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Research Article

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Posted Date: July 24th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-10218700/v1

Additional Declarations: No competing interests reported.

Culturable endophytic fungi from tree peony *Paeonia suffruticosa* 'Luoyang Hong': diversity, identification, and biocontrol potential against plant pathogenic fungi

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Abstract

Endophytic fungi confer diverse fitness benefits to host plants and play a critical role in sustaining plant microecological balance. In this study, we investigated the community composition and tissue distribution of endophytic fungi in the tree peony cultivar *Paeonia suffruticosa* 'Luoyang Hong' (PSLH), screened endophytic strains with antagonistic activity against common fungal pathogens of tree peony and wheat, and further explored the physiological biocontrol mechanism of elite antagonistic strains. A total of 146 endophytic fungal strains were isolated from 480 PSLH tissue segments, with an overall colonization rate of 25.20% and isolation rate of 30.42%. Fungal colonization and isolation rates varied significantly among tissues, reaching the highest levels in roots (56.25% and 80%, respectively) while no endophytic fungi were obtained from seeds. Based on morphological characteristics and ITS sequence analysis, 139 strains were taxonomically classified into 23 species across 17 genera, 17 families, nine orders, four classes, and two phyla. *Fusarium*, Graphiopsis, *Cephalosporium*, and *Paraconiothyrium* dominated the endophytic community of PSLH, with relative abundances of 17.81%, 11.64%, 10.96%, and 6.85%, respectively. The calculated Shannon diversity index ($H' = 2.83$) and Margalef richness index ($D = 3.21$) revealed a relatively high endophytic fungal biodiversity in PSLH. Dual-culture assays against five phytopathogens (the tree peony pathogens *Alternaria alternata* and *A. suffruticosa*, and wheat pathogens *Fusarium pseudograminearum*, *Gaeumannomyces graminis* var. tritici (Ggt), and *Rhizoctonia cerealis*) yielded ten antagonistic strains, including eight antibiotic-producing isolates and two mycoparasitic isolates. The strain *Bartalinia robillardoides* T2-07 exhibited the most prominent biocontrol potential, forming a 15 mm inhibition zone against the wheat take-all pathogen Ggt. Physiological assays demonstrated that the fermentation filtrate of T2-07 significantly increased the relative electrical conductivity and malondialdehyde content in Ggt, inhibited the activities of antioxidant enzymes (SOD, POD, CAT), and suppressed key energy metabolic enzymes (HK, PK, SDH). These findings indicate that strain T2-07 inhibits Ggt via a synergistic multi-mechanism, including cell membrane damage, antioxidant system disruption, and energy metabolism blockade. This study enriches the germplasm resources of tree peony endophytic fungi and provides a valuable strain resource and theoretical basis for the development of microbial biocontrol agents against crop fungal diseases.

Keywords

Endophytic fungi; *Paeonia suffruticosa*; Biodiversity; Biocontrol potential; Physiological mechanism

Introduction

Plant endophytic fungi constitute a core component of plant microecosystems, which colonize asymptomatically in the internal tissues of healthy plants throughout all or part of their life cycles (Guo et al., 2000). Through long-term coevolution and symbiosis with host plants, endophytic fungi exert multiple beneficial regulatory effects on host growth and stress adaptation. They can promote plant growth and fitness, enhance host resistance to biotic and abiotic stresses, and facilitate the accumulation of plant secondary metabolites (Mousa et al., 2015; Kaul et al., 2016; Jia et al., 2016; Al-Askar et al., 2022). The community structure and species diversity of endophytic fungi are dynamically modulated by multiple exogenous and endogenous factors, including plant species, tissue specificity, developmental age, geographical location, and climatic conditions (Niem et al., 2020; Zhu et al., 2023). It has been reported that each host plant harbors an average of four to five obligate endophytic fungal species, with the global total number of endophytic fungal resources exceeding 100 million (Guo, 2012). Nevertheless, endophytic fungal communities have only been characterized in a limited number of plant species, and the exploration and sustainable utilization of endophytic fungal resources remain insufficient.

Tree peony, a perennial deciduous shrub of the Paeoniaceae family, is a traditional and culturally famous ornamental flower endemic to China with high economic and ecological value. Notably, its root bark, known as “Danpi” in traditional Chinese medicine, possesses multiple pharmacological activities, including antibacterial, anti-inflammatory, anticancer, hypoglycemic and hypolipidemic effects (Li et al., 2015; Yang et al., 2015). Additionally, tree peony seed oil, rich in diverse nutrients and bioactive components, was officially approved as a novel food resource by the National Health Commission of China in 2011, attracting extensive attention from researchers and consumers (Deng et al., 2022). Collectively, tree peony exhibits broad application prospects in ornamentation, medicine, and edible oil production.

In recent years, the continuous expansion of tree peony cultivation scale and long-term monoculture patterns have aggravated the occurrence of fungal diseases, seriously restricting the healthy development of the tree peony industry. Emerging destructive fungal diseases have been frequently reported, such as tree peony black spot caused by Alternaria alternata and Alternaria suffruticosa, which cause severe yield and quality losses in tree peony production (Hou et al., 2014; Xuan et al., 2017). Given the growing emphasis on ecological environmental protection and green sustainable agriculture, environment-friendly biological pesticides have become a preferred alternative to chemical pesticides for plant disease control, owing to their pollution-free and sustainable advantages.

Paeonia suffruticosa ‘Luoyang Hong’ (PSLH) is the most representative and widely cultivated tree peony cultivar in Luoyang, China. The present study was conducted to collect, isolate, and identify endophytic fungal resources from PSLH, analyze the structural characteristics of its endophytic fungal community, and screen endophytic fungal strains with significant antifungal activity against phytopathogenic fungi. The findings of this study will enrich the germplasm resource bank of tree peony endophytic fungi and provide fundamental materials and theoretical support for the development and application of microbial biocontrol agents for tree peony disease prevention and control.

Materials and Methods

Sample collection

Healthy PSLH plant samples were randomly collected from two typical peony planting bases in Luoyang, China, namely Luoyang East Flower Peony Garden (E112.674548, N34.651611) and the Peony Garden of Henan University of Science and Technology Kaiyuan Campus (E112.452094, N34.641253), during April to May 2018. Fresh healthy tissues including roots, annual twigs, biennial twigs, triennial twigs, leaves, and seeds were sampled. All samples were placed in sterile foam boxes with ice packs and transported to the laboratory within 24 h, and stored at 4°C for subsequent isolation.

Isolation and purification of endophytic fungi

Endophytic fungi were isolated using the tissue surface sterilization method described by Mefteh et al. (2017) with minor modifications. Briefly, surface dust of tissue samples was rinsed with running tap water, and samples were cut into approximately 1 cm segments. All tissue segments were subjected to strict surface sterilization in a laminar flow cabinet: 75% ethanol for 45 s, 3.3% sodium hypochlorite for 3 min, 75% ethanol for 30 s, followed by five rinses with sterile distilled water to remove residual disinfectant. After blotting surface moisture with sterile filter paper, the sterilized tissues were cut into 0.3 cm × 0.3 cm small pieces using a flame-sterilized scalpel. Tissue pieces were evenly placed on potato dextrose agar (PDA) medium supplemented with 100 mg/L streptomycin to inhibit bacterial growth. Each 90 mm Petri dish contained five tissue pieces, with eight replicates for each tissue type. Surface sterilization reliability was verified using two blank controls: spreading 200 μL of the final rinse water on PDA medium and stamping sterilized tissue pieces on PDA medium. All plates were incubated at 28°C in constant darkness for 2–4 weeks and observed daily for fungal growth. Fungal hyphae growing from tissue segments were picked and transferred to antibiotic-free PDA plates for repeated purification. Purified strains were preserved on PDA slants at 4°C for short-term storage and in 20% glycerol at −80°C for long-term preservation.

Identification of endophytic fungi

Morphological identification

Fungal strains were preliminarily grouped into different morphotypes based on colony characteristics, including colony color, texture, surface morphology, margin shape, aerial mycelium growth, and radial growth rate. Microscopic morphological traits, including conidiophore and conidium morphology and size, were observed under an optical microscope. Taxonomic identification was initially determined by comparing morphological characteristics with fungal taxonomic keys (Kirk et al. 2008).

Molecular identification based on ITS sequencing

Fungal genomic DNA was extracted using the BSC14S1 DNA extraction kit (BioFlux, Beijing, China) according to the manufacturer's instructions. The fungal ITS region (ITS1-5.8S-ITS2) was amplified using the universal primer pair ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′). The 25 μL PCR reaction system contained 12.5 μL 2× Taq PCR Green Mix, 0.5 μL ITS1 primer (10 μM), 0.5 μL ITS4 primer (10 μM), 1 μL template DNA, and 10.5 μL sterile double-distilled water. Sterile double-distilled water was used as a negative control. The PCR amplification program was set as follows: initial denaturation at 94°C for 4 min; 35 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 40 s; final extension at 72°C for 10 min (Zhao et al. 2020).

PCR products were detected via 1% agarose gel electrophoresis stained with GoldView nucleic acid dye. Single bright and specific DNA bands were recovered and sequenced by Sangon Biotech

(Shanghai, China). Obtained ITS sequences were blasted against the NCBI GenBank database, and sequences with similarity >99% were selected for species confirmation, combined with morphological characteristics. Sequence alignment was performed using Clustal W in MEGA 7.0 software, and a phylogenetic tree was constructed via the Neighbor-Joining method with 1000 bootstrap replicates (Tamura et al. 2011; Al-Askar et al. 2022). All obtained sequences were deposited in the NCBI GenBank database to acquire accession numbers.

Screening of antagonistic endophytic fungi

Five phytopathogenic fungi were used for antagonistic screening, including two tree peony pathogens (*Alternaria alternata*, *A.* suffruticosa) and three wheat pathogens (*Fusarium pseudograminearum*, *Gaeumannomyces graminis* var. tritici (Ggt), *Rhizoctonia cerealis*). All pathogen strains were provided by the Plant Pathology Research Center, College of Horticulture and Plant Protection, Henan University of Science and Technology. The dual-culture method was adopted for antagonistic assays (Zhou et al. 2022). All endophytic fungal strains and pathogens were pre-cultured on PDA medium at 28°C for 7 d. Mycelial plugs (5 mm diameter) of endophytic fungi and pathogens were symmetrically inoculated 4 cm apart on 90 mm PDA plates. Plates inoculated with only pathogen mycelial plugs were set as blank controls. All treatments were performed in triplicate and incubated at 28°C. The radial growth of pathogen colonies and inhibition zone width were measured daily until pathogen growth stabilized or the size of the control colony was close to that of the plate. The percentage inhibition of radial growth (PIRG) was calculated using the formula: PIRG = (R1 − R2)/R1 × 100%, where R1 represents the radial growth of pathogen colonies in the control group, and R2 represents the radial growth of pathogen colonies in the treatment group.

Physiological and biochemical mechanism of antagonistic strain T2-07 against Ggt

Preparation of cell-free fermentation filtrate

The strain *Bartalinia robillardoides* T2-07 with the strongest antagonistic activity against Ggt was selected for mechanism analysis. Five 5 mm mycelial plugs of T2-07 were inoculated into 100 mL potato dextrose broth (PDB) medium and cultured at 28°C with shaking at 180 rpm for 16 d. The fermentation broth was centrifuged at 12,000 × g for 10 min at 4°C, and the supernatant was filtered through a 0.22 μm sterile microporous membrane to obtain cell-free fermentation filtrate, which was stored at 4°C for subsequent experiments (Li et al. 2022).

Determination of cell membrane permeability

Five mycelial plugs of Ggt were inoculated into 8 mL of PDB medium and cultured at 28 °C with continuous shaking at 180 rpm for 12 h. Subsequently, 600 μL of T2-07 fermentation filtrate was supplemented to the treatment group, whereas an equal volume of sterile PDB medium was added to the control group. After continuous co-incubation, culture broth was collected at 2, 4, 8, 12, 24, and 48 h post treatment. All samples were heat-treated at 42 °C for 4 h, cooled to room temperature 25°C, and centrifuged at 8000 × g for 10 min. The electrical conductivity of the resulting supernatant was determined using a conductivity meter. All experiments were performed in biological triplicate (Li et al., 2022; Liu et al., 2024).

Determination of enzyme activities and MDA content

Ggt mycelia were harvested from the T2-07 co-culture system at 2, 4, 8, 12, 24, and 48 h post treatment, using the identical incubation and sampling protocols described for the electrical conductivity assay. The collected mycelial samples were vacuum-filtered and dried. For enzyme extraction, 0.1 g of dried mycelia was ground thoroughly with liquid nitrogen and quartz sand, homogenized in 1 mL of pre-cooled 0.2 mol/L phosphate buffer (pH 7.0) on ice, and centrifuged at

8000 × g for 10 min at 4 °C. The resultant supernatants were collected for subsequent measurements of MDA content and enzyme activities. The activities of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and malondialdehyde (MDA) content were determined using commercial assay kits (Tianjin Huasheng Chemical Reagent Co., Ltd., China). The activities of hexokinase (HK), pyruvate kinase (PK), and succinate dehydrogenase (SDH) were measured using kits from Nanjing Jiancheng Bioengineering Institute (China). All experimental operations strictly followed the kit instructions, with three biological replicates for each treatment (Yang et al. 2024).

Data statistical analysis

Colonisation rate (CR) (de Siqueira et al., 2011) of fungal diversity was calculated as percentage of segments colonised by one or more fungal isolates from the total number of segments of each tissue.

Isolation rate (IR) (de Siqueira et al., 2011) was evaluated as the number of isolates obtained from plant segments divided by the total number of segments × 100.

Relative abundance (RA) (de Siqueira et al., 2011) was calculated as the number of strains of one type of endophytic fungus divided by the total number of strains isolated × 100.

Fungal dominance was determined according to the method of Camargo (Camargo, 1992): a species was defined as dominant if Pi > (1/S) and as subordinate if Pi < (1/S), where Pi is the RA of a species and S is the number of different endophytic species in a sample (in this case, the number of genus).

Species richness amongst the isolated endophytic fungi was determined by calculating the Shannon diversity index (H') using the following equation (Kusari et al., 2013):

$$H' = -\sum_{i=1}^{k} (Pi \times \ln Pi)$$

Where H' values can start from 0 (only one species present with no uncertainty as to what species each individual will be) and increase, revealing high uncertainty as species become relatively evenly distributed. Here, k is the number of taxa (internal transcribed spacer ITS genotype), which is the same as S, which was mentioned above and means the number of different endophytic species in a sample.

Furthermore, the endophytic fungal diversity of PLSH in different tissues was quantified using Margalef's richness index D (Zhu et al., 2023), which was calculated using the following equation: $D = (S - 1)/\ln N$, where N is the number of individuals (defined by the number of endophytic fungal isolates) and S is the number of taxa (ITS genotype). All the experiments were repeated at least twice, and three replicates were taken into consideration.

All data are presented as the mean ± standard deviation (SD) from at least three independent experiments. Normality and homogeneity of variance were tested before analysis. One-way Analysis of Variance (ANOVA) or Student's t-test was performed using SPSS (Version 22.0) to determine significant differences ($p < 0.05$, $p < 0.01$). The Least Significant Difference (LSD) test was used for multiple comparisons. Graphs were generated using GraphPad Prism (version 9.4.1).

Results

Isolation of endophytic fungi

A total of 146 endophytic fungal strains were isolated from 480 PSLH tissue segments, with an overall colonization rate of 25.20% and isolation rate of 30.42%. Fungal isolation exhibited obvious tissue specificity. Roots yielded the maximum number of strains (64 strains), with the highest colonization rate (56.3%) and isolation rate (80%). Triennial twigs and biennial twigs produced 38 and 29 strains, respectively, while annual twigs and leaves yielded only 10 and 5 strains.

Notably, no endophytic fungi were isolated from seed tissues. The isolation rate was generally higher than the colonization rate in most tissues, especially in roots. The colonization and isolation rates of roots and triennial twigs were significantly higher than the average level, whereas those of annual twigs and leaves were significantly lower (Fig. 1).

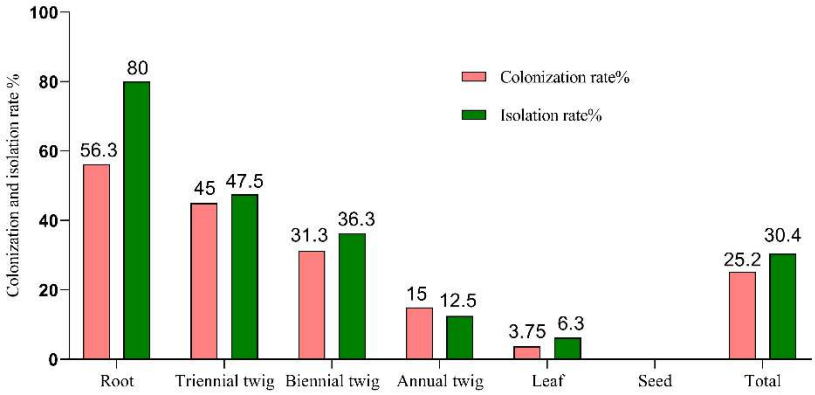

Figure 1. Colonization rate and isolation rate of endophytic fungi in different tissues of *Paeonia suffruticosa* 'Luoyang Hong'

Community composition of endophytic fungi

A total of 139 strains were successfully identified, belonging to 2 phyla, 4 classes, 9 orders, 17 families, 17 genera, and 23 species. Ascomycota dominated the endophytic fungal community, accounting for 95.1% of total strains, while Basidiomycota accounted for only 4.9%. At the class level, Dothideomycetes (50%) and Sordariomycetes (43.7%) were the dominant groups, followed by Agaricomycetes (4.9%) and Eurotiomycetes (1.4%). At the order level, Hypocreales (29.6%), Pleosporales (28.2%), and Capnodiales (14.8%) were predominant. Nectriaceae (18.3%) was the most abundant family, while Aspergillaceae and Phyllostictaceae (1.4% each) were the least abundant (Fig. 2). Seventeen representative strains were selected for ITS sequence analysis and phylogenetic tree construction (Table 1 and Fig. 3).

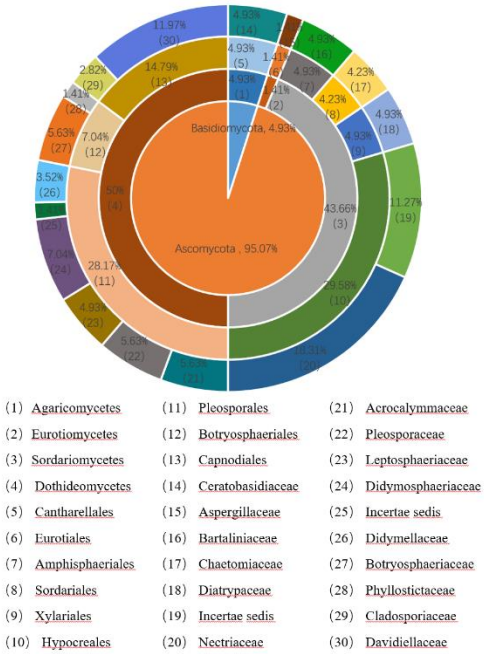

Figure 2. Taxonomic distribution of endophytic fungi associated with *Paeonia suffruticosa* 'Luoyang Hong'

Note: The Arabic numerals in the pie chart represent the different classification status of the strains, and the corresponding percentage represents the percentage of that type of strain in the same classification level.

Table 1. Summary of representative endophytic fungi in each genus of *Paeonia suffruticosa* 'Luoyang Hong' according to ITS analysis in the GenBank.

Taxon	Strain number	Most closely related strain (Accession number)	Maximum identity %	GenBank Accession number
Acrocalymma	R-02	(*Acrocalymma vagum*)KF494165	100.00	OP060693
Alternaria	T1-02	(*Alternaria alternata*)KF380814	99.45	OP060694
Bartalinia	T2-07	(*Bartalinia robillardoides*)LT853104	99.44	OP060696
Botryosphaeria	T2-19	*(Botryosphaeria dothidea)*KR709075	100.00	OP060698
Cephalosporium	T3-10	*(Cephalosporium gramineum)*MF574268	100.00	OP060699
Chaetomium	T3-22	*(Chaetomium globosum)*MT529803	100.00	OP060702
Cladosporium	T2-03	*(Cladosporium paeoniae)*FJ549442	100.00	OP060700
Diatrypella	T3-15	*(Diatrypella vulgaris)*KP050595	100.00	OP060704
Fusarium	R-09	*(Fusarium proliferatum)*MG543732	100.00	OP060705
Graphiopsis	T3-03	*(Graphiopsis chlorocephala)*MF776044	100.00	OP060709
Leptosphaeria	R-26	*(Leptosphaeria biglobosa)*KY221834	99.82	OP060710
Paraconiothyrium	R-13	*(Paraconiothyrium brasiliense)*JF502455	99.48	OP060711
Paraphoma	R-07	*(Paraphoma radicina)*MH429796	99.07	OP060712
Phoma	T1-08	(*Phoma fungicola*)KF293780	100.00	OP060713
Phyllosticta	L-03	(*Phyllosticta ericarum*)KR025424	100.00	OP060714
Rhizoctonia	R-43	(*Rhizoctonia fragariae*)MG547903	100.00	OP060701
Talaromyces	T3-07	(*Talaromyces assiutensis*)KM458833	98.94	OP060715

Note: ① The letters in the strain number represent different tissues of plant. R, T, L represent root, twig, leaf respectively.

② Numbers in "T1", "T2" and "T3" represent "annual twig", "biennial twig" and "triennial twig" respectively.

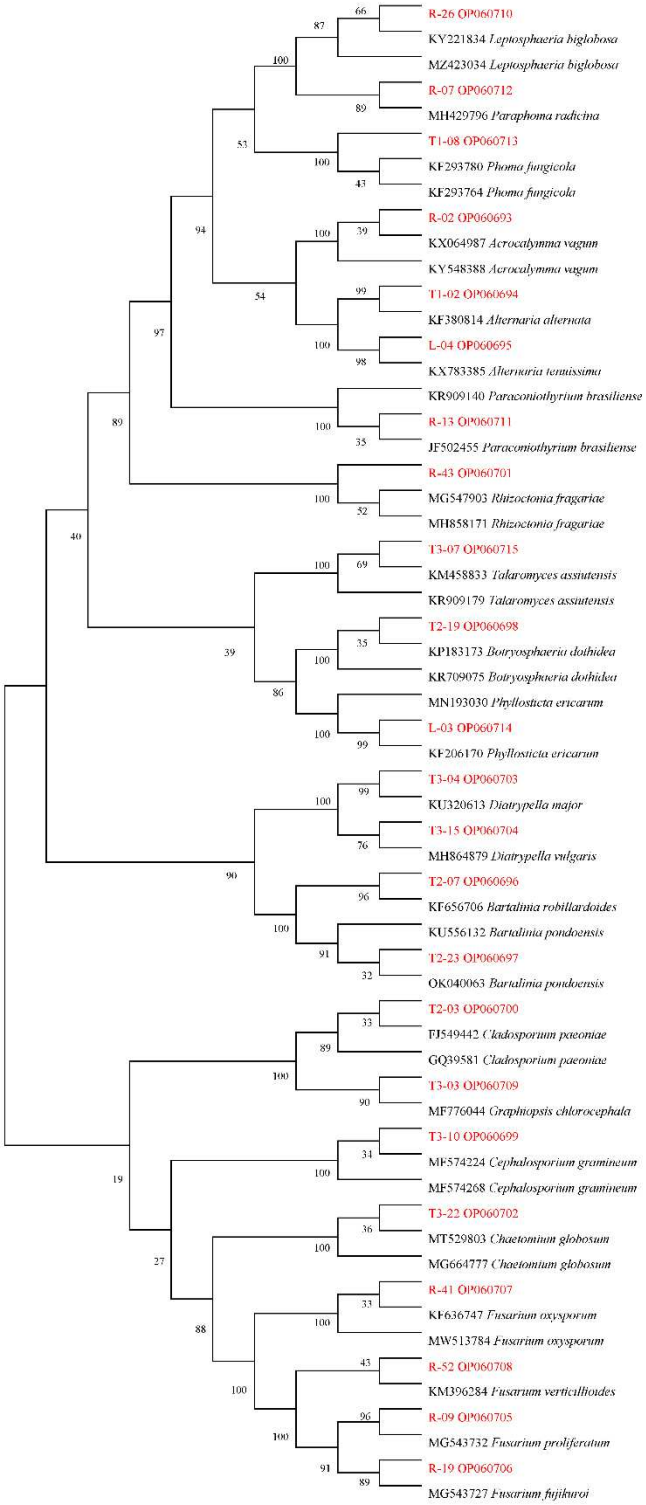

Figure 3. The ITS phylogenetic tree of 23 identified endophytic fungi associated with *Paeonia suffruticosa* 'Luoyang Hong'

Note: The fungal sequences highlighted in red are isolated from *Paeonia suffruticosa* 'Luoyang Hong', while those in black are close relative sequences in GenBank.

Distribution of endophytic fungi

Based on the tissue distribution of the 139 identified endophytic fungal strains, the roots

yielded the highest strain abundance (n = 59), comprising 11 species from eight genera. Triennial twigs (n = 36, seven species belonging to six genera) and biennial twigs (n = 30, six species belonging to six genera) represented the second and third most colonized tissues, respectively. In contrast, annual twigs and leaves harbored fewer endophytes, with four species from three genera and two species from two genera, respectively. At the genus level, *Fusarium* exhibited the highest species richness (four species), while *Alternaria*, *Bartalinia*, and *Diatrypella* each contained two species. The remaining 13 genera were each represented by a single species.

Tissue specificity of endophytic fungi

Endophytic fungal genera displayed distinct tissue specificity across PSLH tissues, with most genera colonizing only one or two tissue types. Eight genera (*Alternaria, Botryosphaeria, Cephalosporium, Chaetomium, Graphiopsis, Paraconiothyrium, Phoma,* and *Talaromyces*) were recovered from two different tissues, whereas the other nine genera were tissue-specific. Specifically, five genera (*Acrocalymma, Fusarium, Leptosphaeria, Paraphoma,* and *Rhizoctonia*) were exclusively isolated from roots; *Bartalinia* and *Cladosporium* were restricted to biennial twigs; *Diatrypella* and *Phyllosticta* were uniquely distributed in triennial twigs and leaves, respectively.

Dominant genera of endophytic fungi

Quantitative analysis of strain abundance indicated that *Fusarium* was the most dominant genus (n = 26), followed by *Graphiopsis* (n = 17), *Cephalosporium* (n = 16), and *Paraconiothyrium* (n = 10). Their corresponding Pi values were 0.1781, 0.1164, 0.1096, and 0.0685, all exceeding the threshold of 0.058 (1/S); while the remaining genera were classified as non-dominant. Tissue-specific dominant fungal assemblages were also observed. Tissue-specific dominant fungal assemblages were also observed. Roots were dominated by *Fusarium* and *Acrocalymma*, both of which were root-specific taxa. Triennial twigs were dominated by *Cephalosporium*, *Graphiopsis*, and *Diatrypella*, with *Diatrypella* occurring exclusively in triennial twigs. Biennial twigs were mainly colonized by *Bartalinia, Graphiopsis,* and *Botryosphaeria,* and *Bartalinia* was specific to biennial twigs. *Alternaria* served as the dominant genus in annual twigs and leaves and showed tissue specificity to these two organs. The detailed genus composition and relative abundance of endophytic fungi among different tree peony tissues are illustrated in Fig. 4.

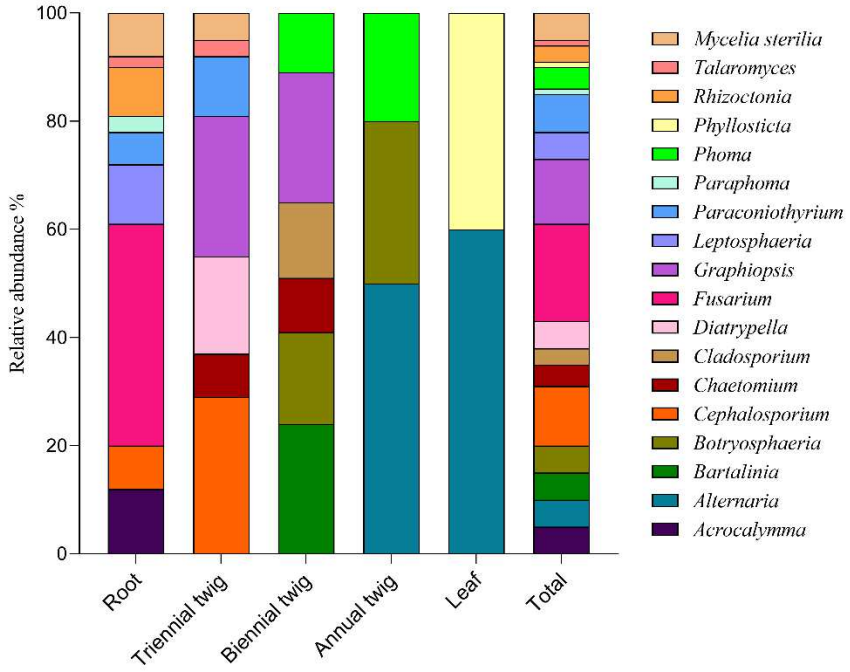

Figure 4. Distribution and relative abundance (RA) of endophytic fungi genera across different tissues in *Paeonia suffruticosa* 'Luoyang Hong'

Biodiversity of endophytic fungi in different tissues

The Shannon diversity index (H′) and Margalef richness index (D) confirmed significant differences in fungal diversity among different PSLH tissues. Roots possessed the highest fungal diversity and richness, followed by triennial twigs and biennial twigs. Annual twigs and leaves showed the lowest diversity. The overall H′ and D values of PSLH endophytic fungi were 2.83 and 3.21, respectively, indicating a high level of endophytic fungal biodiversity in the cultivar (Fig. 5).

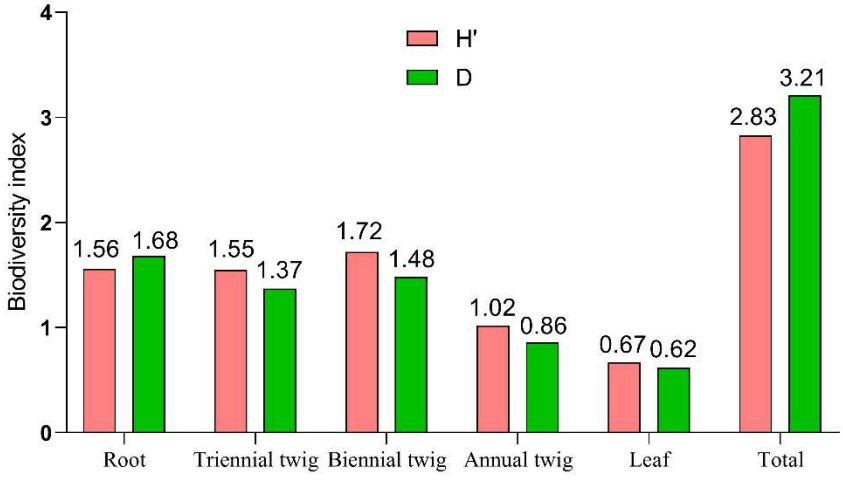

Figure 5. Shannon diversity index (H') and Margalef's richness index D of endophytic fungi in different tissues of *Paeonia suffruticosa* 'Luoyang Hong'

Antifungal activity of endophytic fungi

Dual-culture screening showed that the antagonistic modes of endophytic fungi against phytopathogens included nutritional competition, antibiosis, and mycoparasitism. Due to the rapid growth of tested pathogens, the competitive inhibition effect of most endophytic strains was weak, with most inhibition rates below 50%. Only *Cephalosporium gramineum* T3-13 and *Fusarium proliferatum* R-21 exhibited strong competitive inhibition against *F. pseudograminearum* (63.2%) and *R. cerealis* (60.5%), respectively.

Eight strains exhibited significant antibiotic activity, producing stable inhibition zones against at least two pathogens (Table 2). Among them, *Bartalinia robillardoides* T2-07 showed the strongest specific antagonism against Ggt, with a maximum inhibition zone width of 15 mm (Fig. 6A). *Talaromyces assiutensis* T3-07 possessed broad-spectrum antifungal activity, producing 2–6 mm inhibition zones against all five tested pathogens.

Table 2. Antibiosis of eight endophytic fungi in *Paeonia suffruticosa* 'Luoyang Hong' against five pathogenic fungi after 5 d of confrontation culture

Strain number	GenBank accession number	Species	Width of inhibition zone (mm)					
			A1	A2	R	G	F	Mean±SD
T2-07	OP060696	*Bartalinia robillardoides*	3.98±0.22	/	7.02±0.16	14.84±0.27	/	8.61±4.73A
T2-23	OP060697	*Bartalinia pondoensis*	3.10±0.12	/	2.98±0.23	5.06±0.19	/	3.71±1.00C
T3-10	OP060699	*Cephalosporium gramineum*	/	/	4.86±0.18	3.82±0.19	/	4.34±0.58B
T3-03	OP060709	*Graphiopsis chlorocephala*	/	2.94±0.29	/	4.06±0.27	/	3.50±0.65D
T2-03	OP060716	*Graphiopsis chlorocephala*	/	3.16±0.17	/	3.76±0.15		3.46±0.35D
R-13	OP060711	*Paraconiothyrium brasiliense*	1.96±0.15	2.90±0.20	2.98±0.28	/	/	2.61±0.52F
T3-02	OP060717	*Paraconiothyrium brasiliense*	2.88±0.13	2.86±0.26	3.86±0.19	/	/	3.20±0.52E
T3-07	OP060715	*Talaromyces assiutensis*	5.02±0.23	3.86±0.22	6.06±0.21	5.02±0.25	2.16±0.17	4.42±1.37B
Mean±SD			3.39±1.07c	3.14±0.44d	4.63±1.56b	6.09±4.02a	2.16±0.17e	

Note: ① The letters and numbers in the strain number have the same meanings as in Table 1.
② The letters in the header represent different peony diseases: A1, *Alternaria alternata*; A2, *Alternaria suffruticosa*; R, *Rhizoctonia cerealis*; G, *Gaeumannomyces graminis* var. tritici; F, *Fusarium pseudograminearum*.
③ The different capital letters "ABCDEF" after "Mean±SD" indicate that the width of inhibition zone produced by different endophytic fungi is significantly different; The different lowercase letters "abcde" after "Mean±SD" indicate that the width of inhibition zone produced by different pathogenic fungi is significantly different.
④The symbol "/" indicates that the endophytic fungi tested cannot produce a inhibition zone against the pathogenic fungi, and don't have antibiosis.

Two strains displayed mycoparasitic activity. *Diatrypella major* T3-04 showed broad-spectrum parasitic ability against all tested pathogens, though the parasitic effect was weak for most pathogens. *Fusarium proliferatum* R-09 parasitized *A. alternata* and *Ggt*, with a significantly stronger parasitic effect on *Ggt*. Strain R-09 grew faster than Ggt, gradually colonized and covered Ggt mycelia, and

completely occupied the plate after 15 d of co-culture (Fig. 6C).

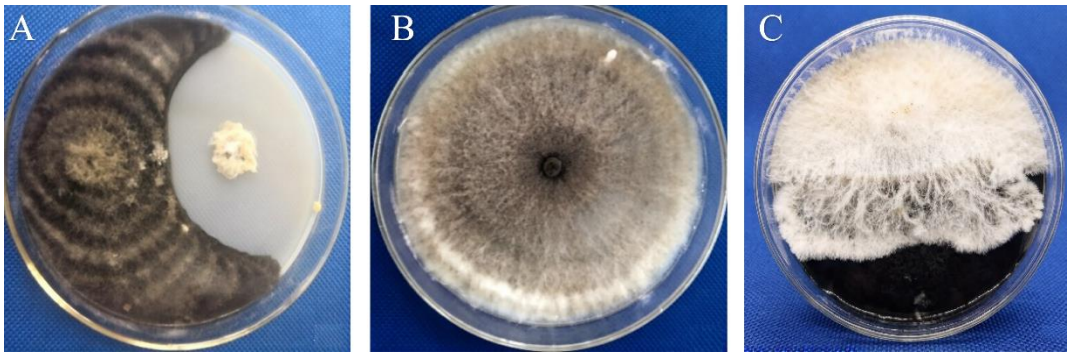

Figure 6. Antagnism of *Bartalinia robillardoides* T2-07 against *Gaeumannomyces graminis* var. tritici (Ggt) on PDA plates

Note: Fig 6A pepresents the antibiosis of T2-07 against Ggt after 5 d of confrontation culture. Fig 6C pepresents the parasitism of T2-07 on Ggt after 11 d of confrontation culture. Fig 6B pepresents the control.

Physiological mechanism of strain T2-07 inhibiting Ggt

The cell-free fermentation filtrate of T2-07 significantly inhibited the growth of Ggt mycelia via multiple synergistic pathways (Fig. 7). First, the filtrate significantly increased the relative electrical conductivity of Ggt culture solution, indicating severe damage to pathogen cell membrane integrity and increased intracellular substance leakage. Second, T2-07 filtrate treatment significantly reduced the activities of antioxidant enzymes (SOD, POD, CAT) in Ggt mycelia and increased MDA accumulation, leading to disrupted intracellular ROS homeostasis and aggravated oxidative damage. Third, the activities of key energy metabolism enzymes (HK, PK, SDH) were significantly suppressed, which blocked glycolysis and TCA cycle pathways, resulting in energy metabolism disorder and growth inhibition of Ggt.

In summary, strain T2-07 achieves efficient biocontrol against wheat take-all disease through the synergistic effects of membrane damage, antioxidant system inhibition, and energy metabolism disruption, rather than relying on a single inhibitory pathway.

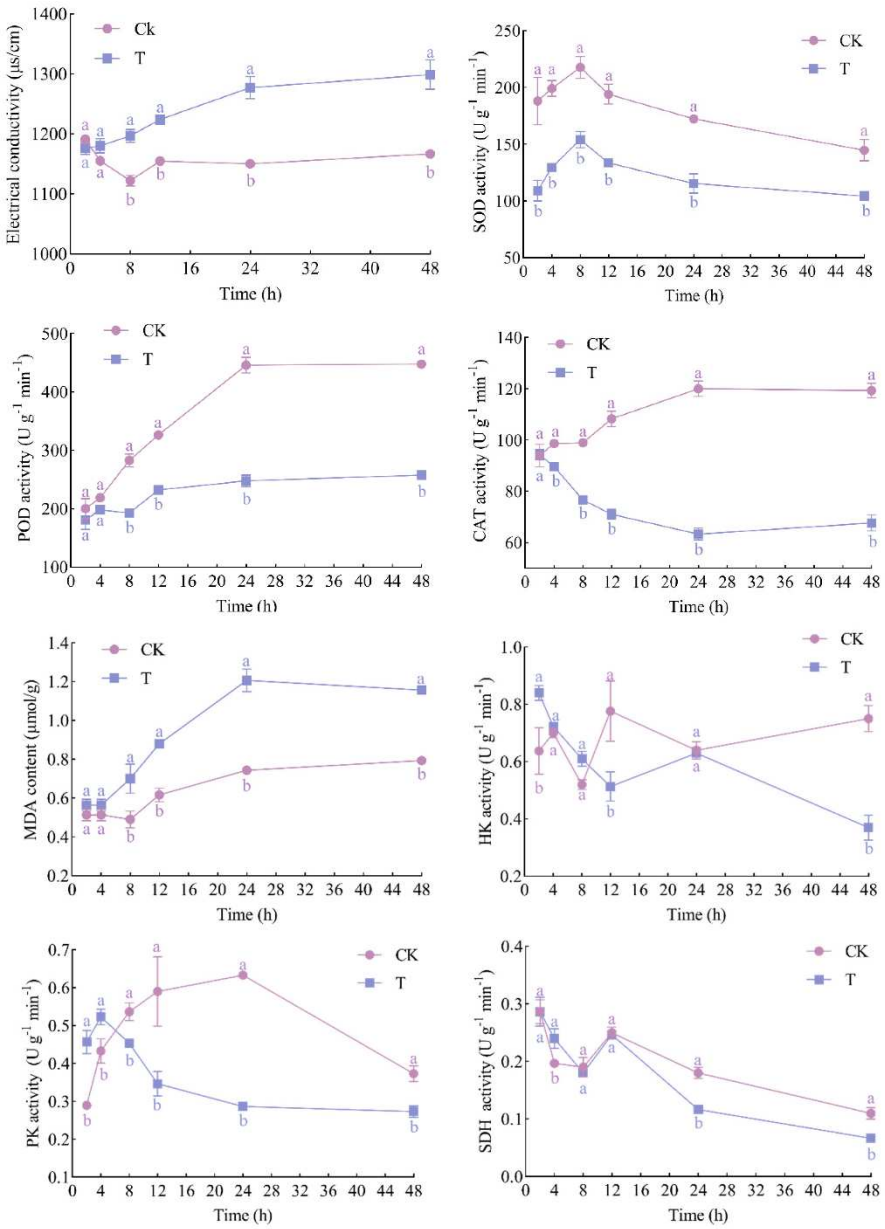

Figure 7. Effects of cell-free fermentation filtrate from Bartalinia robillardoides T-20 on physiological and biochemical characteristics of Fusarium pseudograminearum over time

Note: The pathogen was cultured without (CK) or with (T) fermentation filtrate of strain T-20.

Discussion

Peony in China holds both medicinal and ornamental value. Current research has primarily focused on the detection of active ingredients such as paeoniflorin and the molecular mechanisms underlying flower forms and colors, while systematic studies on its endophytic fungi remain limited. Notably, the community structure of endophytic fungi is highly dependent on host species and geographic location (López-Menchero et al., 2026; Kumar and Nautiyal, 2026). Although China possesses abundant tree peony resources with a wide variety of cultivars, these resources are predominantly distributed across several key cultivation regions—including Luoyang (Henan), Heze (Shandong), Pengzhou (Sichuan), and Tongling (Anhui)—where they have long been shaped by unique local microecosystems. This has resulted in distinct "Dao-di" microhabitats within

different ecological regions, further adding to the complexity and specificity of endophytic fungal research. In recent years, a limited number of studies have preliminarily revealed the diversity and potential functions of endophytic fungi in peony. For example, in Tongling, Anhui Province, a genuine production area of ‘Cortex Moutan’, the endophytic fungi in ‘Feng Dan’ plants not only exhibit high richness but also show the highest detection rates of polyketide synthase (PKS) and non-ribosomal peptide synthase (NRPS) genes, suggesting that endophytic fungi may contribute to the quality formation of genuine medicinal materials (Yang et al., 2018). Additionally, fermented broth of Fusarium, the dominant fungal genus isolated from the roots of "Feng Dan" in Tongling, achieved inhibition rates of over 80% against certain pathogenic fungi (Zheng et al., 2016). Moreover, an endophytic fungus named J1-2, potentially capable of producing paeonol, was screened from 57 fungal strains isolated from P. ostia and identified as Chaetomium sp. based on morphological characteristics and ITS sequence analysis (Li et al., 2015), further indicating the potential of peony endophytic fungi in biocontrol and bioactive compound synthesis. A recent study on endophytic fungi in wild Paeonia delavayi across eight altitude gradients in Lijiang, Yunnan, also demonstrated that altitude and soil nutrients are key factors driving the community structure of endophytic fungi (Han et al., 2026).

However, systematic comparisons of endophytic fungal communities across different geographic origins and cultivars remain scarce, particularly regarding tissue-specific composition and function. To address this, we conducted the first systematic characterization of culturable endophytes in the elite cultivar 'Luoyang Hong', analyzing their distribution across roots, stems, and leaves. We identified the dominant taxa and diversity indices specific to each tissue. This study establishes a crucial localized dataset and microbial resource for future cross-regional and cross-cultivar comparisons, as well as for functional strain screening and elucidating the role of endophytes in shaping the "Dao-di" quality of tree peony.

Although endophytic fungi were successfully isolated from the roots, stems, and leaves using identical protocols, no strains were recovered from the seed samples. While high-throughput sequencing has detected endophytic fungi in tree peony seeds, reports on the isolation of culturable microorganisms from these seeds remain absent (Zhang et al., 2025). This absence does not imply sterility; rather, it highlights a fundamental difference in endophyte colonization patterns between seeds and vegetative organs. This outcome is primarily constrained by two factors: sampling timing and isolation methodology. First, sampling was conducted in April–May, coinciding with the peak metabolic activity of the host plant, which favors fungal colonization in roots, stems, and leaves. Conversely, during this period, the seeds were still in early development—tender and physiologically immature—resulting in negligible or zero endophyte infection rates. Second, the tissue separation method employed proved inadequate for delicate seeds, failing to capture potential trace microbiota. In contrast, a grinding method might be more effective in releasing internal tissues and enhancing isolation efficiency. In summary, successful isolation of culturable endophytes from tree peony seeds is expected by shifting the sampling window to the physiological maturity stage (August–September) and optimizing the protocol with a grinding method.

Bartalinia robillardoides is a fungus with a wide range of ecological origins. It can be isolated from various environments such as freshwater, seawater, and sponges, and also exists as an endophytic fungus in multiple plant hosts (Nguyen et al., 2019; Wang YM et al., 2016; Jansen et al., 2013). Current research has primarily focused on its isolation and identification, with limited exploration of its biocontrol potential. Although it has been reported to be isolated from plants such

as *Azadirachta indica, Ephedra intermedia,* and leaf litter *of Celtis formosana, Ficus ampelas, F. septica, Macaranga tanarius,* and *Morus australis* collected from Taiwan, its antimicrobial activity and related mechanisms remain unclear (Tejesvi et al., 2009; Wang Y et al., 2016; Tennakoon et al., 2021). In this study, a strain of *Bartalinia robillardoides* T2-07 was isolated for the first time from the twigs of tree peony. Its metabolites were confirmed to produce distinct inhibition zones, demonstrating broad-spectrum antimicrobial activity against multiple plant pathogenic fungi, with particularly significant inhibitory effects against Ggt. Further research revealed that its cell-free fermentation filtrate does not act through a single mechanism but employs a synergistic approach involving three physiological pathways: disrupting cell membrane stability, weakening the antioxidant capacity of the pathogen, and blocking energy metabolism. Together, these pathways achieve efficient inhibition against Ggt. This study not only reports for the first time a strain of *Bartalinia robillardoides* with significant antimicrobial activity isolated from tree peony but also preliminarily elucidates its multi-pathway synergistic antimicrobial physiological mechanism. It provides new research materials and theoretical foundations for developing novel biocontrol agents and exploring fungal antibiotic mechanisms.

Conclusion

This study highlights the diversity and community structure of culturable endophytic fungi associated with the tree peony cultivar 'Luoyang Hong' and identifies several strains with strong antagonistic activity against fungal pathogens of tree peony and wheat. Notably, *Bartalinia robillardoides* T2-07 effectively suppresses the wheat take-all pathogen through a synergistic mechanism involving membrane damage, oxidative stress imbalance, and energy metabolism disruption. These findings expand the endophytic fungal resources of tree peony and support the potential of T2-07 as a sustainable biocontrol agent.

Acknowledgments

We gratefully acknowledge the Henan Provincial Science and Technology Tackling Program, China (Grant Nos. 262102110177 and 252102110148).

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