Diversity Analysis of Endophytic Fungi from Camellia luteoflora and Preliminary Screening for Antimicrobial Activity (Postprint)

Authors: Yi Hang, He Jing, Yang Xi, Rong Shutian, Wang Li

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Abstract

To investigate the species composition and population distribution patterns of endophytic fungi in *Camellia luteoflora* and their inhibitory effects on plant pathogenic fungi, this study employed tissue isolation method to isolate and purify endophytic fungi from *C. luteoflora*, identified them based on morphology and molecular biology, evaluated their diversity using statistical analysis, and screened for strains with antifungal activity through dual culture method. The results showed that: (1) A total of 261 endophytic fungal isolates were obtained from 324 tissue segments of *C. luteoflora*, belonging to 1 phylum, 5 classes, 9 orders, and 22 genera. The dominant genera included *Colletotrichum*, *Diaporthe*, and *Pestalotiopsis*, with isolation frequencies of 21.84%, 16.86%, and 10.34%, respectively. (2) The study revealed that endophytic fungi in *C. luteoflora* exhibited different distribution patterns across seasons. Winter yielded the highest number of isolates (72, accounting for 27.59%), distributed across 16 genera; spring yielded 62 isolates across 13 genera; summer yielded 59 isolates across 15 genera; and autumn yielded 68 isolates across 13 genera. The Shannon-Wiener index (H), Simpson index (D), evenness index (E), and richness index (M) were highest in winter. The similarity of endophytic fungal species was higher between spring and winter, and between summer and autumn. (3) Endophytic fungi in *C. luteoflora* showed different distribution patterns among plant tissues. The stem harbored the highest number of endophytic fungi (102 isolates, accounting for 39.08%), distributed across 15 genera; roots yielded 61 isolates across 10 genera; and leaves yielded 98 isolates across 15 genera. The Shannon-Wiener index, Simpson index, evenness index, and richness index were highest in stems, and the similarity of endophytic fungal species was highest between leaves and stems. (4) Dual culture results demonstrated that among the 35 tested endophytic fungal isolates, 26 exhibited inhibitory effects against at least one plant pathogenic fungus, accounting for 74.29%. Among these, CJ-II-2, XY-V-3, QY-II-4, QJ-III-2, and DJ-I-2 showed varying degrees of inhibition against eight plant pathogenic fungi. XY-V-3 demonstrated the best inhibitory effect against the eight plant pathogenic fungi, with inhibition rates exceeding 50% for all. XY-V-3 and QJ-III-2 exhibited inhibition rates higher than 50% against two *C. luteoflora* pathogenic fungi, indicating potential for controlling diseases in *C. luteoflora* itself. The endophytic fungi of *Camellia luteoflora* exhibit rich diversity, and some strains demonstrate promising inhibitory effects against plant pathogenic fungi, laying a foundation for the development of biological control products and the management of *C. luteoflora* diseases.

Full Text

Preamble

Diversity Analysis of Endophytic Fungi and Preliminary Screening of Antimicrobial Activity in *Camellia luteoflora*

YI Hang, HE Jing, YANG Xi, RONG Sutian, WANG Li*

(College of Life Sciences, Sichuan University, Chengdu 610065, China)

Abstract

To investigate the species composition and population distribution patterns of endophytic fungi in *Camellia luteoflora* and their inhibitory effects on plant pathogenic fungi, this study employed tissue isolation methods to isolate and purify endophytic fungi from *C. luteoflora*. The isolates were identified based on morphological and molecular biological characteristics, and their diversity was evaluated through statistical analysis. Strains with antimicrobial activity were screened using the plate confrontation method. The results showed: (1) A total of 261 endophytic fungal strains were isolated from 324 tissue samples of *C. luteoflora*, belonging to 1 phylum, 5 classes, 9 orders, and 22 genera. The dominant genera included *Colletotrichum*, *Diaporthe*, and *Pestalotiopsis*, with isolation frequencies of 21.84%, 16.86%, and 10.34%, respectively. (2) The distribution of endophytic fungi in *C. luteoflora* varied across seasons. The highest number of strains was isolated in winter (72 strains, accounting for 27.59%), distributed across 16 genera; 62 strains were isolated in spring (13 genera); 59 strains in summer (15 genera); and 68 strains in autumn (13 genera). The Shannon-Wiener index (H), Simpson index (D), evenness index (E), and richness index (M) were highest in winter. The similarity of endophytic fungal species between spring and winter was relatively high, as was that between summer and autumn. (3) Endophytic fungi also showed differential distribution among plant parts. The stem harbored the most abundant endophytic fungi, with 102 strains (39.08%) belonging to 15 genera; 61 strains were isolated from roots (10 genera); and 98 strains from leaves (15 genera). The Shannon-Wiener, Simpson, evenness, and richness indices were highest in stems, and the endophytic fungal species in leaves were most similar to those in stems. (4) Plate confrontation results demonstrated that among 35 tested endophytic fungal strains, 26 exhibited inhibitory effects on at least one plant pathogenic fungus (74.29%). Strains CJ-II-2, XY-V-3, QY-II-4, QJ-III-2, and DJ-I-2 showed varying degrees of inhibition against all eight plant pathogenic fungi tested. XY-V-3 demonstrated the best inhibitory effect, with inhibition rates exceeding 50% against all eight pathogens. Both XY-V-3 and QJ-III-2 showed inhibition rates higher than 50% against two pathogenic fungi of *C. luteoflora*, indicating potential for controlling the plant's own diseases. These findings reveal that *C. luteoflora* harbors rich endophytic fungal diversity, with some strains exhibiting promising antifungal activity against plant pathogens, thus laying a foundation for developing biological control products and managing diseases in *C. luteoflora*.

Keywords: *Camellia luteoflora*, endophytic fungi, isolation and identification, diversity, antimicrobial activity

Introduction

Endophytic fungi are microorganisms that reside within healthy plant tissues during certain or all stages of their life cycle without causing obvious disease symptoms in the host plant (Zhao et al., 2011). These fungi form mutualistic symbioses with their host plants: while plants provide nutrients and habitat, endophytic fungi produce antimicrobial and growth-promoting substances that protect the host from pests and diseases and promote plant growth, resulting in co-evolution and reciprocal interactions (Martina et al., 2014). Research has shown that endophytic fungi are highly diverse and widely distributed. Their diversity is reflected in the fact that different ages, tissues, and seasons of the same plant species in the same region yield different types and quantities of endophytic fungi. Wang et al. (2017) found that the diversity and similarity of endophytic fungi in the scale leaves of *Platycladus orientalis* varied among different age groups. Li et al. (2018) reported that both the diversity and similarity of endophytic fungi in walnut (*Juglans regia*) from Yijun, Shaanxi differed across seasons and tissue types. Furthermore, the same plant species from different geographical regions also harbors different endophytic fungal communities. Zhou et al. (2023) analyzed the endophytic fungal diversity of wild *Sinopodophyllum hexandrum* from Honghe Valley and Haoping, finding that the diversity was higher in the Haoping population. Therefore, analyzing endophytic fungal diversity according to local plant growth characteristics and screening for dominant and unique strains forms the basis for exploring fungi with biocontrol and other functional properties.

Endophytic fungi can produce secondary metabolites with antifungal properties, such as terpenoids, alkaloids, and flavonoids (Aly et al., 2010), and can also inhibit pathogenic fungal growth through competition for ecological niches and nutrients (Song and Tian, 2020). Biological control using plant endophytes offers advantages of being pollution-free, avoiding resistance development, and providing safe and sustainable protection.

Camellia luteoflora is a rare and endemic plant species in China belonging to the family Theaceae and genus *Camellia*, currently distributed in Chishui City, Guizhou Province, and Gulin County and Changning County in Sichuan Province (Yang, 2014; Chen and Wang, 2016). With its golden-yellow flowers and broad, thick leaves, *C. luteoflora* possesses unique characteristics and high ornamental value, showing promising prospects for landscaping, potted plants, and cut flowers (Zou, 2000). However, the plant suffers from severe diseases, with camellia gall disease and witch's broom disease being the main pathogens that seriously impact its economic and ornamental value (Liu et al., 2005). In-depth research on disease control for *C. luteoflora* has been lacking, and developing safe and effective disease management strategies without damaging its habitat represents an urgent challenge. As an endangered species, studying the diversity and antimicrobial activity of endophytic fungi in *C. luteoflora* serves two purposes: first, to identify the composition of its endophytic fungal community, understand the distribution and diversity of endophytic fungi across different tissues and seasons, and determine which strains are dominant, thereby enhancing our understanding of this resource and paving the way for its exploitation; second, given the plant's rarity, disease control is particularly critical. Screening for strains with biocontrol potential through antimicrobial assays can open new avenues for disease management in *C. luteoflora* while mitigating environmental impacts from chemical control methods.

Therefore, this study focused on *C. luteoflora* to isolate and identify endophytic fungi using tissue isolation and molecular biology methods, analyze their diversity, screen for dominant and unique strains, and investigate the inhibitory effects of these fungi against plant pathogens. The goal was to identify strains with broad-spectrum antimicrobial activity and provide a reference basis for disease control and further utilization of endophytic fungal resources in *C. luteoflora*.

1.1.1 Plant Materials

Camellia luteoflora samples were collected during four seasons: spring (April 2021), summer (July 2021), autumn (October 2021), and winter (January 2022) from Hanxi Village, Guihua Township, Gulin County, Sichuan Province (altitude 901 m, 105°41 17 E, 28°10 52 N). Five healthy *C. luteoflora* plants were randomly selected, and three samples each of roots, branches, and leaves were collected from each plant. Samples were placed in sterile bags, stored in an ice box, transported to the laboratory, and stored at 4°C.

1.1.2 Pathogenic Fungi

Eight plant pathogenic fungal strains were used: *Pestalotiopsis theae* (tea leaf blight), *Fusarium oxysporum*, *F. moniliforme*, *Colletotrichum fragariae* (strawberry anthracnose), *Sclerotinia sclerotiorum* (rapeseed sclerotinia), *Fusarium oxysporum* f. sp. *niveum* (watermelon wilt), and two pathogens of *C. luteoflora* (*Colletotrichum cliviicola* and *Alternaria alternata*). All pathogens were provided by the Microbiology Laboratory of the College of Life Sciences, Sichuan

University.

1.1.3 Major Equipment

Vertical pressure steam sterilizer (LDZX-50KBS, Shanghai), full-temperature oscillator (HZQ-QX, Harbin), intelligent biochemical incubator (SPX, Ningbo), electric constant-temperature water bath (HSG-IC-2, Zhejiang), benchtop high-speed centrifuge (H1650-W, Hunan), and rotary evaporator (RE-5522, Shanghai).

1.1.4 Major Reagents

Absolute ethanol, sodium hypochlorite solution, lactophenol cotton blue stain, potato dextrose water (PDW), fungal genomic DNA rapid extraction kit (Sangon Biotech, Shanghai), Taq PCR Master Mix (2X with blue dye), universal primers ITS1 and ITS4 (Sangon Biotech, Shanghai), and potato dextrose agar (PDA) medium (Solarbio, USA).

1.2.1 Isolation and Purification of Endophytic Fungi from *C. luteoflora*

Roots, stems, and leaves of *C. luteoflora* were washed and surface-sterilized. The sterilized tissues were cut into segments and inserted obliquely into PDA medium, then incubated at 28°C in darkness. After colonies emerged, hyphae with different morphological textures from the colony margins were transferred to fresh PDA medium for individual cultivation. This process was repeated 2–3 times until pure colonies were obtained, which were photographed and their colony characteristics recorded. Finally, the mycelia were transferred to PDA slants, cultured at 28°C for a period, and stored at 4°C.

Detection of surface sterilization effectiveness: The final rinse solution was spread on PDA medium and incubated at 28°C for 7 days. No colony growth confirmed thorough surface sterilization, indicating that all isolated strains originated from within the plant tissues and were true endophytic fungi.

1.2.2 Identification of Endophytic Fungi

1.2.2.1 Morphological Identification The growth status and morphological characteristics of colonies on PDA medium were observed regularly, including shape, texture, color, and growth rate. During the early growth stage, hyphal spores from the colony margin were collected on glass slides, stained with lactophenol cotton blue, and examined under an optical microscope to observe hyphal morphology, sporulation, sporulation structure morphology, sporulation patterns, and spore morphology, size, and color. Based on these observations and with reference to *Identification Manual of Fungi* (Wei, 1979) and *Illustrated Genera of Imperfect Fungi* (Barnett & Hunter, 1998), combined with the methods of Zhang et al. (2010), the fungi were identified. Identified endophytic fungi were coded with a system where the first two letters represent season and tissue abbreviations, Roman numerals indicate the plant individual number, and the final digit represents the tissue segment number.

1.2.2.2 Molecular Identification Endophytic fungal DNA was extracted using a fungal genomic DNA rapid extraction kit and stored at -20°C. Universal primers ITS1 (5 -TCCGTAGGTGAACCTGCGC-3) and ITS4 (5 -TCCTCCGCTTATTGATATGC-3) were used for PCR amplification of the extracted DNA. The PCR products were subjected to agarose gel electrophoresis, and products with bright, clear bands were sent to Sangon Biotech (Shanghai) Co., Ltd. for sequencing.

1.2.3 Phylogenetic Analysis of Endophytic Fungi from *C. luteoflora*

The ITS sequences of endophytic fungi obtained from sequencing were subjected to BLAST analysis in the NCBI database (https://blast.ncbi.nlm.nih.gov/Blast.cgi). A phylogenetic tree was constructed using the neighbor-joining (NJ) method in MEGA 11 software with 1,000 bootstrap replicates to determine the taxonomic status of the fungi (Kumar et al., 2016).

1.2.4 Diversity Analysis of Endophytic Fungi from *C. luteoflora*

The colonization rate (CR), isolation rate (IR), isolation frequency (IF), Simpson's diversity index (D), Shannon-Weiner diversity index (H), Pielou's evenness index (E), and Margalef's richness index (M) of endophytic fungi from *C. luteoflora* were calculated. Based on the species composition, cluster analysis of endophytic fungi from different seasons and tissues was performed using SPSS 24.0 software. These analyses evaluated the diversity, dominant populations, distribution uniformity, richness, and similarity levels among different seasons and tissues.

Colonization rate (CR): The ratio of tissue segments with fungal growth to the total number of tissue segments isolated, reflecting the degree of fungal infection in the plant.

Isolation rate (IR): The ratio of the number of a particular strain type to the total number of tissue segments isolated, measuring the abundance of fungi in tissues and the frequency of tissue infection.

Isolation frequency (IF): The ratio of the number of a particular strain type to the total number of strains isolated, reflecting the dominance of different endophytic fungal species.

Simpson index (D): Reflects the dominance or diversity of species in a community.

Shannon-Weiner index (H): Reflects the diversity of species in a community. $= 1 - =1$

Richness index (M): Reflects the richness of species in a community. $=- =1$

Evenness index (E): Reflects the uniformity of species distribution in a community.

Where: Ni represents the number of endophytic fungus i in different seasons or tissues; N is the total number of strains; S is the number of species.

1.2.5 Antimicrobial Activity of Endophytic Fungi from *C. luteoflora*

The antimicrobial activity of endophytic fungi was determined using the plate confrontation method. Mycelial plugs (6 mm diameter) of 35 endophytic fungal strains and eight pathogenic fungal strains were prepared. Each pathogen plug was paired with each endophytic fungus plug and inoculated onto opposite sides of the same PDA plate. Each treatment was replicated three times, with PDA plates inoculated only with the pathogen serving as controls. Plates were incubated at 28°C in darkness for 7 days. The radial growth of pathogens in confrontation (r) and in monoculture (R) was measured using the cross method, and the inhibition rate was calculated to screen for antifungal endophytic fungi. 100%.

Grading criteria: Inhibition rate >75% = strong; 50%–75% = moderate; 10%–50% = low; <10% = none.

2.1 Isolation of Endophytic Fungi from *C. luteoflora*

A total of 261 endophytic fungal strains were isolated from 324 tissue segments of *C. luteoflora*. As shown in Table 1 , the overall colonization and isolation rates were 71.30% and 80.55%, respectively. Colonization rates varied among tissues, with stems showing the highest rate (88.89%), followed by leaves (81.48%), and roots the lowest (43.52%). Seasonal colonization rates also differed, with winter showing the highest (75.31%), followed by autumn (72.84%), spring (69.14%), and summer the lowest (67.90%). Isolation rates from high to low were stems (94.44%) > leaves (90.74%) > roots (56.48%), and by season: winter (88.89%) > autumn (83.95%) > spring (76.54%) > summer (72.83%). Isolation frequency was highest in stems (39.08%), followed by leaves (37.55%), and lowest in roots (23.37%). Seasonal isolation frequencies from high to low were winter (27.59%) > autumn (26.05%) > spring (23.75%) > summer (22.61%).

2.2 Population Composition of Endophytic Fungi from *C. luteoflora*

Fungal genomic DNA was extracted and amplified using ITS1 and ITS4 primers, yielding fragments approximately 500–750 bp in length. After sequencing, the results were subjected to BLAST analysis in the GenBank database. Based on the alignment results, a phylogenetic tree was constructed using MEGA 11 software with sequences showing >99% similarity (Figure 1 [Figure 1: see original paper]). Combined with colony color, morphology, texture, and microscopic observations of spore and hyphal morphology, 35 species were identified, belonging to 1 phylum, 5 classes, 9 orders, and 22 genera (microscopic morphology and colony characteristics are shown in Figures 2 [Figure 2: see original paper] and 3 [Figure 3: see original paper]).

As shown in Table 2 , all endophytic fungi from *C. luteoflora* belonged to the phylum Ascomycota. At the class level, Sordariomycetes was the dominant class with 197 strains (75.48% of the total), followed by Eurotiomycetes (20 strains, 7.66%), Dothideomycetes (21 strains, 8.05%), Leotiomycetes (20 strains, 7.66%), and Chaetothyriomycetes (3 strains, 1.15%). At the order level, nine orders were identified: Xylariales, Hypocreales, Diaporthales, Glomerellales, Eurotiales, Capnodiales, Pleosporales, Helotiales, and Chaetothyriales. The dominant orders were Xylariales (26.47%), Glomerellales (21.84%), and Diaporthales (18.77%). At the genus level, 22 genera were identified: *Pestalotiopsis*, *Hypoxylon*, *Annulohypoxylon*, *Xylaria*, *Chaetomium*, *Nodulisporium*, *Arthrinium*, *Fusarium*, *Ilyonectria*, *Diaporthe*, *Phomopsis*, *Colletotrichum*, *Penicillium*, *Aphanoascus*, *Cadophora*, *Phyllosticta*, *Cladosporium*, *Acrocalymma*, *Hymenoscyphus*, *Scytalidium*, *Pezicula*, and Herpotrichiellaceae. The dominant genera were *Colletotrichum* (21.84% of total strains), *Diaporthe* (16.86%), and *Pestalotiopsis* (10.34%).

2.3.1 Seasonal Distribution of Endophytic Fungi

As shown in Figure 4 [Figure 4: see original paper], endophytic fungi isolated in spring comprised 13 genera; summer, 15 genera; autumn, 13 genera; and winter, 16 genera. The genera shared across all four seasons were *Aphanoascus*, *Colletotrichum*, *Diaporthe*, *Fusarium*, *Hypoxylon*, *Xylaria*, *Pestalotiopsis*, *Pezicula*, and *Phyllosticta*. Unique genera found only in spring tissues were *Hymenoscyphus* (1 genus); only in summer: *Scytalidium*, *Acrocalymma*, and Herpotrichiellaceae (3 genera); only in autumn: *Annulohypoxylon*; and only in winter: *Chaetomium* and *Arthrinium* (2 genera). Unique strains isolated in spring were *Helotiales* sp. and *Diaporthe discoidispora*; in summer: *Herpotrichiellaceae* sp. 2 KO-2013, *Scytalidium auriculariicola*, and *Acrocalymma medicaginis*; in autumn: *Annulohypoxylon stygium* and *Colletotrichum camelliae*; and in winter: *Chaetomium subaffine*, *Arthrinium arundinis*, *Pestalotiopsis chamaeropis*, and *P. microspora*. Spring and summer shared 12 species; spring and autumn shared 16 species; spring and winter shared 15 species; summer and autumn shared 11 species; summer and winter shared 14 species; and autumn and winter shared 14 species.

2.3.2 Tissue-Specific Distribution of Endophytic Fungi

As shown in Figure 4, endophytic fungi isolated from stems comprised 15 genera; leaves, 18 genera; and roots, 10 genera. The genera shared across all three tissues were *Aphanoascus*, *Colletotrichum*, *Diaporthe*, *Hypoxylon*, *Ilyonectria*, *Pezicula*, and *Xylaria*. Unique genera found only in roots were *Hymenoscyphus* and Herpotrichiellaceae; only in stems: *Acrocalymma*; and only in leaves: *Scytalidium*, *Annulohypoxylon*, *Chaetomium*, and *Arthrinium*. Unique strains from roots were *Helotiales* sp. and *Herpotrichiellaceae* sp. 2 KO-2013; from stems: *Diaporthe discoidispora*, *Acrocalymma medicaginis*, and *Colletotrichum pseudomajus*; and from leaves: *Scytalidium auriculariicola*, *Annulohypoxylon stygium*, *Chaetomium subaffine*, and *Arthrinium arundinis*. Roots and stems shared 10 species; roots and leaves shared 8 species; and stems and leaves shared 20 species.

2.3.3 Diversity Analysis of Endophytic Fungi from *C. luteoflora*

Based on diversity indices of endophytic fungi across seasons (Figure 5 [Figure 5: see original paper]), Simpson's index ranked from high to low as winter (0.944) > spring (0.929) > autumn (0.926) > summer (0.919). Shannon-Wiener index values were winter (3.027) > spring (2.820) > autumn (2.813) > summer (2.670). Richness index values were winter (3.728) > spring (3.359) > autumn (3.285) > summer (2.890). Evenness index values were winter (0.952) > spring (0.926) > summer (0.923) > autumn (0.906). For different tissues, Simpson's index ranked stems (0.947) > leaves (0.936) > roots (0.887). Shannon-Wiener index values were stems (3.116) > leaves (2.958) > roots (2.337). Richness index values were stems (3.897) > leaves (3.628) > roots (2.023). Evenness index values were stems (0.945) > leaves (0.919) > roots (0.911). These results indicate that among seasons, winter had the most abundant and evenly distributed endophytic fungal populations; among tissues, stems harbored the most abundant and evenly distributed populations, while roots had the simplest and least stable endophytic fungal community.

2.3.4 Similarity Analysis of Endophytic Fungi from *C. luteoflora*

Based on the species composition of endophytic fungi from *C. luteoflora*, cluster analysis was performed using squared Euclidean distance and between-groups linkage to assess similarity among seasons and tissues. As shown in Figure 6 [Figure 6: see original paper], J, Y, and G represent stems, leaves, and roots, respectively, while C, D, X, and Q represent spring, winter, summer, and autumn, respectively. The analysis revealed two major clusters: one for tissues and one for seasons, indicating significant differences in endophytic fungal composition between these categories. Within the tissue cluster, stems and leaves showed the highest similarity, while roots differed significantly from both. Within the season cluster, spring and winter showed higher similarity, as did summer and autumn, while clear differences existed between the spring-winter and summer-autumn groups.

2.4 Antimicrobial Activity of Endophytic Fungi from *C. luteoflora*

Plate confrontation results (Table 3) showed that among 35 tested endophytic fungal strains, 26 exhibited inhibitory effects on at least one pathogenic fungus (74.29%). The proportions of strains showing antimicrobial activity against *Pestalotiopsis theae*, *Fusarium moniliforme*, *Colletotrichum fragariae*, *Fusarium* *oxysporum*, *Fusarium oxysporum* f. sp. *niveum*, *Sclerotinia sclerotiorum*, *Colletotrichum cliviicola*, and *Alternaria alternata* were 17.14%, 25.71%, 37.14%, 25.71%, 20.00%, 34.29%, 42.86%, and 25.71%, respectively.

Five endophytic strains showed strong antimicrobial activity with broad-spectrum effects: CJ-II-2, XY-V-3, QY-II-4, QJ-III-2, and DJ-I-2. Strain XY-V-3 exhibited inhibition rates exceeding 50% against all eight pathogenic fungi, with the strongest inhibition against *C. fragariae* (76.31%), *F. oxysporum* f. sp. *niveum* (80.64%), and *A. alternata* (77.49%). Strain DJ-I-2 showed the strongest inhibition against *S. sclerotiorum* (82.16%) and *C. fragariae* (78.43%). Strain QJ-III-2 demonstrated the strongest inhibition against *F. moniliforme* with a rate of 75.41% (Figure 7 [Figure 7: see original paper]).

Note: +++ indicates inhibition rate \$ 75 *inhibitionrate* 75 \$inhibition rate < 50%; - indicates no inhibition.

3 Discussion and Conclusion

This study isolated 261 endophytic fungal strains from roots, stems, and leaves of *C. luteoflora* across four seasons, identifying them as 22 genera with *Colletotrichum*, *Diaporthe*, and *Pestalotiopsis* as dominant genera. These results are similar to those reported for endophytic fungi in *Camellia oleifera*, another species in the Theaceae family (Zhang et al., 2017). *Camellia luteoflora* shares eight genera with the sympatric tree fern *Alsophila spinulosa* from the Chishui Nature Reserve in Guizhou, including *Arthrinium*, *Cladosporium*, *Colletotrichum*, *Fusarium*, *Hypoxylon*, *Pestalotiopsis*, *Phomopsis*, and *Xylaria* (Liu et al., 2021). However, only four genera were shared with tea plants (*Camellia sinensis*) from Zigong (You, 2009), likely due to greater geographical distance. The more similar endophytic fungal composition between *C. luteoflora* and the co-occurring *A. spinulosa* suggests that environment significantly influences endophytic fungal community composition. Among the 35 strains isolated from *C. luteoflora*, 33 have been reported in other plant endophytic fungal communities, except for *Aphanoascus verrucosus* (QY-II-6) and *Scytalidium auriculariicola* (XY-V-3).

The overall colonization and isolation rates of endophytic fungi in *C. luteoflora* were 71.30% and 80.55%, respectively, similar to those reported for *Datura metel* and *Taxus wallichiana* var. *mairei* in southern China (Zang et al., 2014; Feng, 2021), but slightly higher than those for plants in northern regions. This may be related to higher precipitation and humidity in southern tropical and subtropical regions, which favor endophytic fungal colonization (Porras-Alfaro & Bayman, 2011). The colonization and isolation rates in leaves and stems were similar and higher than in roots, with diversity indices ranking stems > leaves > roots, indicating tissue-specific preferences of endophytic fungi in *C. luteoflora*. This may be because leaves and stems provide more abundant nutrients and space, creating more suitable conditions for endophytic fungal colonization. Among seasons, colonization and isolation rates were higher in autumn and winter than in spring and summer, with diversity indices also higher in autumn and winter. These findings align with studies on *Pinus massoniana* and *Blumea balsamifera* in Guizhou (Luo & Yu, 2021; Tang et al., 2017). *Camellia luteoflora* prefers shade and moisture, growing in the region bordering Gulin County, Sichuan and Chishui City, Guizhou, which experiences heavy autumn rainfall and average temperatures of 17°C from September to December (Weng et al., 2010). Additionally, autumn and winter are the flowering and fruiting periods for *C. luteoflora*, during which significant changes in internal nutrient accumulation and hormone levels occur (Sun et al., 2017), potentially contributing to more active endophytic fungal growth during these seasons.

Cluster analysis revealed higher similarity between spring and winter endophytic fungal communities, as well as between summer and autumn communities, with clear differences between these two seasonal groups. This reflects the long-term co-evolutionary adaptation of endophytic fungi to seasonal environmental changes within the plant (Zhang et al., 2013). Stems and leaves showed the highest similarity in endophytic fungal composition, while roots differed significantly from both, likely because different plant organs have distinct microenvironments, including chemical composition, space availability, and aeration status, which directly affect endophytic fungal community composition (Vesterlund et al., 2011).

Plate confrontation assays revealed that 26 endophytic fungal strains exhibited inhibitory effects on at least one plant pathogen, with five strains showing strong, broad-spectrum activity: *Diaporthe phaseolorum* (CJ-II-2), *Scytalidium auriculariicola* (XY-V-3), *Hypoxylon fragiforme* (QJ-III-2), *Fusarium graminearum* (DJ-I-2), and *Annulohypoxylon stygium* (QY-II-4). *Scytalidium auriculariicola* showed strong inhibition against all eight pathogenic fungi, with rates exceeding 50%. Ahmad et al. (2020) found that *S. parasiticum* produces alkaloids, flavonoids, and fatty acids with antifungal activity, suggesting that *Scytalidium* species produce diverse secondary metabolites with strong antifungal properties. This study also found that *F. graminearum* strongly inhibited *S. sclerotiorum* and *C. fragariae*, with an 82.16% inhibition rate against *S. sclerotiorum*, consistent with findings by Fu et al. (2006). Jin et al. (2022) reported that *Hypoxylon* spp. from hickory (*Carya cathayensis*) produced secondary metabolites inhibiting three plant pathogens, while Becker et al. (2020) found that *A. viridistratum*, a congeneric species of *A. stygium*, produced antimicrobial compounds active against *Candida albicans* and *Mucor hiemalis*. These findings suggest that *Annulohypoxylon* and *Hypoxylon* species possess biocontrol potential through their secondary metabolites. Further research on the antimicrobial mechanisms of these five endophytic strains could provide theoretical support for their development and application in *C. luteoflora* disease management.

Among the eight plant pathogens tested, *Colletotrichum cliviicola* and *Alternaria alternata* are pathogens of *C. luteoflora*, causing yellowish-white spots and black patches on leaves, respectively (Dai, 2021). Both *Scytalidium* *auriculariicola* and *Hypoxylon fragiforme* showed inhibition rates exceeding 50% against these two pathogens, demonstrating promising potential for controlling diseases in *C. luteoflora* itself and warranting further investigation for biocontrol development.

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