108, KD4-96, *Rokubacteriales*, Subgroup_7, Subgroup_10, *Latescibacterota*, and *Haliangium*. RB41, MB-A2-108, and *Rokubacteriales* were less abundant in JG1-3, while *Vicinamibacteraceae* and RB41 were reduced in HQ1. Fungal genera such as *Lectera*, *Tausonia*, *Fusarium*, *Cladosporium*, *Doratomyces*, *Cladorrhinum*, *Verticillium*, *Alternaria*, *Aspergillus*, and *Pyrenochaetopsis* showed considerable variation among samples. JG1-3 had notably higher *Cladorrhinum* and *Pseudeurotium*, whereas HQ1 showed lower *Tausonia* and higher *Lectera* and *Alternaria* abundances.

LEfSe analysis was employed to identify potential biomarkers in the rhizosphere microbiota (Fig. 6). At the bacterial phylum level, *Bacteroidota* exhibited the highest LDA score and was identified as a key taxon in *S. baicalensis*. Among bacterial genera, the most discriminative features were *Acinetobacter*, *Sphingomonas*, and *Inquilinus*, listed in descending order of LDA score. For *P. grandiflorus*, the fungal phylum *Basidiomycota* played a

major discriminative role, while at the genus level, *Cladosporium*, *Ophiocordyceps*, *Thanatephorus*, and *Articulospora* showed the highest LDA scores.

Prediction of microbial community functional potential

Differential microbial communities enriched in the rhizosphere soils of *P. grandiflorus* and *S. baicalensis* were functionally annotated against the MetaCyc database to categorize associated metabolic processes (Fig. 7). Bacterial metabolic pathways were classified into seven major categories: Biosynthesis, Degradation/Utilization/Assimilation, Detoxification, Generation of Precursor Metabolite and Energy, Glycan Pathways, Macromolecule Modification, and Metabolic Clusters. Among these, Biosynthesis represented the most abundant functional category in both plant species, followed by Generation of Precursor Metabolite and Energy. Fungal pathways were grouped into five categories—Biosynthesis, Degradation/Utilization/Assimilation, Generation of Precursor Metabolite and Energy, Glycan Pathways, and Metabolic Clusters—with Biosynthesis and Generation of Precursor Metabolite and Energy also being the most predominant. Key pathways such as Amino Acid Biosynthesis, Cofactor, Prosthetic Group, Electron Carrier, and Vitamin Biosynthesis, Fatty Acid and Lipid Biosynthesis, Nucleoside and Nucleotide Biosynthesis, and Respiration are essential for fundamental plant life processes and growth, indicating that rhizosphere microorganisms play a critical role in plant development.

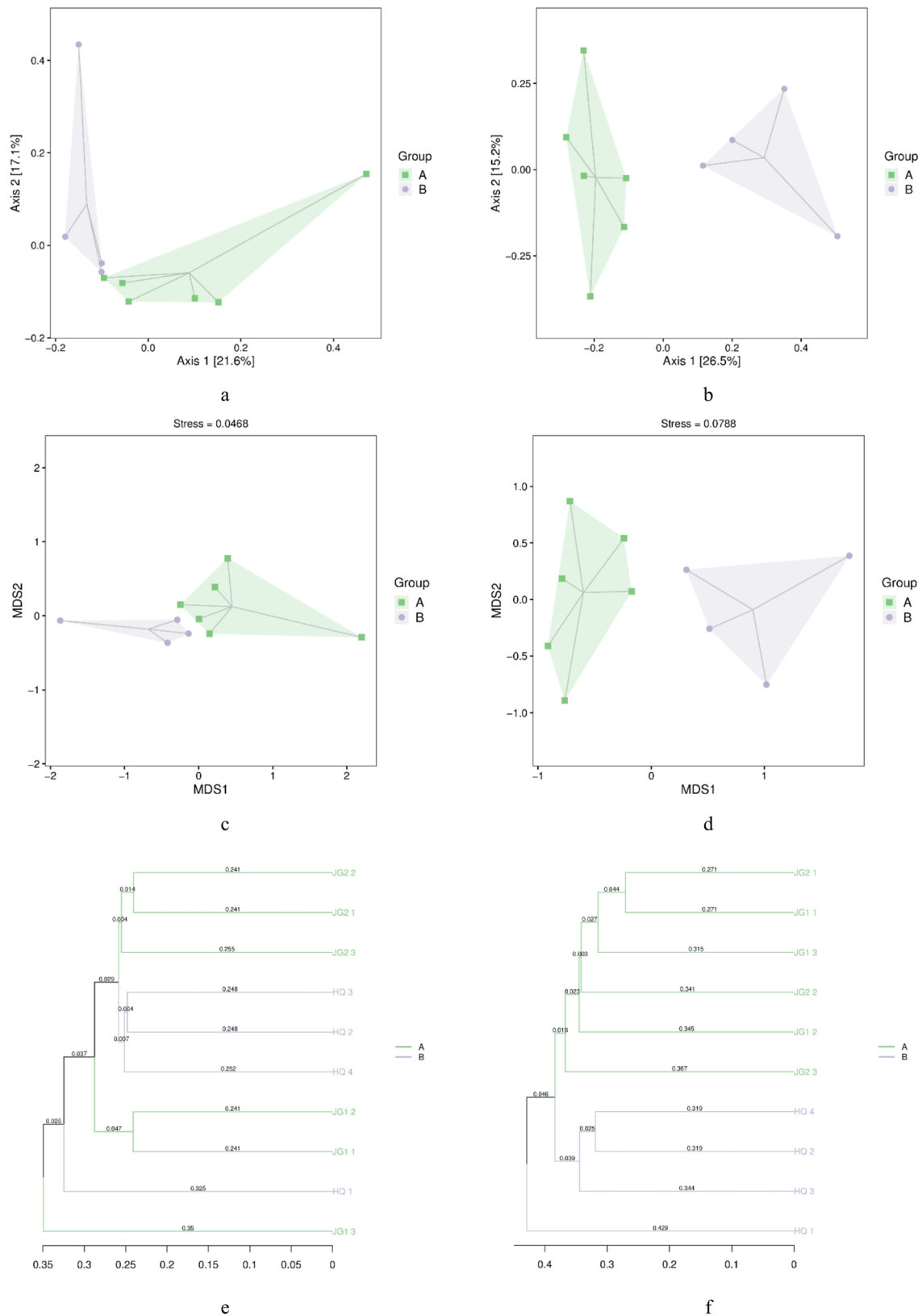


Fig. 4 Beta Diversity Index Results. **a** PCoA analysis of soil bacterial communities, **(b)** PCoA analysis of soil fungal communities, **(c)** NMDS analysis of soil bacterial communities, **(d)** NMDS analysis of soil fungal communities, **(e)** UPGMA clustering analysis of soil bacterial communities, **(f)** UPGMA clustering analysis of soil fungal communities

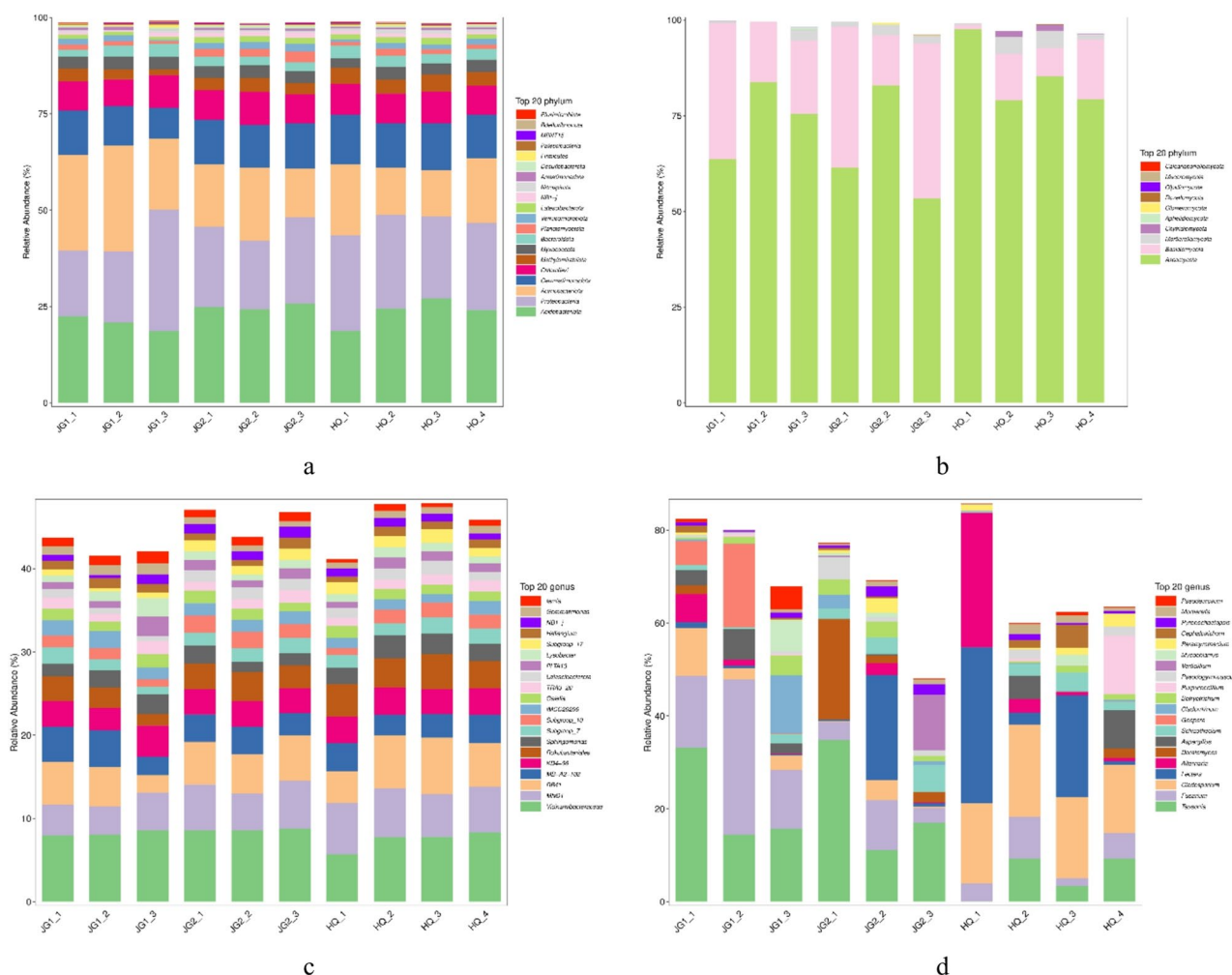


Fig. 5 Analysis of Species Composition by Bar Chart. **a** Bacterial Phylum, **(b)** Bacterial Genus, **(c)** Fungal Phylum, **(d)** Fungal Genus

Effects of *B. subtilis* inoculation on metabolic variations in the soil

To mechanistically elucidate microbial community differences between the two medicinal plants, a comparative metabolomics analysis was conducted on the rhizosphere soils of *P. grandiflorus* and *S. baicalensis* under the conditions of only applying organic fertilizer (without applying microbial fertilizer GYH20240327-1) and applying both organic fertilizer+compound microbial fertilizer. Based on UHPLC-MS/MS analysis, 216 metabolites were detected across all soil samples. Orthogonal partial least squares-discriminant analysis (OPLS-DA) was applied to identify differential metabolites between the two plant species. Model reliability was assessed via cross-validation parameters R^2 (goodness of fit) and Q^2 (predictive ability), where values closer to 1 indicate greater model stability and robustness. The permutation test confirmed the model was not overfitted, supporting the validity of metabolite selection based on variable importance in projection (VIP) scores (Fig. 8). Under positive and

negative ionization modes, the OPLS-DA model yielded R^2X values of 0.444 and 0.591, R^2Y values of 0.990 and 0.997, and Q^2 values of 0.610 and 0.819, respectively, indicating acceptable model stability and reliability.

Analysis of differential metabolites in soil

A total of 4,905 metabolites were identified, from which 216 significantly differential metabolites were screened based on $VIP > 1$ and $P < 0.05$. As shown in Fig. 9a, compared to JG2, JG1 exhibited 11 up-regulated and 33 down-regulated metabolites; compared to HQ, JG1 showed 38 up-regulated and 23 down-regulated metabolites; while only 5 up-regulated and 6 down-regulated metabolites were observed between JG2 and HQ, suggesting relatively minor metabolic differences between these two groups. Figure 9b illustrates the differential metabolites between *P. grandiflorus* and *S. baicalensis*, revealing higher similarity in metabolite profiles between HQ (annual *S. baicalensis*) and JG2 (biennial *P. grandiflorus*) than between JG2 and JG1 (annual *P. grandiflorus*).

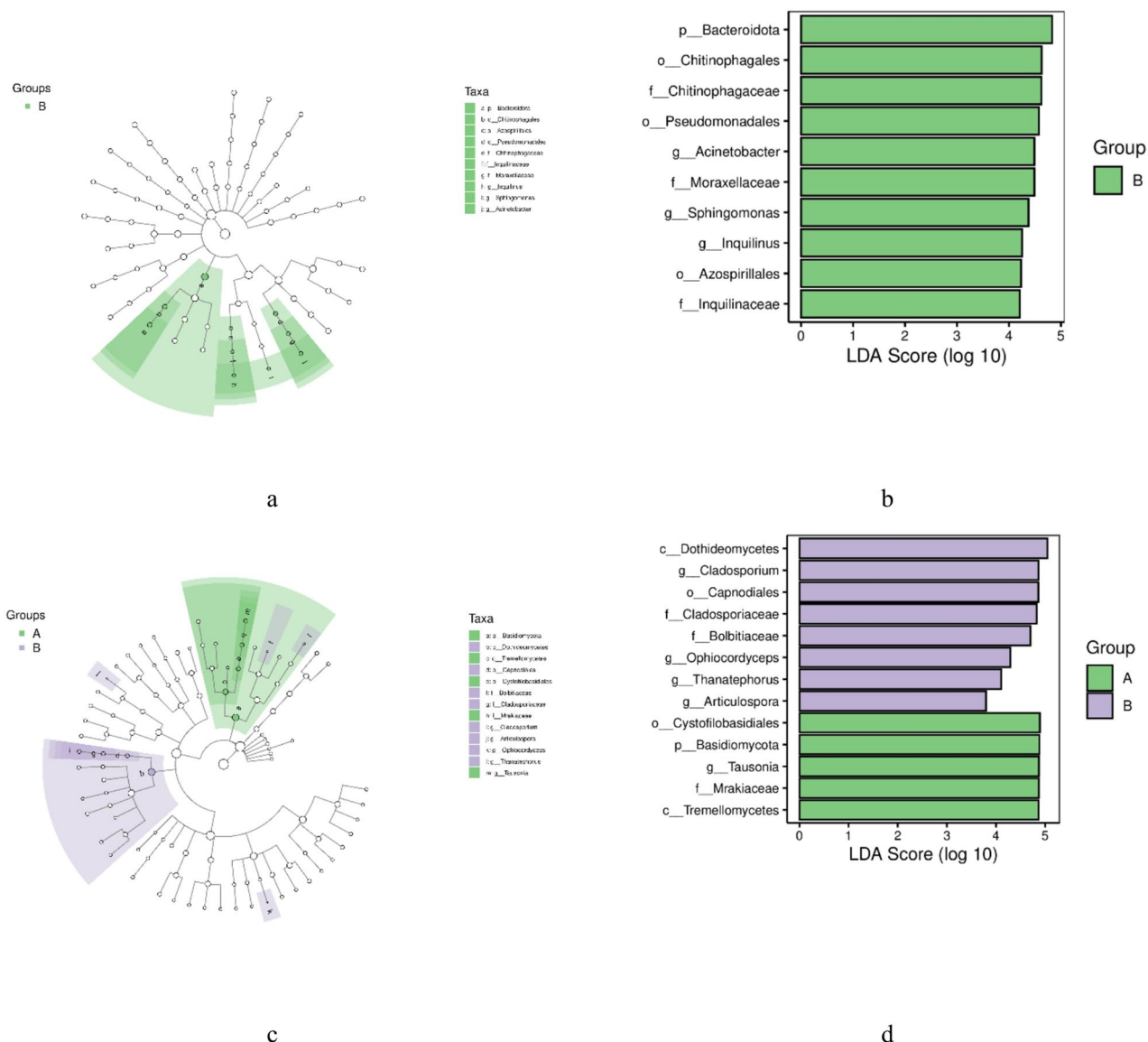


Fig. 6 The linear discriminant analysis effect size (LEfSe). Cladogram of bacterial (a) and fungal (c) taxa that differ significantly in abundance among different oolong tea varieties. Indicator bacterial (b) and fungal (d) with LDA scores in microbial communities among different oolong tea varieties

In Fig. 9c, stearolic acid was notably more abundant in JG samples, particularly in JG1. The top five differentially expressed metabolites between JG1 and JG2 (Fig. 9d) were adenine, 3-dehydrosphinganine, epiandrosterone, 2-pyrocatechuic acid, and sphinganine. Between JG1 and HQ (Fig. 9e), the top five differential metabolites were aesculin, deoxycorticosterone acetate, epiandrosterone, D-ribose 5-phosphate, and stearolic acid. Between JG2 and HQ (Fig. 9f), the top-ranking differential metabolites included anabasine, linoleic acid, epiandrosterone, LysoPA(16:0/0:0), and stearolic acid.

Metabolic pathway analysis

KEGG enrichment analysis of differential metabolites revealed distinct pathway profiles across the comparisons

(Fig. 10). In the JG1 vs. JG2 comparison, “biosynthesis of plant hormones” was the most significantly enriched pathway, with differential metabolites also exerting a notable influence on the “Asthma” pathway (Fig. 10a). For JG1 vs. HQ, “linoleic acid metabolism” was the top enriched pathway, and the associated metabolites similarly impacted the “Asthma” pathway (Fig. 10b). In JG2 vs. HQ, “biosynthesis of plant secondary metabolites” represented the most enriched pathway, while the “steroid degradation” pathway was also substantially affected (Fig. 10c). Collectively, these findings suggest that several core metabolic pathways—biosynthesis of plant hormones, plant hormone signal transduction, synaptic vesicle cycle, linoleic acid metabolism, biosynthesis of plant secondary metabolites, steroid degradation, and vitamin

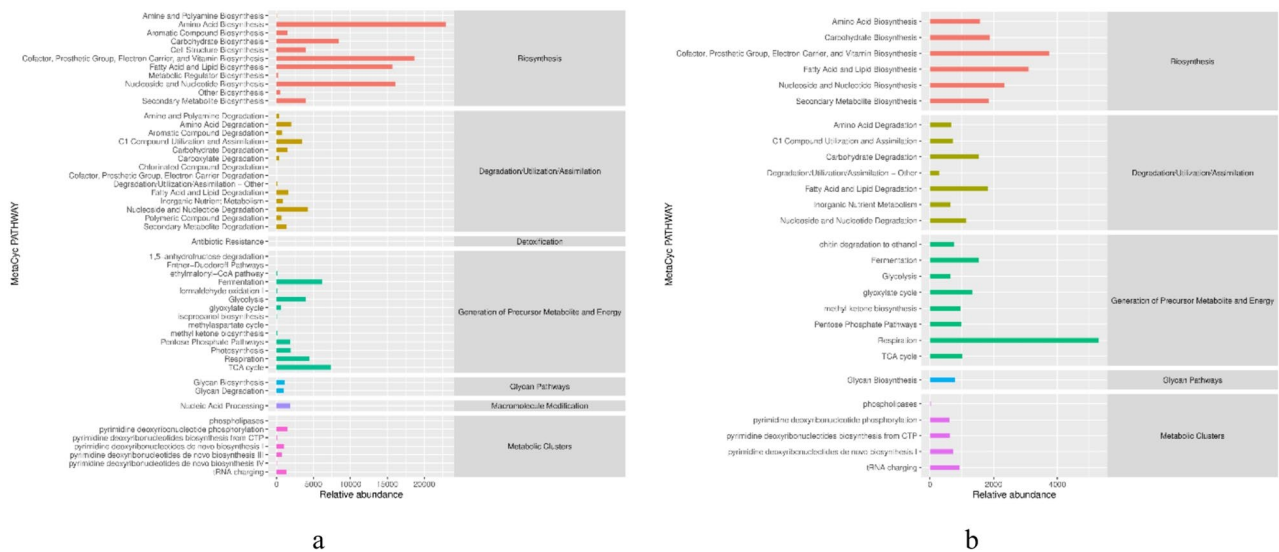


Fig. 7 Predicted MetaCyc secondary functional pathway abundance map. **a** Bacteria **(b)** Fungi

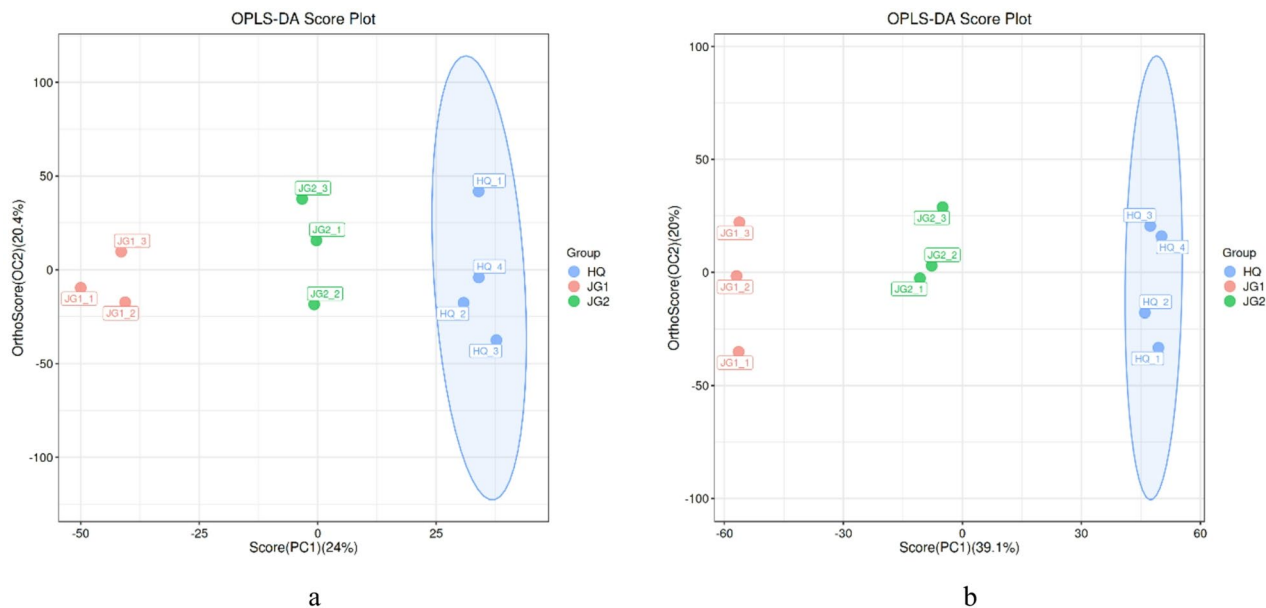


Fig. 8 OPLS-DA Score Plot. **a** Positive Ion Mode **(b)** Negative Ion Mode

B6 metabolism—may play pivotal roles in shaping the metabolic divergence between the two plant species.

In the JG1 vs JG2 comparison, key metabolic pathways and their associated differential metabolites were identified. The “biosynthesis of plant hormones” pathway included four metabolites: up-regulated trans-Cinnamate and down-regulated Jasmonic acid, Indoleacetic acid, and beta-Carotene. The “plant hormone signal transduction” pathway involved three down-regulated metabolites: Jasmonic acid, Indoleacetic acid, and beta-Carotene. Other pathways also showed distinct regulation: Phenylpropanoid biosynthesis: trans-Cinnamate (up), 4-Hydroxystyrene (down), Chavicol (down). Sphingolipid

metabolism: sphinganine (up), 3-dehydrosphinganine (down). Purine metabolism: 2'-Deoxyadenosine (up), adenine (down), dGMP (down). Pentose phosphate pathway: Ribose 1,5-bisphosphate (up), 6-Phosphogluconic acid (down). Biosynthesis of phenylpropanoids: Biochanin A (up), Genistein (down), 2-pyrocatechuic acid (down). Synaptic vesicle cycle: Norepinephrine (down), Histamine (down). Asthma pathway: Histamine (down). Primary bile acid biosynthesis: 25-Hydroxycholesterol (up), Allocholic acid (down).

In the JG1 vs HQ comparison, several key metabolic pathways exhibited distinct differential metabolite profiles: Biosynthesis of plant hormones: trans-Cinnamate

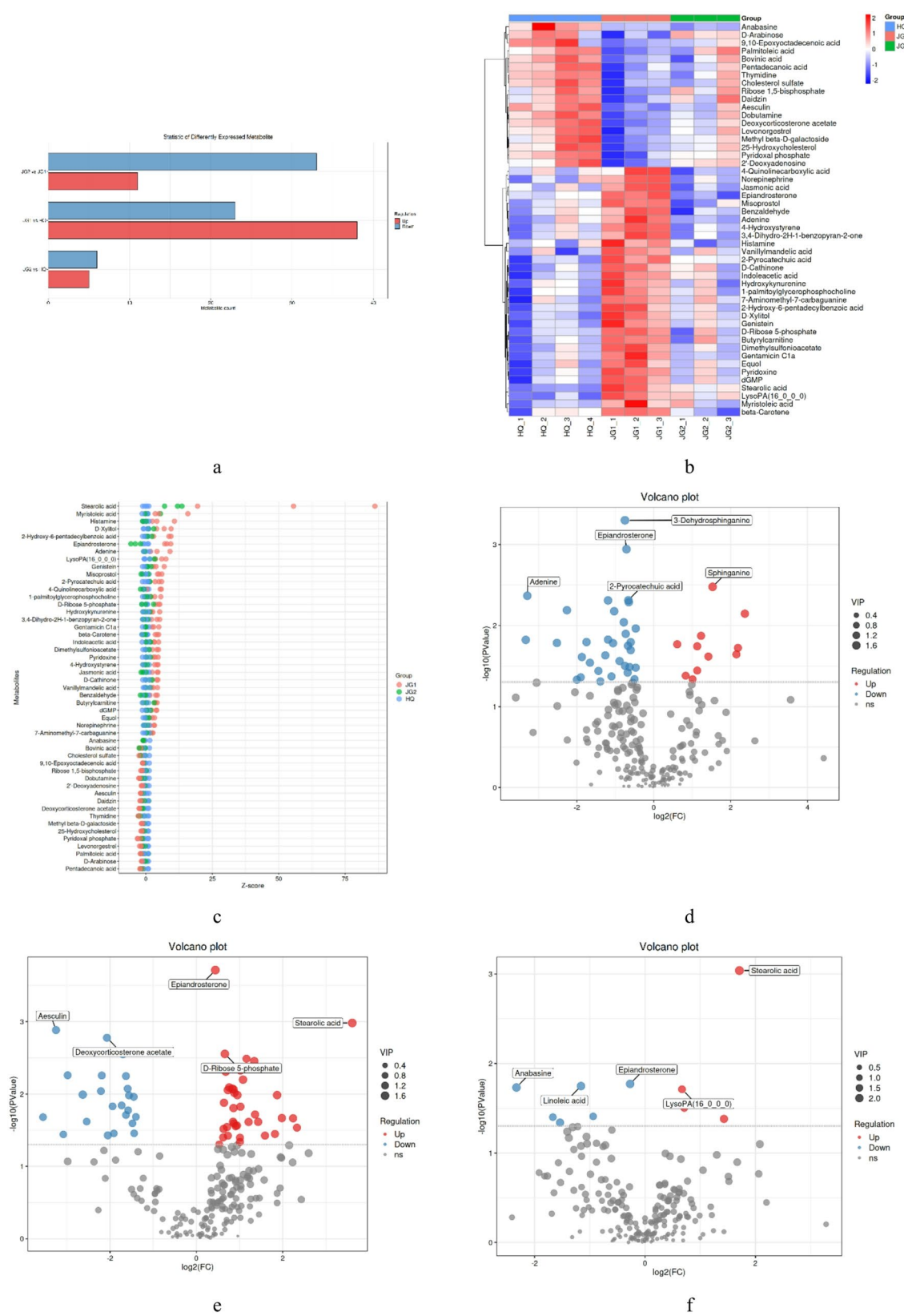


Fig. 9 Analysis of Differential Metabolites. **a** Bar chart of differential metabolites; **(b)** Cluster heatmap of differential metabolites; **(c)** Z-score plot; **(d)** Volcano plot of differential metabolites: JG1 vs JG2; **(e)** Volcano plot of differential metabolites: JG1 vs HQ; **(f)** Volcano plot of differential metabolites: JG2 vs HQ

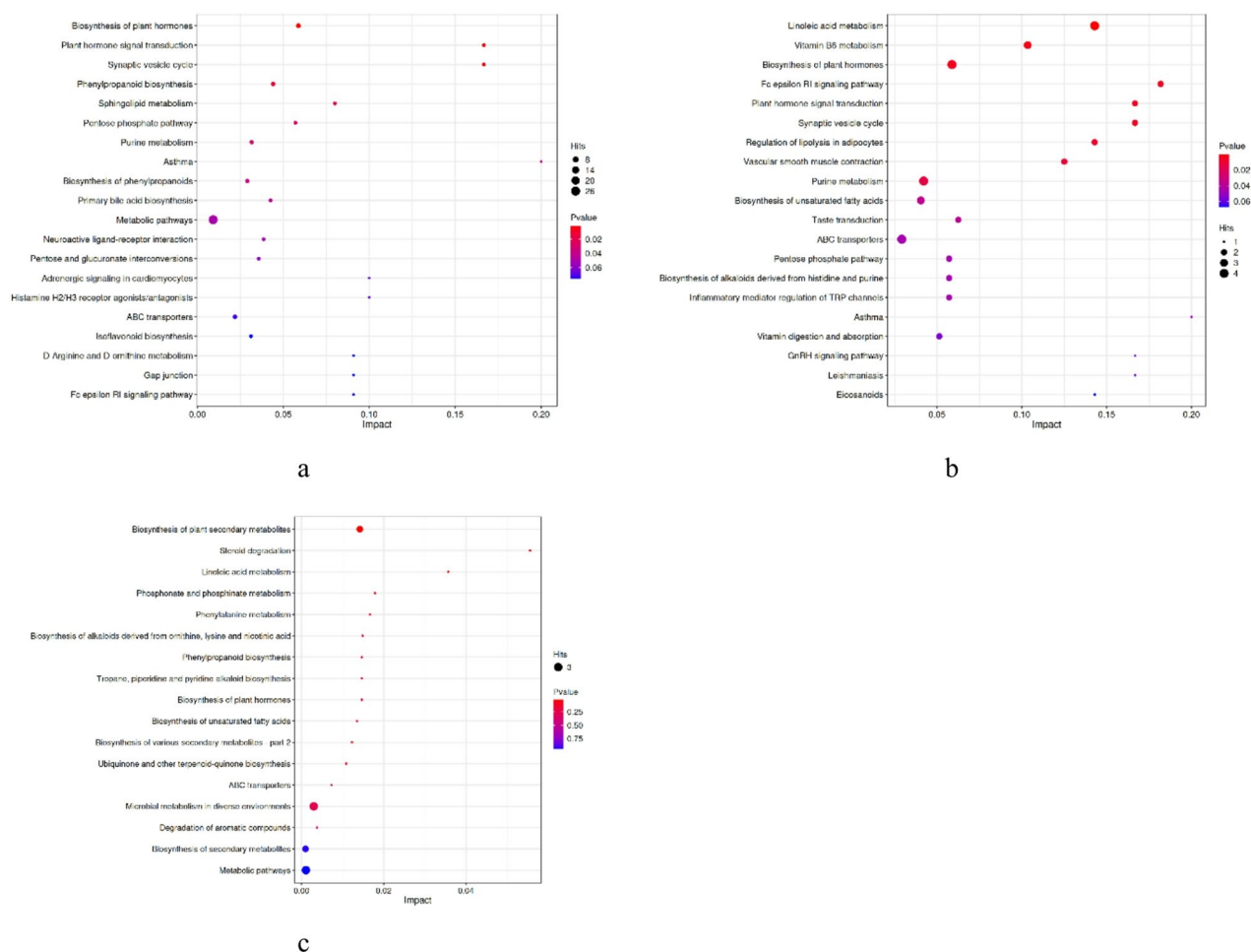


Fig. 10 Bubble Chart of Metabolic Pathway Impact Factors. Top 10 KEGG pathway impact factor bubble plots of differential metabolites enriched between (a) JG1 vs JG2, (b) JG1 vs HQ, and (c) JG2 vs HQ

(down-regulated), Jasmonic acid, Indoleacetic acid, and beta-Carotene (all down-regulated). Vitamin B6 metabolism: Pyridoxine and D-ribose 5-phosphate (up-regulated), Pyridoxal phosphate (down-regulated). Purine metabolism: adenine, D-ribose 5-phosphate, and dGMP (up-regulated), 2'-Deoxyadenosine (down-regulated). Linoleic acid metabolism: Arachidonic acid (up-regulated), 9,10-Epoxyoctadecenoic acid, Bovinic acid, and 9(S)-HPODE (down-regulated). Regulation of lipolysis in adipocytes: Norepinephrine and Arachidonic acid (up-regulated). Vascular smooth muscle contraction: Norepinephrine and Arachidonic acid (up-regulated). Synaptic vesicle cycle: Norepinephrine and Histamine (up-regulated). Fc epsilon RI signaling pathway: Arachidonic acid and Histamine (up-regulated). Biosynthesis of unsaturated fatty acids: Arachidonic acid, Erucic acid, and Docosaheptaenoic acid (up-regulated).

In the JG2 vs. HQ comparison, five key metabolites exhibited differential regulation across multiple pathways: Linoleic acid was down-regulated in biosynthesis of plant secondary metabolites, linoleic acid metabolism,

and biosynthesis of unsaturated fatty acids. Anabasin was down-regulated in alkaloid biosynthesis pathways. Epiandrosterone was down-regulated in steroid degradation. trans-Cinnamate was up-regulated in pathways related to phenylpropanoid and plant hormone biosynthesis. Ciliatine was up-regulated in phosphonate and phosphinate metabolism.

Discussion

B. subtilis possesses significant potential in promoting plant growth, enhancing plant stress responses, wastewater treatment, and soil remediation (Wang et al. 2025b; Diniz et al. 2025). As bio-fertilizers and bio-inoculants, *B. subtilis* can significantly improve the growth and development of *Brassica juncea* (Ndeddy Aka And Babalola 2016), tomato (Qiao et al. 2017), maize (Mumtaz et al. 2017), capsicum (Panneerselvam et al. 2019), wheat and Chinese cabbage (Zhang et al. 2019; Hussain et al. 2020), black pepper (Lau et al. 2020), citrus (Ezrari et al. 2021), sugarcane (Chandra et al. 2021), rice (Panneerselvam et al. 2019; Mahmud et al. 2023), chickpea growth in

calcareous soils (Nabati et al. 2025). Research shows that *B. subtilis* SB008 can influence the growth and development of soybean plants through multiple mechanisms, including the production of indole-3-acetic acid (IAA), exopolysaccharides, siderophores, and amino acids, along with hydrolytic enzymes (amylase, cellulase, protease, and chitinase) (dos Santos Oliveira et al., 2025). Studies by Mykola et al. (Patyka et al. 2025) indicate that inoculation with *B. subtilis* H38 can affect soybean phenotypic data, while significantly restructuring the rhizosphere bacterial community and metagenome, and also mention its ability to increase IAA and key siderophore levels. In this study, we also reached similar conclusions, showing that *B. subtilis* is beneficial to the growth of *P. grandiflorus* and *S. baicalensis*. Furthermore, *B. subtilis* can inhibit pathogens such as *Rhizoctonia solani*, *Fusarium proliferatum*, *Athelia rolfsii*, *Colletotrichum gloeosporioides*, and *Fusarium oxysporum* f.sp. *ciceris*, thereby controlling diseases like dry root rot and *Fusarium* head blight, and enhancing salt stress tolerance (Panneerselvam et al. 2019; Mukherjee et al. 2020; Li et al. 2021; Ezrari et al. 2021; Hadj Brahim et al. 2022), demonstrating its bio-controlling role. The *Acidobacteriota* phylum is a highly diverse and abundant bacterial group, playing a key role in maintaining ecosystem stability and is particularly important in soil ecology. *Acidobacteriota* participates in various biogeochemical cycles such as carbon, nitrogen, and sulfur, and contributes to organic matter decomposition and nutrient turnover (Gonçalves et al. 2025). The abundance of *Acidobacteriota* is often higher in healthy, mature plants compared to some diseased plants (Wu et al. 2025b). The *Gemmatimonadota* phylum is found in various environments including soil, freshwater, wastewater treatment plants, and oceans. Its relative abundance is higher in semi-arid/arid soils and deserts compared to other environments, and it responds positively to soil moisture changes under drought conditions (Cary et al. 2010; Gong et al. 2024). In this study, the application of GYH20240327-1 biofertilizer increased the relative abundance of *Acidobacteriota* in the rhizosphere soil of both *P. grandiflorus* and *S. baicalensis* plants, and increased the relative abundance of *Gemmatimonadota* in *P. grandiflorus* plants. The *Proteobacteria* phylum is a group of Gram-negative bacteria that includes various species harmful to humans and animals, such as *Escherichia coli* and *Helicobacter pylori*, drawing significant attention (Zeng et al. 2025). The application of GYH20240327-1 biofertilizer reduced the relative abundance of *Proteobacteria* in *P. grandiflorus*, optimizing the soil micro-environment for its growth. The *Ascomycota* and *Basidiomycota* phyla are key soil decomposers, contributing to nutrient cycling within microbial and protist communities (Wang et al. 2025a; Stanić et al. 2025). *Mortierellomycota* is also associated with soil nutrient

cycling; it can convert soil phosphorus into available forms, promoting plant uptake and thereby enhancing soil productivity (Nyimbo et al. 2025). After applying the GYH20240327-1 biofertilizer, the relative abundance of *Ascomycota* decreased in *S. baicalensis*, while the relative abundances of *Basidiomycota* and *Mortierellomycota* increased, indicating that the GYH20240327-1 biofertilizer contributes to the growth and development of *S. baicalensis*.

In this study, the identified PGPR was an allochthonous *B. subtilis*. There are limitations in the study of *B. subtilis* GYH20240327-1 as a plant biofertilizer. As a preliminary study, the high-throughput sequencing and metabolomics data were limited due to insufficient sample size, which somewhat weakens the persuasive power of GYH20240327-1's effect as a biofertilizer. This study is only a preliminary investigation of GYH20240327-1 as a biofertilizer, and there are still many aspects worth exploring in depth. For example, regarding GYH20240327-1 itself, we still need to explore its value through whole-genome, transcriptome, and proteome sequencing. In the production of biofertilizers containing GYH20240327-1, the effect of the carrier was not ruled out, so whether the plant growth-promoting effect of the biofertilizer comes from the carrier, such as cyclodextrin and humic acid, or from the combined effect of the carrier and GYH20240327-1, requires further mechanistic study. In future experimental design and grouping, biofertilizer carriers containing organic fertilizer but not containing GYH20240327-1 will be used as independent groups for study. At the same time, the colonization effects of GYH20240327-1 in the rhizosphere of two medicinal plants are also worth studying. Subsequently, our research group will use marker genes to modify GYH20240327-1 into an engineered strain, measure the colonization of the engineered strain GYH20240327-1 in the rhizosphere soil of the two medicinal plants, and use metagenomic whole-genome sequencing technology to analyze more deeply the impact of strain GYH20240327-1 on the rhizosphere soil microecology of *P. grandiflorus* and *S. baicalensis*. This project will also study the nutrients and basic physicochemical properties of the plant rhizosphere soil, such as soil pH, radioactivity, exchangeable sodium, and soil enzyme activity. In terms of sample testing, sufficient experimental groups and sample sizes will be added to enhance the reliability of the data statistics. In summary, based on this study, future research could explore the colonization ability of the GYH20240327-1 biofertilizer in the cultivation of *P. grandiflorus* and *S. baicalensis*, as well as its effects on various functional indicators of plant growth, including plant growth hormones, compounds with pharmacological activity, and tolerance to biotic and abiotic stresses. This will help to gain a more comprehensive

understanding of the potential of GYH20240327-1 as a biofertilizer.

Conclusions

Microbial fertilizers have important economic and environmental value for a variety of cash crops, including medicinal plants, as they can improve soil structure and physicochemical properties, enhance crops' stress resistance and quality. This study examined the role of *B. subtilis*, isolated from yogurt, as a PGPR functioning as a biofertilizer, and did a preliminary investigation into its effects on the growth and development of *Platycodon grandiflorus* and *Scutellaria baicalensis*, as well as on their rhizosphere microbes and metabolites. In summary, soil microbial community analysis showed that after the application of microbial compound fertilizer, there was no significant difference in the microbial community diversity or composition in the rhizosphere soil of the two medicinal plants, *P. grandiflorus* and *S. baicalensis*, which may be related to the limitation of insufficient sample size in this study. The most dominant bacterial phyla across all samples were *Acidobacteriota*, *Proteobacteria*, *Actinobacteriota*, *Gemmatimonadota*, and *Chloroflexi*, while the most prominent fungal phyla were *Ascomycota*, *Basidiomycota*, and *Mortierellomycota*. LEfSe analysis of bacterial and fungal communities indicated that the key fungal biomarkers differed between *P. grandiflorus* and *S. baicalensis*. The main differentially expressed metabolites identified included adenine, 3-dehydrosphinganine, epiandrosterone, 2-pyrocatechuic acid, sphinganine, aesculin, deoxycorticosterone acetate, D-ribose 5-phosphate, stearolic acid, anabasine, linoleic acid, and lysoPA(16:0/0:0). The soil metabolites of *P. grandiflorus* and *S. baicalensis* were significantly enriched in several metabolic pathways, including biosynthesis of plant hormones, plant hormone signal transduction, synaptic vesicle cycle, linoleic acid metabolism, biosynthesis of plant secondary metabolites, steroid degradation, and vitamin B6 metabolism. This study focused exclusively on the application of *B. subtilis* as a PGPR for fertilizing *P. grandiflorus* and *S. baicalensis*, preliminarily exploring the effects of *B. subtilis* strain GYH20240327-1 on the growth and development of these two medicinal plants, and investigating the differences in soil metabolites and microbial communities of the two plants. Further research is necessary to explore the relationships between key metabolites and microbial communities in *P. grandiflorus* and *S. baicalensis*, and to fully evaluate the potential of *B. subtilis* GYH20240327-1 as a biofertilizer.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s13213-026-01850-0>.

Supplementary Material 1.

Authors' contributions

All authors contributed to the study conception and design. Conceptualization: Haibin Guan, Jian Shen; Methodology: Haibin Guan, Jian Shen, Ying Zhang, Xiao Qi, Ruiwen Shi; Formal analysis and investigation: Jing He, Na Sai, Yang Cao, Yue Zhang, Jing Zhao, Jianxia Gao; Writing - original draft preparation: Jing He, Na Sai, Yang Cao; Writing - review and editing: Jing He, Na Sai, Yang Cao, Ying Zhang, Xiao Qi, Ruiwen Shi, Haibin Guan, Jian Shen; Funding acquisition: Haibin Guan, Fengyu Han, Lingjiang Meng, Xiuling Yu; Resources: Haibin Guan, Fengyu Han, Lingjiang Meng, Xiuling Yu; Supervision: Ying Zhang, Xiao Qi, Ruiwen Shi. Data curation: Jing He, Na Sai, Yang Cao, Yue Zhang, Jing Zhao, Jianxia Gao. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding

This work was supported by the Inner Mongolia Autonomous Region Science and Technology Plan (Grant numbers 2022YFSJ0006), Inner Mongolia Autonomous Region Science and Technology Innovation Guidance Program (Grant numbers kcj-202212), the 2022 Inner Mongolia Health Science and Technology Plan Project (Grant numbers 202201184), the Inner Mongolia Medical University student innovation and entrepreneurship project (Grant numbers S202410132009 and S202410132012), and Inner Mongolia Medical University's 2024 University Student Scientific and Technological Innovation "Talent Cultivation" School of Pharmacy Sponsored Projects (Grant numbers ycpyxm202401 and ycpyxm202402).

Data availability

Data sets generated during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests

The authors have no relevant financial or non-financial interests to disclose.

Author details

¹College of Pharmacy, Inner Mongolia Medical University, Hohhot 010000, China

²Inner Mongolia Tianqi Zhong meng Pharmaceutical Co, Ltd, Chifeng 024000, China

Received: 15 November 2025 / Accepted: 16 March 2026

Published online: 30 March 2026

References

- Abdelrazig S, Safo L, Rance GA, Fay MW, Theodosiou E, Topham PD, Kim DH, Fernández-Castan   A (2020) Metabolic characterisation of *Magnetospirillum gryphiswaldense* MSR-1 using LC-MS-based metabolite profiling. *RSC Adv* 10(54):32548–32560. <https://doi.org/10.1039/d0ra05326k>
- Akinsemolu AA, Onyeaka H, Odion S, Adebajo I (2024) Exploring *Bacillus subtilis*: ecology, biotechnological applications, and future prospects. *J Basic Microbiol* 64(6):2300614. <https://doi.org/10.1002/jobm.202300614>
- Ali S, Akhtar MS, Siraj M, Zaman W (2024) Molecular communication of microbial plant biostimulants in the rhizosphere under abiotic stress conditions. *Int J Mol Sci*. <https://doi.org/10.3390/ijms25212424>
- An H, Liu L, He T, Chen X, Jin X, Zhou X, Gao W (2025) Preclinical evaluation of baicalin for the treatment of diabetic nephropathy: a systematic review and meta-analysis. *Planta Med*. <https://doi.org/10.1055/a-2615-8249>
- Bhardwaj I, Kumar V, Singh S, Sharma AJ, Kumari S, Bhardwaj N, Dulta K, Peter L, Verma R, Kumar N, Ahlawat YK, Malik A, Okla MK, Porcel R, Mulet JM, Jayabalan K (2025) Evaluation of stress-tolerant *Serratia* and *Enterobacter* as PGPR for nutrient solubilization and dose-dependent bioformulation to enhance tomato seedlings. *Plants*. <https://doi.org/10.3390/plants14142154>
- Bokulich NA, Kaehler BD, Rideout JR, Dillon M, Bolyen E, Knight R, Huttley GA, Gregory Caporaso J (2018) Optimizing taxonomic classification of marker-gene

- amplicon sequences with QIIME 2's q2-feature-classifier plugin. *Microbiome* 6:90. <https://doi.org/10.1186/s40168-018-0470-z>
- Cary SC, McDonald IR, Barrett JE, Cowan DA (2010) On the rocks: the microbiology of Antarctic Dry Valley soils. *Nat Rev Microbiol* 8(2):129–138. <https://doi.org/10.1038/nrmicro2281>
- Chandra A, Chandra P, Tripathi P (2021) Whole genome sequence insight of two plant growth-promoting bacteria (*B. subtilis* BS87 and *B. megaterium* BM89) isolated and characterized from sugarcane rhizosphere depicting better crop yield potentiality. *Microbiol Res* 247:126733. <https://doi.org/10.1016/j.micres.2021.126733>
- Dang M, Zhang J, Ren RX, Fu MM, Li KJ, Gao HQ, Zhang Q, Wang J, Sun J, Zhang XF, Wang CL, Zhang MM, Zhao CB (2025) *Platycodon grandiflorum* attenuates non-alcoholic fatty liver disease in rats through regulating inflammatory response via PPAR γ and TLR4/NF- κ B signaling pathway. *Biomed Chromatogr* 39(7):e70111. <https://doi.org/10.1002/bmc.70111>
- Diniz FV, Scherwinski-Pereira JE, Costa FHS, Carvalho CM (2025) Effects on plant physiology in response to inoculation of growth-promoting bacteria: systematic review. *Braz J Biol* 85:e287279. <https://doi.org/10.1590/1519-6984.287279>
- Dong W, Zang Q, Wang Y, Ma E, Ding W, Yan L, Hao F (2025) Synergistic effects of *Paenibacillus polymyxa* NBmelon-1 inoculation and grafting restructure of rhizosphere microbiome and enhanced disease resistance in melon self-rootstocks. *Microorganisms*. <https://doi.org/10.3390/microorganisms13061172>
- dos Santos Oliveira JAL, Matos EF, Giovana Gomes Ferreira Corniani, Natieli Jenifer Mateus, Coneglian CfdSR, Maria Eduarda Viero, Patricia Marcusso, Luana Thais Varize da Silva, Carlos Augusto Corniani Marcusso, Willian Martire (2025) Dose-dependent effects of *Bacillus subtilis* SB008 on root development, photosynthetic efficiency, and antioxidant activity in soybean plants. *Biocatal Agric Biotechnol* 70:103878. <https://doi.org/10.1016/j.bcab.2025.103878>
- Ezrari S, Mhidra O, Radouane N, Tahiri A, Polizzi G, Lazraq A, Lahlali R (2021) Potential role of rhizobacteria isolated from Citrus rhizosphere for biological control of Citrus dry root rot. *Plants (Basel)*. <https://doi.org/10.3390/plants10050872>
- Fan J, Rao W, Peng D, Wei T, Xing S (2025) Genome-wide identification of UGT gene family and functional analysis of PgUGT29 in *Platycodon grandiflorus*. *Int J Mol Sci*. <https://doi.org/10.3390/ijms26104832>
- Gao J, Wang H, Sun J, Tang H, Yang Y, Li Q (2025a) Highly efficient genome editing in *Bacillus subtilis* via miniature DNA nucleases IscB. *Synth Syst Biotechnol* 10(4):1215–1223. <https://doi.org/10.1016/j.synbio.2025a.06.012>
- Gao JX, Yue MY, Huang JZ, Guo ZY, Wang H, Deng LW, Ding WJ, Zhou H (2025b) The HIF-1 α signaling pathway: a key approach to revealing the effects of *Scutellaria baicalensis* Georgi against lipopolysaccharide-induced spontaneous abortion. *Phytomedicine* 145:157013. <https://doi.org/10.1016/j.phymed.2025b.157013>
- Gharsallah H, Cheffi M, Mallek R, Omri N, Triki MA, Öztöp MH, Zarai Z (2025) Anti-fungal potential of *Bacillus* strains: implications for biocontrol strategies in food safety and sustainable agriculture. *Front Microbiol* 16:1615252. <https://doi.org/10.3389/fmicb.2025.1615252>
- Goginyan V, Harutyunyan S, Stepanyan T, Khachatryan G, Bagiyany V, Hovhannisyan R, Harutyunyan B, Kinosyan M, Ghazanchyan N, Davidyany T, Verdyan A, Chitchyan K (2025) Effect of complex microbial preparation of free-living and symbiotic nitrogen-fixing bacteria for agricultural crops. *Braz J Biol* 85:e292171. <https://doi.org/10.1590/1519-6984.292171>
- Gonçalves OS, Fernandes AS, de Sousa TGG, Santana MF, Barreto CC (2025) From cultivation challenges of Acidobacteriota to biotechnological promises - unveiling what is needed to fully harness their potential. *World J Microbiol Biotechnol* 41(7):208. <https://doi.org/10.1007/s11274-025-04433-4>
- Gong X, Xu L, Langwig MV, Chen Z, Huang S, Zhao D, Su L, Zhang Y, Francis CA, Liu J, Li J, Baker BJ (2024) Globally distributed marine Gemmatimonadota have unique genomic potentials. *Microbiome* 12(1):149. <https://doi.org/10.1186/s40168-024-01871-4>
- Gupta R, Kumari A, Sharma S, Alzahrani OM, Noureldeen A, Darwish H (2022) Identification, characterization and optimization of phosphate solubilizing rhizobacteria (PSRB) from rice rhizosphere. *Saudi J Biol Sci* 29(1):35–42. <https://doi.org/10.1016/j.sjbs.2021.09.075>
- Hadjj Ibrahim A, Ben Ali M, Daoud L, Jilidi M, Akremi I, Hmani H, Feto NA, Ben Ali M (2022) Biopriming of durum wheat seeds with endophytic diazotrophic bacteria enhances tolerance to Fusarium head blight and salinity. *Microorganisms*. <https://doi.org/10.3390/microorganisms10050970>
- Horai H, Arita M, Kanaya S, Nihei Y, Ikeda T, Suwa K, Ojima Y, Tanaka K, Tanaka S, Aoshima K, Oda Y, Kakazu Y, Kusano M, Tohge T, Matsuda F, Sawada Y, Hirai MY, Nakanishi H, Ikeda K, Akimoto N, Maoka T, Takahashi H, Ara T, Sakurai N, Suzuki H, Shibata D, Neumann S, Iida T, Tanaka K, Funatsu K, Matsuura F, Soga T, Taguchi R, Saito K, Nishioka T (2010) MassBank: a public repository for sharing mass spectral data for life sciences. *J Mass Spectrom* 45(7):703–714. <https://doi.org/10.1002/jms.1777>
- Hussain A, Ahmad M, Nafees M, Iqbal Z, Luqman M, Jamil M, Maqsood A, Mora-Poblete F, Ahmar S, Chen JT, Alyemeni MN, Ahmad P (2020) Plant-growth-promoting *Bacillus* and *Paenibacillus* species improve the nutritional status of *Triticum aestivum* L. *PLoS One* 15(12):e0241130. <https://doi.org/10.1371/journal.pone.0241130>
- Ji MY, Bo A, Yang M, Xu JF, Jiang LL, Zhou BC, Li MH (2020) The pharmacological effects and health benefits of *Platycodon grandiflorus*-a medicine food homology species. *Foods*. <https://doi.org/10.3390/foods9020142>
- Johnson W Jr., Bergfeld WF, Belsito DV, Klaassen CD, Liebler DC, Marks JG Jr., Peterson LA, Shank RC, Slaga TJ, Snyder PW, Fiume MM, Heldreth B (2025) Safety assessment of *Scutellaria baicalensis*-derived ingredients as used in cosmetics. *Int J Toxicol* 44(2_suppl):124s–138s. <https://doi.org/10.1177/10915818251350378>
- Khan N, Ali S, Tariq H, Latif S, Yasmin H, Mehmood A, Shahid MA (2020) Water conservation and plant survival strategies of rhizobacteria under drought stress. *Agronomy* 10(11):1683. <https://www.mdpi.com/2073-4395/10/11/1683>
- Kieffer DA, Piccolo BD, Vaziri ND, Liu S, Lau WL, Khazaeli M, Nazertehrani S, Moore ME, Marco ML, Martin RJ, Adams SH (2016) Resistant starch alters gut microbiome and metabolomic profiles concurrent with amelioration of chronic kidney disease in rats. *Am J Physiol-Ren Physiol* 310(9):F857–871. <https://doi.org/10.1152/ajprenal.00513.2015>
- Korenblum E, Dong Y, Szymanski J, Panda S, Jozwiak A, Massalha H, Meir S, Rogachev I, Aharoni A (2020) Rhizosphere microbiome mediates systemic root metabolite exudation by root-to-root signaling. *Proc Natl Acad Sci U S A* 117(7):3874–3883. <https://doi.org/10.1073/pnas.1912130117>
- Kuwar OK, Kalia N (2025) Anti-inflammatory and antioxidant effects of baicalein: targeting Nrf2, and NF κ B in neurodegenerative disease. *Inflammopharmacology* 33(3):1303–1310. <https://doi.org/10.1007/s10787-025-01698-x>
- Kwon H, Moon J, Kim JH, Hillman PF, Velina S, Nam JW, Nam SJ, Choi I, Song KS, Kim GJ, Choi H (2025) Structure characterization and antibacterial, antifungal, and antiquorum-sensing activity of new metabolites from the lipophilic fractions of *Platycodon grandiflorum* root. *Arch Pharm (Weinheim)* 358(7):e70043. <https://doi.org/10.1002/ardp.70043>
- Lau ET, Tani A, Khew CY, Chua YQ, Hwang SS (2020) Plant growth-promoting bacteria as potential bio-inoculants and biocontrol agents to promote black pepper plant cultivation. *Microbiol Res* 240:126549. <https://doi.org/10.1016/j.micres.2020.126549>
- Li C, Cheng P, Zheng L, Li Y, Chen Y, Wen S, Yu G (2021) Comparative genomics analysis of two banana Fusarium wilt biocontrol endophytes *Bacillus subtilis* R31 and TR21 provides insights into their differences on phyto-beneficial trait. *Genomics* 113(3):900–909. <https://doi.org/10.1016/j.ygeno.2021.02.006>
- Liu PF, Zhao YK, Ma JN, Cao Y, Zhang MX, Yu J, Guan HB, Xing YS, Wang XQ, Jia X (2025) Impact of various intercropping modes on soil quality, microbial communities, yield and quality of *Platycodon grandiflorum* (Jacq.) A. DC. *BMC Plant Biol* 25(1):503. <https://doi.org/10.1186/s12870-025-06544-4>
- Liu Y, Dong Y, Shen W, Du J, Sun Q, Yang Y, Yin D (2023) *Platycodon grandiflorus* polysaccharide regulates colonic immunity through mesenteric lymphatic circulation to attenuate ulcerative colitis. *Chin J Nat Med* 21(4):263–278. [https://doi.org/10.1016/s1875-5364\(23\)60435-2](https://doi.org/10.1016/s1875-5364(23)60435-2)
- Lopes M, Dias-Filho MB, Gurgel ESC (2021) Successful plant growth-promoting microbes: inoculation methods and abiotic factors. *Front Sustain Food Syst*. <https://doi.org/10.3389/fsufs.2021.606454>
- Lu S, Brown RW, Li M, Chen M, Huang C, Lian J, Wu D, Jones DL (2025) Soil application of PE and PLA microplastics alter earthworm (*Eisenia nordenskiöldi*) gut bacterial community and soil microbiome-metabolome dynamics. *Environ Pollut* 383:126895. <https://doi.org/10.1016/j.envpol.2025.126895>
- Mahmud FMA, Islam MA, Rubel MH, Mukharjee SK, Kumar M, Bhattacharya P, Ahmed F (2023) Effects of halotolerant rhizobacteria on rice seedlings under salinity stress. *Sci Total Environ* 892:163774. <https://doi.org/10.1016/j.scitoten.2023.163774>
- Ma J, Jiang S, Kong L, Ma L, Wang X, Pan M, Li C, Huang S, Liu X, Ma W, Ren W (2025) Genome-wide analysis of the caffeoyl coenzyme A-O-methyltransferase (CCoAOMT) gene family in *Platycodon grandiflorus* (Jacq.) A. DC. and the potential regulatory mechanism in response to copper stress. *Int J Mol Sci*. <https://doi.org/10.3390/ijms26104709>
- Mukherjee A, Singh BK, Verma JP (2020) Harnessing chickpea (*Cicer arietinum* L.) seed endophytes for enhancing plant growth attributes and bio-controlling against *Fusarium* sp. *Microbiol Res* 237:126469. <https://doi.org/10.1016/j.micres.2020.126469>

- Mumtaz MZ, Ahmad M, Jamil M, Hussain T (2017) Zinc solubilizing *Bacillus* spp. potential candidates for biofortification in maize. *Microbiol Res* 202:51–60. <https://doi.org/10.1016/j.micres.2017.06.001>
- Nabati J, Yousefi A, Hasanfar A, Nemati Z, Kahrom N, Malakshahi Kurdestani A (2025) Enhancing chickpea yield through the application of sulfur and sulfur-oxidizing bacteria. *Sci Rep* 15(1):1169. <https://doi.org/10.1038/s41598-024-84971-3>
- Navarro-Reig M, Jaumot J, García-Reiriz A, Tauler R (2015) Evaluation of changes induced in rice metabolome by Cd and Cu exposure using LC-MS with XCMS and MCR-ALS data analysis strategies. *Anal Bioanal Chem* 407(29):8835–8847. <https://doi.org/10.1007/s00216-015-9042-2>
- Ndeddy Aka RJ, Babalola OO (2016) Effect of bacterial inoculation of strains of *Pseudomonas aeruginosa*, *Alcaligenes faecalis* and *Bacillus subtilis* on germination, growth and heavy metal (Cd, Cr, and Ni) uptake of *Brassica juncea*. *Int J Phytorem* 18(2):200–209. <https://doi.org/10.1080/15226514.2015.1073671>
- Nilsson RH, Ryberg M, Kristiansson E, Abarenkov K, Larsson KH, Kõljalg U (2006) Taxonomic reliability of DNA sequences in public sequence databases: a fungal perspective. *PLoS One* 1(1):59. <https://doi.org/10.1371/journal.pone.0000059>
- Nyimbo WJ, Fallah N, Mutombo AM, Xu H, Bin Q, Makungu JS, Lin W (2025) Exploring the change of coastal saline agroecosystem diversity, composition and predicted function of soil microbial community mediated by soybean and corn intercropping. *Front Plant Sci* 16:1427196. <https://doi.org/10.3389/fpls.2025.1427196>
- Ogata H, Goto S, Sato K, Fujibuchi W, Bono H, Kanehisa M (1999) KEGG: kyoto encyclopedia of genes and genomes. *Nucleic Acids Res* 27(1):29–34. <https://doi.org/10.1093/nar/27.1.29>
- Panneerselvam P, Senapati A, Kumar U, Sharma L, Lepcha P, Prabhukarthikeyan SR, Jahan A, Parameswaran C, Govindharaj GPP, Lenka S, Nayak PK, Mitra D, Sagarika MS, Thangappan S, Sivakumar U (2019) Antagonistic and plant-growth promoting novel *Bacillus* species from long-term organic farming soils from Sikkim, India. *3 Biotech* 9(11):416. <https://doi.org/10.1007/s13205-019-1938-7>
- Patyka M, Wang R, Honchar A, Patyka T, Khablak S (2025) Modulation of the rhizosphere microbiome structure and optimization of beneficial functions in winter wheat induced by *Bacillus subtilis*: a metagenomic and phenotypic study. *FEMS Microbiol Ecol* 101(11). <https://doi.org/10.1093/femsec/fiaf097>
- Qiao J, Yu X, Liang X, Liu Y, Borriss R, Liu Y (2017) Addition of plant-growth-promoting *Bacillus subtilis* P15-394 on tomato rhizosphere has no durable impact on composition of root microbiome. *BMC Microbiol* 17(1):131. <https://doi.org/10.1186/s12866-017-1039-x>
- Rasmussen JA, Villumsen KR, Ernst M, Hansen M, Forberg T, Gopalakrishnan S, Gilbert MTP, Bojesen AM, Kristiansen K, Limborg MT (2022) A multi-omics approach unravels metagenomic and metabolic alterations of a probiotic and synbiotic additive in rainbow trout (*Oncorhynchus mykiss*). *Microbiome* 10(1):21. <https://doi.org/10.1186/s40168-021-01221-8>
- Saeed Q, Xiukang W, Haider FU, Kucerik J, Mumtaz MZ, Holatko J, Naseem M, Kintl A, Ejaz M, Naveed M, Brtnicky M, Mustafa A (2021) Rhizosphere bacteria in plant growth promotion, biocontrol, and bioremediation of contaminated sites: a comprehensive review of effects and mechanisms. *Int J Mol Sci*. <https://doi.org/10.3390/ijms221910529>
- Shahid M, Singh UB, Khan MS, Singh P, Kumar R, Singh RN, Kumar A, Singh HV (2023) Bacterial ACC deaminase: insights into enzymology, biochemistry, genetics, and potential role in amelioration of environmental stress in crop plants. *Front Microbiol* 14:1132770. <https://doi.org/10.3389/fmicb.2023.1132770>
- Shen W, Jia W, Wu Q, Shen L, Wang Y (2025) Exosomes derived from baicalin-treated bone marrow mesenchymal stem cells inactivate the TLR4/MyD88/NF- κ B pathway to improve asthma. *Immunobiology* 230(4):153103. <https://doi.org/10.1016/j.jimbio.2025.153103>
- Song ZY, Yu WJ, Jin YJ, Zhou MF, He CX, Li Z, He JW, Chen Q, Li P, Yi Y, Cheng SW (2025) Baicalin alleviates pathological changes in Alzheimer's disease comorbid with type 2 diabetes: targeting metabolic dysregulation and neuroinflammation. *J Ethnopharmacol* 120352. <https://doi.org/10.1016/j.jep.2025.120352>
- Souza R, Ambrosini A, Passaglia LM (2015) Plant growth-promoting bacteria as inoculants in agricultural soils. *Genet Mol Biol* 38(4):401–419. <https://doi.org/10.1590/s1415-475738420150053>
- Stanić I, Čačković A, Orlić S (2025) Salinity negatively impacts protistan and fungal community stability and shapes assembly processes in freshwater ecosystems. *Environ Microbiol Rep* 17(5):e70209. <https://doi.org/10.1111/1758-2229.70209>
- Sud M, Fahy E, Cotter D, Brown A, Dennis EA, Glass CK, Merrill AH Jr, Murphy RC, Raetz CR, Russell DW, Subramaniam S (2007) LMSD: LIPID MAPS structure database. *Nucleic Acids Res* 35(Database issue):D527–532. <https://doi.org/10.1093/nar/gkl838>
- Sun C, Sun X, Chen Y, Wu Y, Xiang C, Wu S (2025) Platycodin D-mediated METTL16 downregulation promotes docetaxel treatment of prostate cancer by regulating ferroptosis. *BMC Cancer* 25(1):1042. <https://doi.org/10.1186/s12885-025-14291-w>
- Sunita R, Saleena LM, Vasudevan P, Nair S (2003) Biological suppression of rice diseases by *Pseudomonas* spp. under saline soil conditions. *Plant Soil* 251(1):73–82. <https://doi.org/10.1023/A:1022950811520>
- Thévenot EA, Roux A, Xu Y, Egan E, Junot C (2015) Analysis of the human adult urinary metabolome variations with age, body mass index, and gender by implementing a comprehensive workflow for univariate and OPLS statistical analyses. *J Proteome Res* 14(8):3322–3335. <https://doi.org/10.1021/acs.jproteome.5b00354>
- Wang N, Ding B, Zhang R, Chen H, Xie T, Bai S, Chen H, Pan X (2025a) The six-year decomposition of coarse woody debris drives shifts in soil fungal communities in subtropical forests. *Front Microbiol* 16:1544163. <https://doi.org/10.3389/fmicb.2025a.1544163>
- Wang Z, Li Z, Gao C, Jiang Z, Huang S, Li X, Yang H (2025b) *Bacillus Subtilis* as an excellent microbial treatment agent for environmental pollution: a review. *Biotechnol J* 20(4):e70026. <https://doi.org/10.1002/biot.70026>
- Want EJ, Masson P, Michopoulos F, Wilson ID, Theodoridis G, Plumb RS, Shockcor J, Loftus N, Holmes E, Nicholson JK (2013) Global metabolic profiling of animal and human tissues via UPLC-MS. *Nat Protoc* 8(1):17–32. <https://doi.org/10.1038/nprot.2012.135>
- Wishart DS, Tzur D, Knox C, Eisner R, Guo AC, Young N, Cheng D, Jewell K, Arndt D, Sawhney S, Fung C, Nikolai L, Lewis M, Coutouly MA, Forsythe I, Tang P, Shrivastava S, Jeroncic K, Stothard P, Amegbey G, Block D, Hau DD, Wagner J, Miniaci J, Clements M, Gebremedhin M, Guo N, Zhang Y, Duggan GE, Macinnis GD, Weljie AM, Dowlatabadi R, Bamforth F, Clive D, Greiner R, Li L, Marrie T, Sykes BD, Vogel HJ, Querengesser L (2007) HMDB: the Human Metabolome Database. *Nucleic Acids Res* 35(Database issue):D521–526. <https://doi.org/10.1093/nar/gkl923>
- Wu J, Qiu Y, Tian M, Wang L, Gao K, Yang X, Jiang Z (2025a) Flavonoids from *Scutellaria baicalensis*: promising alternatives for enhancing swine production and health. *Int J Mol Sci*. <https://doi.org/10.3390/ijms26083703>
- Wu J, Zhou J, Zhao Q, Yang C, Bai Y (2025b) Metagenomic analysis of microbial community dynamics in konjac rhizosphere during soft rot disease progression. *Appl Microbiol Biotechnol* 109(1):212. <https://doi.org/10.1007/s00253-025-13600-4>
- Xiang J, Suo M, Lan J, Ning S, Liu H, Hong W, Liang J, Zheng J, Zhu D, Mo J (2025) Novel carrier-free nanomedicine for regulating macrophage phenotype to amplify anti-tumor photoimmunotherapy. *Small* e05304. <https://doi.org/10.1002/smll.202505304>
- Xie Z, Chen Y, Xie J, Lei Y, Jia C, Liang Y, Wang H, Huang J (2025) Mechanistic insight into the enhanced anti-pulmonary hypertension efficacy of wogonin co-amorphous. *Pharmaceutics*. <https://doi.org/10.3390/pharmaceutics17060724>
- Zelena E, Dunn WB, Broadhurst D, Francis-McIntyre S, Carroll KM, Begley P, O'Hagan S, Knowles JD, Halsall A, Wilson ID, Kell DB (2009) Development of a robust and repeatable UPLC-MS method for the long-term metabolomic study of human serum. *Anal Chem* 81(4):1357–1364. <https://doi.org/10.1021/ac8019366>
- Zeng J, He Z, Wang G, Ma Y, Zhang F (2025) Interaction between microbiota and immunity: molecular mechanisms, biological functions, diseases, and new therapeutic opportunities. *MedComm* (2020) 6(7):e70265. <https://doi.org/10.1002/mco2.70265>
- Zhang H, Liu Y, Wang G (2019) Integrated use of maize bran residue for one-step phosphate bio-fertilizer production. *Appl Biochem Biotechnol* 187(4):1475–1487. <https://doi.org/10.1007/s12010-018-2874-4>
- Zhang J, Wen H, Wang S, Wang Y, Wang Z, Zheng X, Chen J, Yang B, Shan H (2025a) Integrative multi-omics analysis reveals the potential mechanism by which *Streptomyces pactum* Act12 enhances wheat root drought tolerance by coordinating phytohormones and metabolic pathways. *BMC Plant Biol* 25(1):707. <https://doi.org/10.1186/s12870-025-06746-w>
- Zhang J, Zhu X, Chen X, Liu H, Yan Z, Chen Z, Li Q, Yu W (2025b) Multi-target synergy against acute lung injury: systems pharmacology decoding Dahuang-Huangqin herb pair's therapeutic mechanism. *J Inflamm Res* 18:9413–9441. <https://doi.org/10.2147/jir.S527378>
- Zhang X, Dong B, He Q, Tan X, Li Y, Zhao K, Xu X, Zeng J (2025c) Baicalin of *Scutellaria baicalensis* inhibits *Mycobacterium abscessus* biofilm formation to restore

the antibiotics susceptibility. *Biofouling* 41(7):735–750. <https://doi.org/10.1080/08927014.2025c.2531139>

Zhi N, Chang X, Zha L, Zhang K, Wang J, Gui S (2025) *Platycodonis radix* polysaccharides suppress progression of high-fat-induced obesity through modulation of intestinal microbiota and metabolites. *Phytomedicine* 142:156653. <https://doi.org/10.1016/j.phymed.2025.156653>

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.