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Bacillus-Mediated Transcriptional and Metabolic Reprogramming Mitigates Root Rot in Coptis chinensis

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Article

Keywords: Coptis chinensis, Bacillus, Fusarium root rot, Nitrogen assimilation, Priming

Posted Date: January 23rd, 2026

DOI: https://doi.org/10.21203/rs.3.rs-8146810/v1

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Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at Scientific Reports on April 24th, 2026. See the published version at https://doi.org/10.1038/s41598-026-49863-8.

Abstract

The medicinal plant *Coptis chinensis* is highly susceptible to root rot caused predominantly by *Fusarium* spp., threatening yield and medicinal quality. Biological control agents (BCAs) from Bacillus are promising, yet their host mechanisms remain incompletely defined. We evaluated four *Bacillus* strains as BCAs in field-grown *C. chinensis* and profiled host roots by transcriptomics and metabolomics. Pre-inoculation with the BCA mixture reduced disease severity following *Fusarium solani* (Fs) challenge. Multi-omics analyses showed that BCA treatment activated genes linked to nitrogen assimilation, accompanied by increased glutamine (Gln) accumulation. Supplementing potato dextrose agar with exogenous Gln inhibited *F. solani* growth in a concentration-dependent manner at 7 d. Notably, Fs or BCAs alone elicited modest regulation of canonical defense genes, whereas BCA pre-inoculation followed by Fs robustly up-regulated defense modules across MAPK signaling, plant–pathogen interaction, and hormone signaling (JA, ET, SA), consistent with immune priming. BCAs mitigate root rot in *C. chinensis* by reprogramming nitrogen and amino acid metabolism and by priming host immunity. These findings inform BCA-based strategies for sustainable management of *C. chinensis* root rot.

1. Introduction

Rhizoma Coptidis (RC), the rhizome of the perennial medicinal herb *Coptis chinensis*, has been used for millennia in traditional Chinese medicine for heat-clearing, dampness-drying, and detoxification, and is a key component of classic formulae such as Huanglian Tang, Huanglian Jiedu Tang, and Huanglian Ejiao Tang [1, 2]. Modern pharmacology attributes broad activities to RC including antibacterial, antiviral, cardioprotective, anticancer, and potential anti-diabetic and anti-Alzheimer's effects [3, 4]. RC is widely used in East Asian (China, Japan, Korea, Malaysia, and Singapore) [5], with an annual demand of 3,500–4,000 tons that continues to rise [6]. China supplies roughly two-thirds of global output, approximately 90% of which comes from cultivation, with major production centers in Shizhu County (Chongqing) and Lichuan City (Hubei) [7]. As cultivation expands and fields are continuously cropped, disease pressure has intensified. Root rot is among the most severe problems, with reported incidence in Shizhu of ~ 40% and yield losses approaching 67% [8].

Root rot is a globally prevalent soil-borne disease that severely affects the growth and development of many plant species [9]. In China, since first reports in 1989, its distribution has expanded to Xinjiang, Gansu, Heilongjiang, Jilin, Guizhou, and Sichuan [10]. Losses can be substantial, for example, 5–20% annually in *Panax notoginseng* (reaching 70% or complete crop failure in severe cases) and 30–90% in cultivated American ginseng [11, 12]. Typical symptoms include root browning and decay, stunting, leaf chlorosis and wilting, and plant death [9]. Although bacteria and nematodes can contribute, fungi—especially *Fusarium* spp.—are recognized as principal causal agents across many medicinal plants, including *R. glutinosa*, *A. sinensis*, and *C. chinensis* [10].

Integrated management—combining precision field hygiene, judicious fungicide use, and biological control—offers the most practical approach because above-ground symptoms often appear late [9]. Microbial biocontrol has shown particularly promising efficacy and translational potential [13]. For example, strains isolated from *Angelica* rhizosphere (YF, SY89) produce IAA and siderophores, promote root growth, and reduce disease indices by more than 60% [14]. In addition, *Trichoderma harzianum*, *Pseudomonas putida*, *Bacillus pumilus*, and *Burkholderia cenocepaciaand* have been successfully used to suppress root rot in pea, maize, and other crops [15, 16].

Biocontrol agents mitigate disease via direct pathogen suppression and/or enhancement of plant resistance. Beneficial microbes can rapidly colonize plant surfaces, tissues, and the rhizosphere, occupying ecological niches and competing with pathogens for nutrients [17]. Many produce antibiotics, cell-wall-degrading enzymes, and other metabolites that directly inhibit pathogens [18, 19]. Exposure to non-pathogenic microbes commonly triggers defense responses [20, 21]. Microbes can also elicit the accumulation of defensive metabolites (e.g., phenolics, alkaloids, and flavonoids) [22, 23]. Increasingly, primary metabolites are recognized as important in plant defense [24]. For example, galactitol accumulates during pathogen infection and contributes to systemically induced resistance [25], threonine functions as a signaling molecule in *Arabidopsis* defense [26], and glutamate (Glu)—a principal amino-group donor for nucleotide and nitrogen-containing compound biosynthesis—has been implicated as a defense-related signal [27].

Previously, we profiled the rhizosphere and endophytic microbiomes of healthy versus diseased *C. chinensis* plants and observed a negative correlation between *Bacillus* and pathogenic *Fusarium*. Notably, four *Bacillus* strains isolated from healthy *C. chinensis* displayed pronounced antagonism against root rot pathogens including *F. solani* and *F. avenaceum* [28]. Whether these *Bacillus* strains effectively reduce root rot under field conditions, and by what mechanisms, remains unclear. Here, we assess their efficacy in preventing or ameliorating root rot and examine host transcriptomic and metabolomic responses to elucidate the underlying mechanisms.

2. Materials and methods

2.1 In vitro compatibility among four *Bacillus* strains

Four antagonistic strains—*B. mycoides* GJ-LB-021, *B. pseudomycoides* GJ-YEM-005, *B. velezensis* GJ-JM-1, and *B. subtilis* GJ-TR-064—were assayed pairwise on nutrient agar (NA) base plates (10 mL per plate). For each strain, 500 µL of an overnight culture was mixed with 10 mL semi-solid NA, gently swirled, and overlaid to form the indicator lawn [29]. The test strain (3-4 µL overnight culture) was spot-inoculated at the center; assays were performed reciprocally for each pair. Plates were incubated at 37 °C for 24 h and inspected. Compatibility was inferred from the presence or absence of a clear inhibition zone around the central colony.

2.2 Microbial strains and culture conditions

Preparation of *Fusarium solani* (Fs) spore suspension: Fs was inoculated onto potato dextrose agar (PDA) plates and incubated in darkness for 3 days. A single colony was then transferred to potato dextrose broth (PDB) and cultured at 25 °C with shaking at 120 rpm for 4 days. The resulting culture was filtered through four layers of sterilized lens paper to obtain a clarified filtrate. Spores were collected by centrifugation at 6,000 rpm for 3 min, washed twice with sterile sterile PBS buffer to remove residual impurities, and resuspended in sterile PBS to 1×10^7 cfu/mL.

Preparation of the biological control agents (BCAs): Single colonies of each *Bacillus* strain were grown in NA liquid medium broth at 37 °C, 120 rpm for 24 h. Cells were harvested (6,000 rpm), washed twice with sterile PBS, and adjusted to $OD_{600} = 1$. The four strains were then mixed in equal proportions.

2.3 Field site, design, and sampling

The experimental plants were one-year-old cultivated *Coptis chinensis* (voucher specimen deposited in the Artificial Climate Chamber of Chengdu University of Traditional Chinese Medicine, identified by Prof. Yuntong Ma, voucher No. WWS-GP-240608-001), supplied by Wa'wu Mountain Pharmaceutical Co., Ltd. The field trials were conducted at the company's Coptis cultivation base in Group 2, Heshan Village, Gaomiao Town, Hongya County, Meishan City, Sichuan Province, China (29°29'10.91" N, 103°9'39.9" E). The area was partitioned into 12 plots in a 4×3 grid, each containing about 180-200 healthy plants. Four treatments were applied: (1) water control (CK); (2) Fs only; (3) BCAs only (BCA); (4) BCA pre-inoculation followed 7 d later by Fs (BCAFs). Ten milliliters of water or inoculum were injected into the surrounding soil by syringe once per plant [30]. Disease index were recorded at day 7 for Fs and day 14 for CK, BCA, and BCAFs. Fifteen plants per treatment were scored; five plants per treatment were sampled for root transcriptomics and metabolomics.

2.4 Disease index assessment

Following published methods [31], 15 plants per treatment were scored on a six-point scale (0–5):

Grade 0: Rhizomes and fibrous roots intact; no visible lesions; no leaf wilting.

Grade 1: Scattered lesions confined to fibrous roots; trace infection; no leaf wilting.

Grade 2: ≤25% of fibrous roots infected (mild); no leaf wilting.

Grade 3: >25% to ≤50% of fibrous roots infected; no leaf wilting.

Grade 4: >50% to ≤75% of fibrous roots infected, with leaf wilting in ≤25% of leaves.

Grade 5: >75% of fibrous roots infected, rhizomes rotted, with ≥50% of leaves wilted.

The disease index was calculated using the following formula:

$$\text{Disease Index}=\frac{\sum \text{grade}\times\text{number of plants of that grade}}{5\times\text{Total number of plants}}\times 100\% \quad (1)$$

2.5 Metabolomic analysis of *C. chinensis* roots

Metabolites were extracted, identified and quantified by Wekemo Tech Group Co., Ltd. (Shenzhen, China) with five biological replicates per treatment using LC–MS/MS system (Ultra-High Performance Liquid Chromatography, Thermo Vanquish; Mass Spectrometer, Thermo Q Exactive series). The LC and MS conditions, as well as the qualitative and quantitative analyses of metabolites, were set as described by Cao et al. [32]. Principal component analysis (PCA) and orthogonal partial least-squares discriminant analysis (OPLS-DA) were performed to assess global metabolic variations among samples. Differentially accumulated metabolites (DAMs) were selected using the criteria variable importance in projection (VIP)≥1, FDR<0.05 and fold change (|FC|)≥1.2.

2.6 Transcriptomic analysis of *C. chinensis* roots

RNA-seq (Illumina HiSeq) was performed by Wekemo Tech Group Co., Ltd. (Shenzhen, China) on four groups with five biological replicates each. The downstream analyses were performed through the company's online pipeline [33]: (1) adapters and low-quality bases were trimmed with fastp (v0.21.3) to obtain clean data; (2) gene-level read counts were quantified with featureCounts (v2.0.1) and normalized to fragments per kilobase of transcript per million mapped reads (FPKM); (3) differentially expressed genes (DEGs) were identified with DESeq2 (v1.26.0) using |FC|≥1.2 and FDR<0.05; (4) kyoto encyclopedia of genes and genomes (KEGG) was used to analyze pathways mainly involved in the DEGs [34, 35]. All KEGG pathway maps used in this study were obtained from the KEGG database (www.kegg.jp/kegg/kegg1.html).

2.7 The detection of quantitative real-time PCR (qRT-PCR)

Total RNA was extracted from *C. chinensis* roots using a plant RNA extraction kit (Takara Biomedical Technology, Beijing Co., Ltd.) following the manufacturer's instructions. First-strand cDNA was synthesized with HiScript II Q RT SuperMix (Beijing Qingkai Biotechnology Co., Ltd.). RT-qPCR was performed in 2×RealStar Green Fast Mix (BioRad, Shanghai, China) on a CFX96 Touch Real-Time PCR System (Thermo Fisher Scientific). The *C. chinensis* 18S gene was used as an internal reference for normalization. Gene-specific primers (Supplementary Table S1) were designed using Primer 5.0, and relative expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method.

2.8 Effects of metabolites on the growth of Fs

Following the method described by Chen, H. F. et al. [36], L-Glutamine (L-Gln) stock was added to PDA cooled to approximately 60℃, to yield final concentrations of 1.0, 2.0, 3.0, 5.0, and 8.0 mmol/L. Mycelial plugs (8 mm diameter) were cut from the actively growing margin of 7-day Fs cultures and placed on the amended PDA plates and incubated at 28℃. Colony diameters were measured at 3 and 7 days. PDA

without L-Gln served as control. Data are mean ± SD (n = 5). One-way ANOVA (IBM SPSS Statistics 27) was used with significance at $P < 0.05$.

3. Results

3.1 *Bacillus* strains partially attenuate *C. chinensis* root rot in the field

The four *Bacillus* isolates showed no mutual inhibition in vitro (Supplementary Fig. S1), supporting their use as a mixed inoculum. In field trials, above-ground tissues exhibited no visible symptoms across treatments, whereas Fs-inoculated roots displayed browning and decay (Fig. 1). Fs alone caused mild disease (DI = 6.67%; Supplementary Table S2), while BCAs alone caused none. Notably, pre-inoculation with BCAs followed by Fs reduced DI to 4.00%.

Figure 1.

3.2 Metabolomic profiles associated with BCA-mitigated *C. chinensis* root rot

Across treatments, 533 metabolites spanning 13 classes were detected; lipids (18.6%), flavonoids (11.1%), terpenoids (10.7%), carbohydrates and derivatives (9.8%), and organic acids (9.4%) predominated (Fig. 2A). Principal-component analysis (PCA) clearly separated the four treatments (Fig. 2B), with PC1 and PC2 jointly explaining 30.56% of the total variance. Relative to CK, Fs increased 18 metabolites (Fig. 2C, D), notably lipids (e.g., LPA 18:2, LPI 18:3, 9-HpOTrE) and carbohydrates (e.g., D-gossypose, trehalose, stachyose). BCA treatment evelated 23 metabolites (Fig. 2C, E), including amino acids/derivatives (L-Gln, 5-oxoproline, D-phenylalanine), sugars, and lipids (e.g., 13(S)-HOTE, 9-HpODE, 9-HpOTrE, methyl jasmonate), while decreasing certain phenolic acids (e.g., 5-*O*-caffeoylshikimic acid). Compared with Fs alone, BCAFs further increased L-Gln, 5-oxoproline, D-(-)-Lyxose, and N-Acetylglucosamine, while decreasing lipids such as LPA 18:2, arachidonic acid, and eleostearic acid (Fig. 2F).

Figure 2.

3.3 Transcriptomic reprogramming underlies BCAs effects

To further elucidate how biocontrol agents alleviate *C. chinensis* root rot, we performed transcriptome profiling. In total, 20 cDNA libraries were constructed, yielding 40,401,024–74,075,838 clean reads per library (Supplementary Table S3). Q20 and Q30 exceeded 97.29% and 91.51%, respectively, and the GC content ranged from 41.08% to 42.50% (Supplementary Table S3). PCA clearly separated roots transcriptomes by microbial treatment, with PC1 and PC2 explaining 23.5% and 11.06% of the variance, respectively (Supplementary Fig. S2). Differential expression analysis revealed that Fs triggered 469 up-regulated and 277 down-regulated genes (Fig. 3A, Supplementary Table S4). Notably, pre-inoculation

with BCAs followed by Fs (BCAFs) yielded 1,887 up-regulated and 1,279 down-regulated genes (Fig. 3E, Supplementary Table S4), indicating robust transcriptional reprogramming upon sequential exposure to beneficial and pathogenic microbes.

KEGG pathway enrichment showed that Fs predominantly affected the ribosome, flavonoid/flavonol biosynthesis, and fatty-acid biosynthesis/metabolism (Fig. 3B, Supplementary Table S5). In contrast, BCAs enriched monoterpenoid biosynthesis, motor proteins, arginine biosynthesis, and valine/leucine/isoleucine degradation (Fig. 3D, Supplementary Table S5). Relative to Fs alone, BCAFs further enriched terpenoid biosynthesis, alanine/aspartate/glutamate metabolism, valine/leucine/isoleucine degradation, MAPK signaling, and plant-hormone signaling (Fig. 3F, Supplementary Table S5).

We next examined DEGs involved in nitrogen and amino acid metabolism. In the BCA group, genes associated with nitrogen assimilation and Glu metabolism were markedly enriched (Fig. 4A), including *NRT2*, *NR*, *NiR*, and *GDH*, which facilitate primary nitrogen assimilation and Gln synthesis—consistent with the observed Gln accumulation. Following sequential BCA pre-inoculation and subsequent Fs challenge, nitrogen-assimilation genes were down-regulated, whereas *GLU*- and *GAD*-annotated genes (involved in Glu and γ-aminobutyric acid metabolism) were up-regulated, indicating pronounced modulation of N and amino-acid metabolism under sequential inoculations. The qRT-PCR corroborated these trends, showing significantly elevated *NRT2*, *NiR*, and *GDH* transcripts under BCA treatment, whereas *GAD* and *GLU* transcripts were highest in the BCAFs group (Fig. 4B).

Figure 3.

Figure 4.

3.4 Transcriptional-metabolic connectivity elicited by BCA inoculation

Given the central roles of nitrogen and amino acid pathways in disease mitigation, we correlated DAMs with DEGs in these modules. Integrating the transcriptomic and metabolomic datasets (Fig. 5) revealed that the relative abundances of two amino acid related compounds—L-Gln and 5-oxoproline—were significantly correlated with the transcript levels of seven differentially regulated genes: one *NRT2*, one *NR*, three *GDH*, one *GLT1*, and one *NiR*. These metabolites and their cognate genes therefore represent potential hub components of the nitrogen and amino acid metabolism circuitry that modulates the progression of *C. chinensis* root rot.

Figure 5.

3.5 Effect of exogenous glutamine on Fs growth

Building on these results, we assessed the effect of exogenous Gln on Fs colony growth on PDA plates. After 3 d of incubation, colony diameters at all five Gln concentrations did not differ significantly from the

control (CK; Fig. 6, Supplementary Table S6; $P > 0.05$). By day 7, however, the 3.0 mmol/L Gln yielded a significantly smaller colony than CK ($P < 0.05$), and higher concentrations produced progressively stronger inhibition, indicating a concentration-dependent effect.

Figure 6.

4. Discussion

4.1 BCAs confer protective effects against *C. chinensis* root rot

Previous work showed that co-inoculation of Fs with *Bacillus* spp. on detached *C. chinensis* roots reduced disease severity [28]. In the present field study, mild symptoms appeared 7 d after Fs inoculation, whereas pre-inoculation with BCAs 7 d earlier partially alleviated root-rot symptoms, indicating that BCAs can likewise exert protective effects under field conditions. Although a statistically significant reduction in disease was not observed within the limited observation window, the pronounced transcriptomic and metabolomic reprogramming of *C. chinensis* following BCA treatment supports a positive impact on host defense.

The four *Bacillus* strains used here are widely regarded as plant-beneficial. They can suppress pathogens, induce systemic resistance, produce IAA, enhance photosynthetic performance, and mitigate abiotic stress [37–39]. Consistent with these traits, no disease symptoms were observed in plants receiving BCAs alone during the 14-day period. Taken together, our results suggest that BCAs have potential for managing *C. chinensis* root rot, while also highlighting the need for further work—e.g., extended monitoring, additional evaluation metrics, and exploration of growth-promoting outcomes—to comprehensively assess their biocontrol value.

4.2 Positive roles of BCA-induced Gln accumulation in *C. chinensis* defense

In this study, BCA inoculation significantly increased Gln levels together with the up-regulation of related genes. To evaluate the biological relevance of this metabolite, we supplemented Fs culture medium with exogenous Gln; Gln significantly suppressed fungal growth in a concentration-dependent manner. Prior work similarly shows that glutamate (Glu) inhibits multiple phytopathogens [40]. Consistent with these observations, Gln can also restrain pathogen proliferation and virulence: exogenous Gln markedly limits *Magnaporthe oryzae* growth and inhibits appressorium formation [36]. In the cotton-wilt pathogen *F. oxysporum* f. sp. *vasinfectum*, among the amino acids tested, Gln and Glu selectively inhibit the naphthoquinone polyketides bikaverin (a nonaketide) and nectriafurone (a heptaketide) [41]. Moreover, exogenous Gln has been shown to suppress bacterial virulence and growth while bypassing MAMP perception and SA signaling [42]. Because Gln and Glu are coupled through the GS/GOGAT cycle- cytosolic Gln synthetase (*GS1*) assimilates ammonium into Glu to form Gln, which is then converted back to Glu

by glutamate synthase [27]. Given their tight coupling, future work should disentangle their respective contributions to *C. chinensis* resistance.

Beyond direct antifungal effects, Gln likely contributes to energy provisioning, as the GS/GOGAT cycle is central to primary nitrogen metabolism and connects to the TCA cycle [27]. Adequate nitrogen supplies essential building blocks for resisting or recovering from damage, where nitrogen deficiency weakens plants and increases pathogen susceptibility [43]. Many antimicrobial proteins are nitrogen-rich (e.g., PR proteins). Overexpression of chitinase genes enhances resistance to phytopathogens in rice and lily [44, 45], and chitinase activity depends on N availability [46, 47]. In tomato, *PR4* expression is repressed under N limitation and induced under N sufficiency, correlating with resistance to *Botrytis cinerea* [48]. Multiple amino acids feed the TCA cycle as α-ketoglutarate, acetyl-CoA, and succinyl-CoA, supplying substrates for respiration during the energy-intensive defense response [49].

Our data align with this framework: BCA inoculation up-regulated *NRT2*, *NR*, *NiR*, *GS1*, *GLT1*, and *GDH*, indicating activation of primary N assimilation, thereby facilitating nitrogen accumulation. Following subsequent Fs challenge, *GLU* and *GAD*-annotated genes were induced, indicating diversion from Gln to Glu and then to γ-aminobutyric acid (GABA). In tomato ABA-deficient mutants, activation of the GS/GOGAT cycle and the GABA shunt sustains TCA intermediates, maintaining the viability of cells surrounding *B. cinerea* lesions and ultimately strengthening resistance [50]. Collectively, these findings support a model in which BCA pre-inoculation primes the host by accelerating N assimilation and pre-accumulating C–N skeletons and ATP, eenabling a rapid and effective defense upon pathogen attack. Consistent with this model, recent studies show that exogenous Glu alone can reprogram C–N metabolism and induce the accumulation of pathogenesis-related proteins, thereby markedly suppressing microbial pathogens [51, 52]. These observations underscore the need to define, mechanistically, how *Bacillus*-triggered Gln accumulation rewires C–N metabolism to confer disease resistance in *C. chinensis*.

4.3 Pre-inoculation with BCAs primes defense responses in *C. chinensis*

Upon microbial attack, plants typically activate two tiers of innate immunity: PAMP-triggered immunity (PTI) and effector-triggered immunity (ETI) [53]. In this study, the BCAFs treatment exhibited strong up-regulation of multiple genes within the MAPK and plant-pathogen interaction pathways (Supplementary Fig. S3A), eliciting a more robust defense response than inoculation with either Fs or BCAs alone. Pathogens can deploy effectors to suppress PTI and establish infection, whereas beneficial microbes may transiently and locally attenuate PTI to avoid self-targeting and to establish mutualism [54, 55]. In compatible interactions, *Fusarium* can suppress host defenses to facilitate intimate colonization [56], which may explain the relatively weak responses observed with Fs alone. In contrast, colonization by beneficial microbes can shift plants into a primed physiological state, enabling faster and stronger responses to subsequent attack [57].

Consistent with this paradigm, in olive, neither *F. solani* Fso14 nor *Trichoderma harzianum* Ths97 alone markedly altered the expression of many immune-related genes (oxidative-stress responses, phenylpropanoid pathway, PR proteins, and JA/Et-SA hormonal status), whereas co-inoculation significantly up-regulated multiple defense genes [58]. Similarly, we observed enhanced expression of ET-, JA-, and SA-pathway components (e.g., *ERF1*, *PDF1.2*, *MYC2*, *TGA*, *PR1*) in BCAFs plants (Supplementary Fig. S3B). Systemic acquired resistance (SAR) is canonically mediated by SA, whereas induced systemic resistance (ISR) is predominantly governed by JA and ET. Together, these phytohormones constitute a core signaling network that coordinates plant defense against biotic stress by engaging key nodes of the induced-resistance circuitry [59]. Although SA- and JA-dependent signaling have traditionally been viewed as mutually antagonistic [60], accumulating evidence indicates that beneficial microbes can simultaneously activate both pathways, resulting in cooperative engagement of SAR and ISR [61].

Our data support a model in which pre-inoculation with BCAs establishes a root-localized primed state in *C. chinensis* that, upon challenge with Fs, activates multiple defense-signaling cascades. Because defense-gene activation coincided with reduced disease, and ISR often confers broad-spectrum resistance to biotic and abiotic stresses, future work will focus on further evaluating BCA-based immune induction and dissecting the physiology of the *Coptis–Fusarium–Bacillus* tripartite interaction.

5. Conclusion

This study demonstrates that BCA inoculation exerts biocontrol activity against *Fusarium*-induced root rot in *C. chinensis* and provides insight into the underlying mechanisms. We show that BCAs contribute to disease suppression in part by inducing accumulation of Gln, which directly inhibits Fs growth. BCA treatment strengthens host N assimilation, thereby pre-accumulating Gln as a nitrogen source and supporting energy demands of defense. In addition, BCAs appear to act as priming agents that, upon subsequent Fs infection, markedly activate PR proteins and the JA, SA, and ET signaling pathways, thereby enhancing resistance. While these findings provide initial mechanistic evidence, further work is required to assess the robustness of BCA-mediated control across environments and to delineate the detailed regulatory networks involved.

Declarations

Conflict of Interest:

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding:

This work was supported by National Natural Science Foundation of China (82474036) and Sichuan Natural Science Foundation (2024NSFSC2111).

Author Contribution

Y.T.M., N.L, and H.L.L. carried out the design of this research work and writing this paper. N.L, H.L.L, W.J.K., and J.J.D. carried out the transcriptome and metabolome analysis of this work, and C.Z, F.F.X, and Y.X. participated in experiment management and manuscript revision. T.Z. and B.J.X. participated in the revision of the manuscript.

Acknowledgement

We are grateful to all those who contributed to this study and to the funding.

Data Availability

The raw sequence data reported in this paper have been deposited in the Genome Sequence Archive (Genomics, Proteomics & Bioinformatics 2025) in National Genomics Data Center, China National Center for Bioinformation / Beijing Institute of Genomics, Chinese Academy of Sciences (GSA: CRA033710) that are publicly accessible at https://ngdc.cncb.ac.cn/gsa.

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Figures



Figure 1

Preventive efficacy of BCAs against *C. chinensis* root rot caused by Fs. (A) Water-treated for 14 days. (B) Inoculated with *Fusarium solani* (Fs) for 7 days. (C) Treated with biocontrol agents (BCAs) for 14 days. (D) Pre-inoculated with BCAs for 7 days, then challenged with Fs for 7 days.

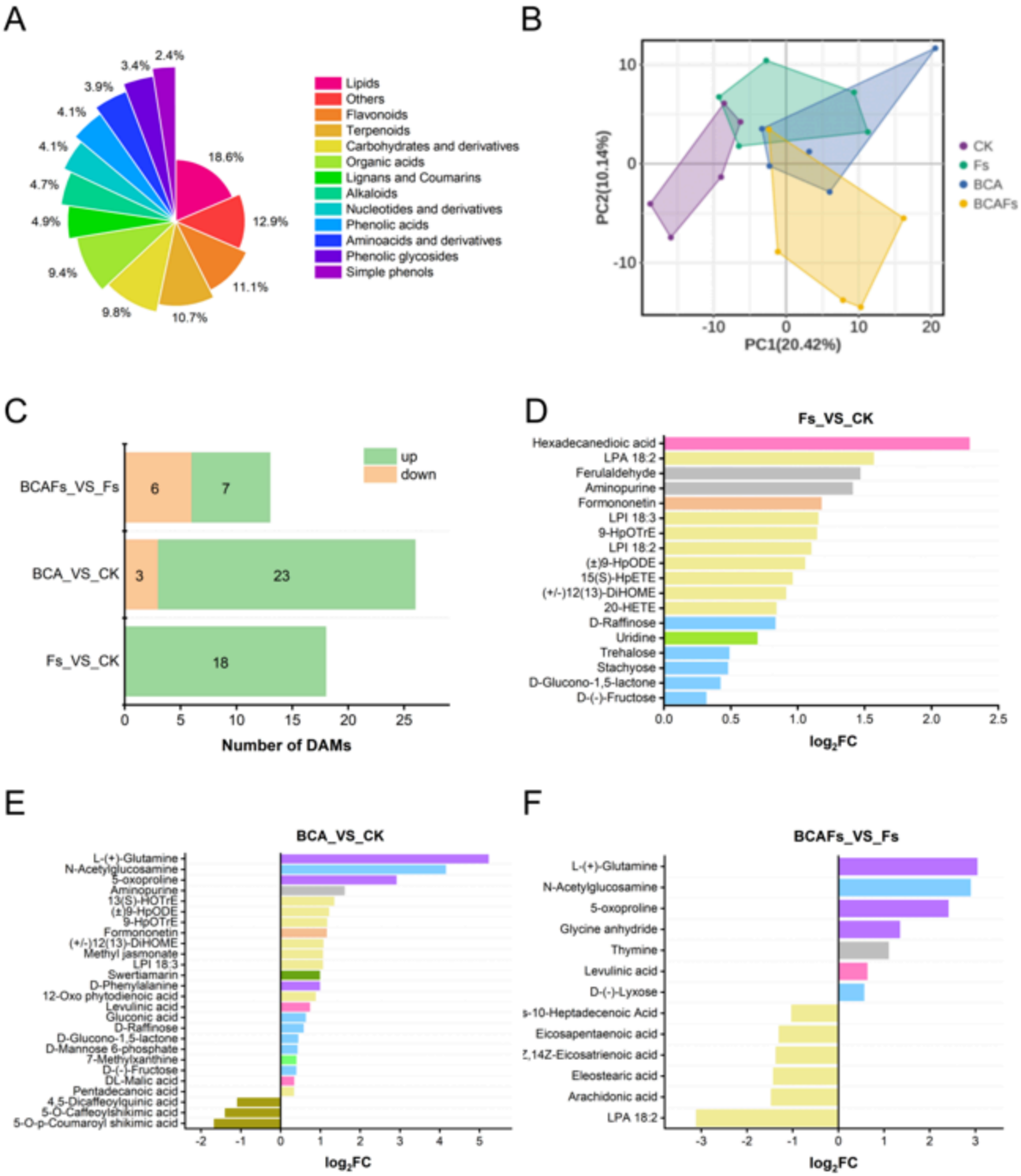

Figure 2

Effects of the inoculation with Fs and BCAs on the metabolism of *C. chinensis* root. (A) Chemical classification of all identified metabolites. (B) Principal component analysis. (C) The numbers of DAMs. (D) Difference multiple analysis of differential metabolites in the comparison group of Fs and CK. (E) Difference multiple analysis of differential metabolites in the comparison group of BCA and CK. (F) Difference multiple analysis of differential metabolites in the comparison group of BCAFs and Fs.

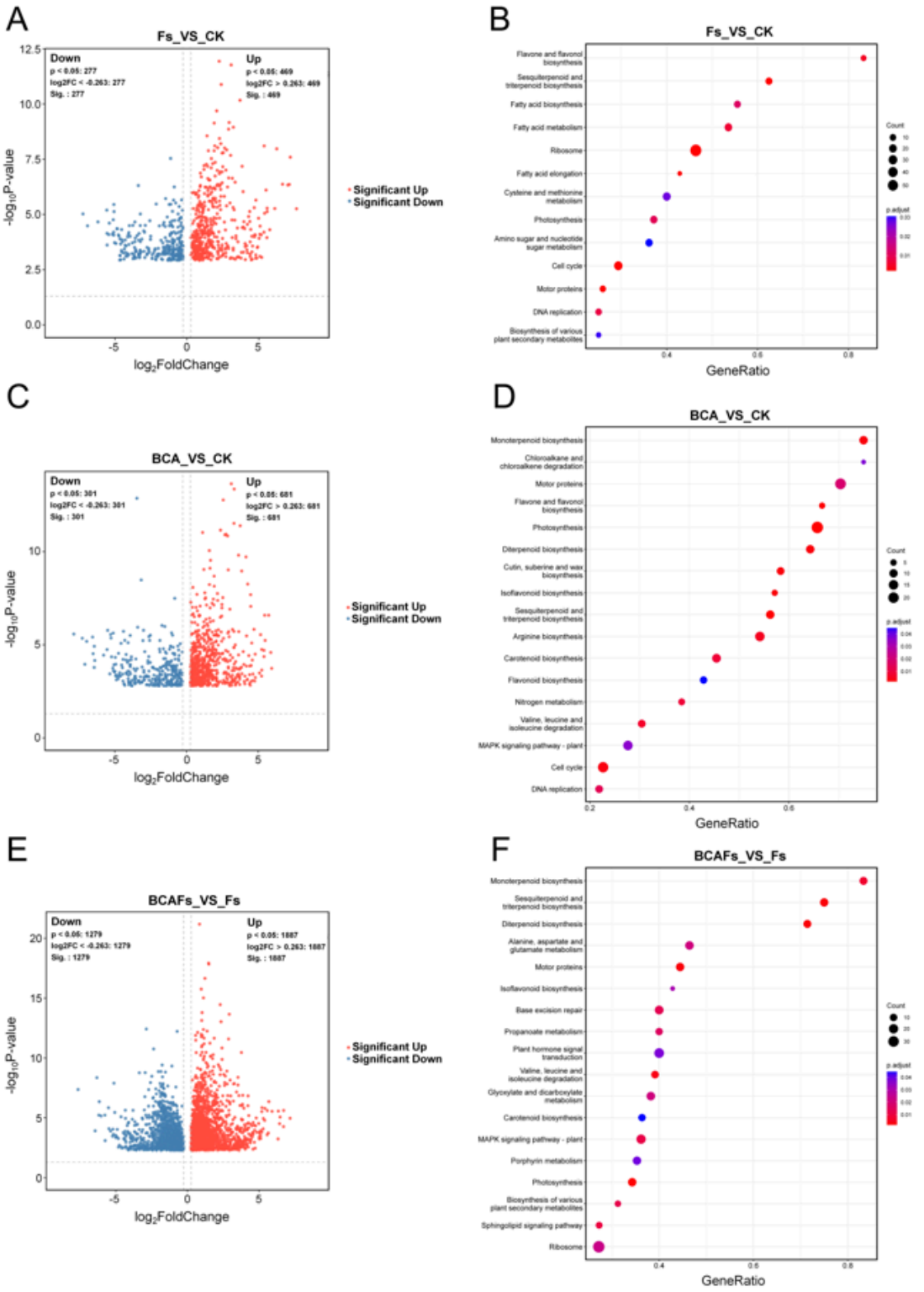

Figure 3

Profile of DEGs in *C. chinensis*responsive to Fs and BCA colonization. (A) Volcano plot of DEGs in Fs vs CK group. (B) DEGs enriched in KEEG pathway in Fs vs CK group. (C) Volcano plot of DEGs in BCA vs CK group. (D) DEGs enriched in KEEG pathway in BCA vs CK group. (E) Volcano plot of DEGs in BCAFs vs Fs group. (F) DEGs enriched in KEEG pathway in BCAFs vs Fs.

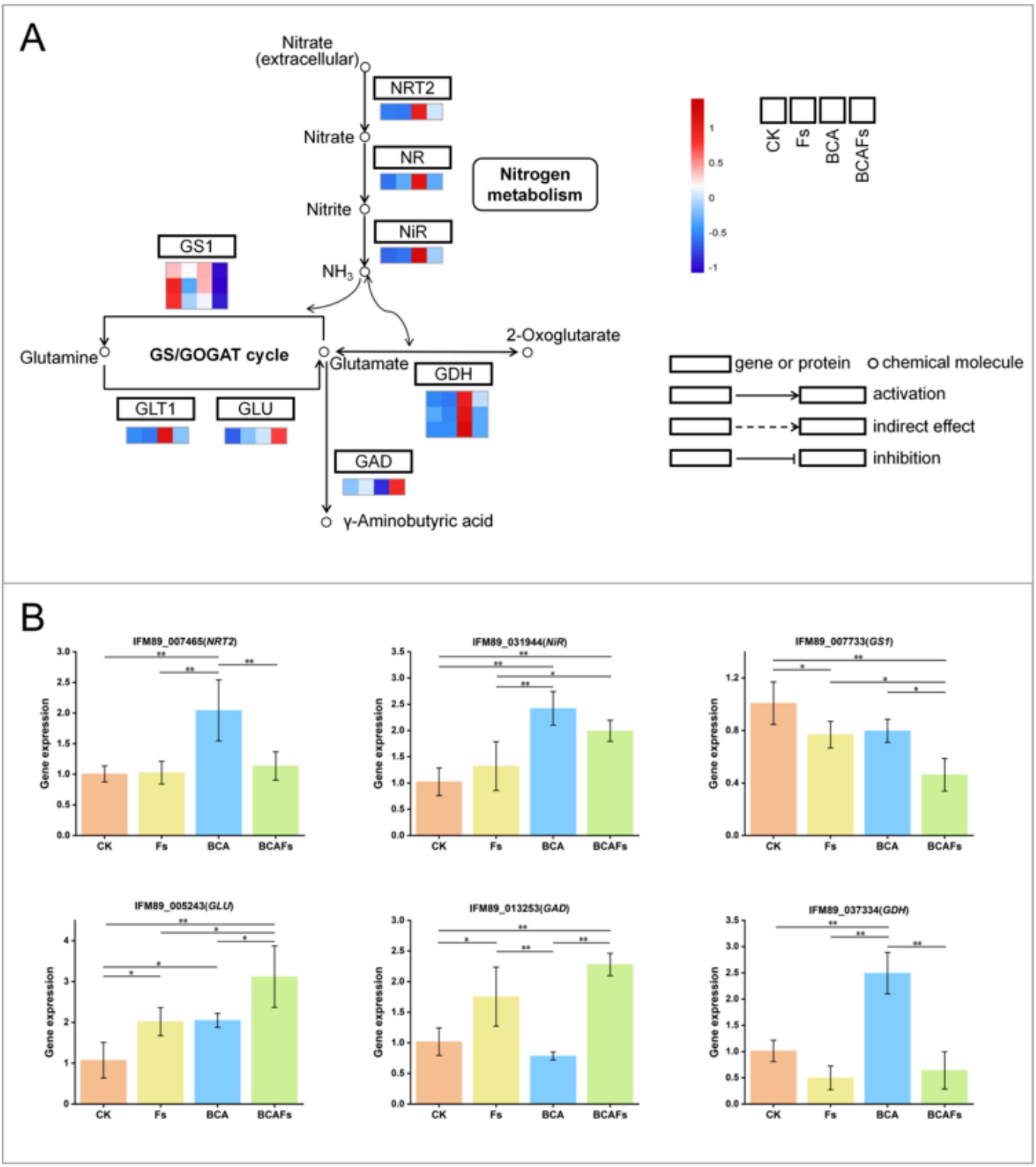

Figure 4

The expression of DEGs is involved in amino acid metabolism and nitrogen metabolism. (A) Amino acid metabolism and nitrogen metabolism signaling pathway. (B) The transcription levels of amino acid and nitrogen related genes analyzed using qRT-PCR. Values are presented as the means ± SD of three independent replicates. *P<0.05, **P<0.01.

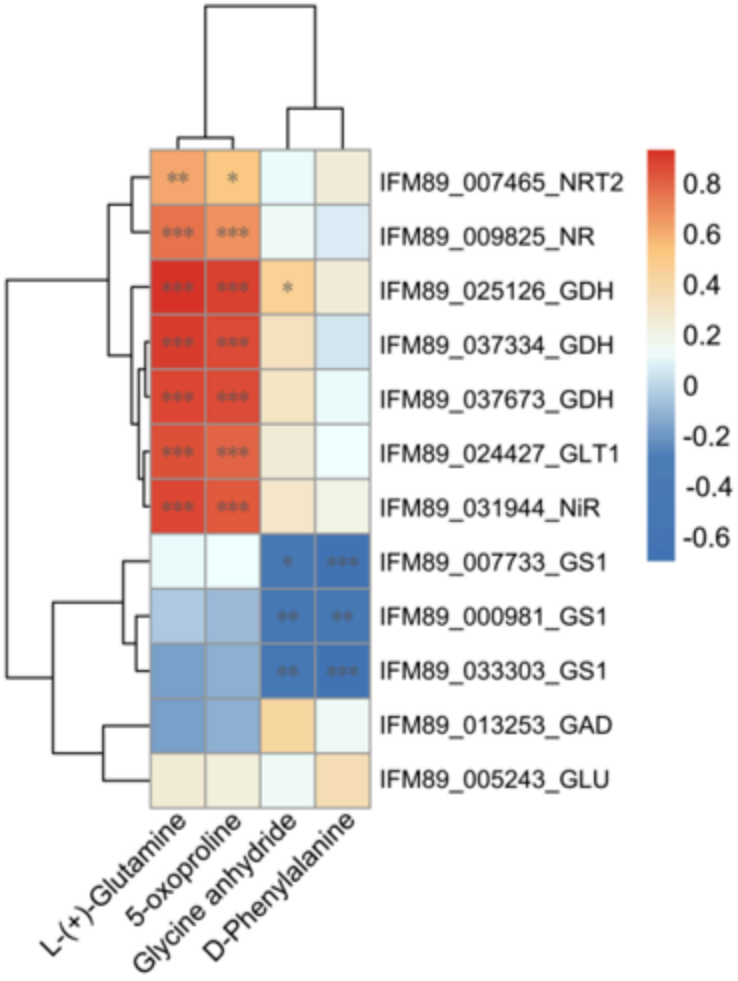

Figure 5

The putative regulatory relationship of key metabolisms in BCA-alleviated root rot development in *C. chinensis*. Pearson's correlation analysis; asterisks indicate significance: **P*<0.05, ***P*<0.01, ****P*<0.001.

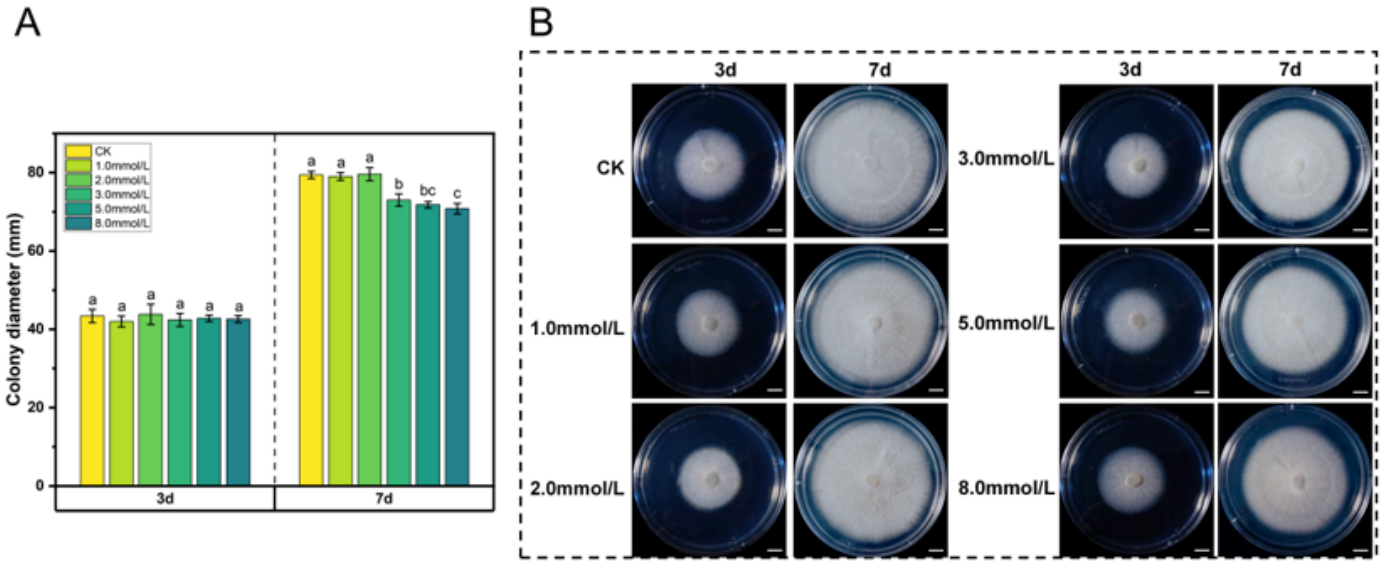

Figure 6

Effects of Gln on the growth of Fs. (A) Colony diameter. (B) Colony growth. Different lowercase letters on the bar indicated significant difference (P<0.05).

Supplementary Files

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