



# Diversity of endophytic fungi in *Coptis chinensis* Franch. and their activity against methicillin-resistant *Staphylococcus aureus*

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## Abstract

This study aimed at investigating the diversity of endophytic fungi from *Coptis chinensis* and their activity against methicillin-resistant *Staphylococcus aureus* (MRSA). Seventy-nine fungal isolates obtained from *C. chinensis* were identified to belong to 27 species based on morphological features and internal transcript spacer (ITS) gene sequencing analysis. Comparing relative frequency values, the most frequent genera were *Colletotrichum* and *Fusarium*, while most frequent species were *C. gloeosporioides* and *F. avenaceum*. Analysis of diversity indices indicated that *C. chinensis* harbored abundant fungal resources. Methanol extracts of fungal endophyte cultures were evaluated for antibacterial activity against *S. aureus* ATCC 25923 and two other MRSA clinical strains. Nine of 27 endophytic fungi exhibited inhibitory activities against *S. aureus* ATCC 25923. Among them, *Paraboeremia litseae* HL-17, *Fusarium* sp. HL-23, and *Fusarium* sp. HL-27 exhibited obvious inhibition against the three *S. aureus* strains. Our findings suggest that the endophytic fungi in *C. chinensis* have a high diversity and an obvious tissue specificity, and could be of potential interest in screening anti-MRSA agents. To the best of our knowledge, this is the first report on the diversity and anti-MRSA activity of fungal endophytes from *C. chinensis*.

**Keywords** *Coptis chinensis* · Endophytic fungi · Diversity · Tissue specificity · Methicillin-resistant *Staphylococcus aureus*

## Introduction

*Coptis chinensis* Franchet (Ranunculaceae), which is known as *Weilian* (one of three kinds of *Huanglian*) in China, is an important and well-known traditional Chinese medicinal plant. Its rhizomes, which contain alkaloids as the main bioactive components and some other types of natural products including organic acids, phenylpropanoids, lignans, flavones, volatile oils, saccharides, and steroids (Rui et al. 2020), have been frequently utilized for the treatment of various diseases including bacillary dysentery, typhoid, tuberculosis, epidemic cerebrospinal meningitis, empyrosis, pertussis, and other illnesses (Meng et al. 2018; Wang et al. 2019). Recent studies have indicated that the rhizome of *C. chinensis* has wide pharmacological activities, including antibacterial, antiviral, antifungal, anti-atherosclerosis, antimyocardial

ischemia/reperfusion injury, anti-hyperlipidemia, antihypertension, anti-obesity, anticancer, anti-Alzheimer's disease, anti-inflammation, antioxidation, and aging-related diseases (Xu et al. 2017; Ran et al. 2019; Wang et al. 2019, 2020a). Due to its age-old use in traditional medicine and perennial dug, the resource of wild *C. chinensis* is extremely rare. Furthermore, the cultivated *C. chinensis* requires long-term cultivation and a strict growth environment to maintain its quality. Therefore, we studied the endophytic fungi as a possible alternative to *C. chinensis*.

Endophytic fungi refer to groups of fungi that inter and/or intracellularly colonize healthy tissues of host plants during all or part of their lifecycle without causing obvious pathogenic symptoms (Aly et al. 2010; Xiao et al. 2021). There is a very complex relationship between endophytic fungi and their host plants (Du et al. 2020). Endophytic fungi have the ability to produce hormones and/or promote the biosynthesis of host plant secondary metabolites (Ming et al. 2013), enabling host plants to grow quickly and resist external biotic and abiotic stresses (Yan et al. 2019), and produce many products with various biological activities (Gupta et al. 2020). These bioactive compounds, which belong to

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various structural groups including alkaloids, peptides, steroids, terpenoids, phenols, quinones, phenols, and flavonoids, possess a variety of biological properties that include antibacterial, antiviral, antifungal, antiprotozoal, antiparasitic, antioxidant, immunosuppressant, and anticancer functions (Manganyi and Ateba 2020).

*Staphylococcus aureus*, the most common pathogen of food contaminants, is recognized for its virulence and its ability to produce staphylococcal enterotoxins and cause a variety of diseases, such as local suppurative infection, osteomyelitis, pneumonia, pseudomembrane, enteritis, meningitis, pericarditis, and toxic shock syndrome (Odeyemi et al. 2018; Wang et al. 2020b; Matias et al. 2021). Antibiotic therapy is an important strategy for *S. aureus* control. However, as a response to the selective pressure of antimicrobials, the multi-drug resistant *S. aureus* appeared and the treatment options for clinicians and veterinarians have been narrowed (Wang et al. 2018). It reported that methicillin-resistant *S. aureus* (MRSA), one kind of multi-drug resistant *S. aureus*, spread across the world causing a variety of nosocomial infections, community infectious diseases and contaminated exceeded 50% of cases in intensive care unit (ICU) patients since 1999 (Matias et al. 2021). Therefore, it is in high demand for developing novel antibacterial agents for MRSA infection treatment.

As a rich source of novel natural products, the endophytic fungus is a treasure house for developing novel antibacterial agents. Moreover, *C. chinensis* was reported to have strong antibacterial properties against MRSA (Kim et al. 2020). Therefore, this study aimed to determine the diversity and anti-MRSA activity of endophytic fungi from the medicinal plant *C. chinensis*. In this research, the diversity of the endophytic fungi isolated from the healthy fibrous root, rhizome, and leaf of *C. chinensis* was evaluated, and the antimicrobial activities against MRSA were examined. To the best of our knowledge, this work is the first report on the biodiversity, phylogeny, and assessment for the anti-MRSA activity of endophytic fungi harbored in *C. chinensis*.

## Materials and methods

### Collection of plant material

Healthy and symptomless 3-year-old *C. chinensis* plants were collected from Huangshui Town, Shizhu County, Chongqing, China. Selected plant samples were immediately transported to the laboratory and preserved at 4 °C. The *C. chinensis* plants were identified by Professor Peng Li from the College of pharmacy at the Army Medical University. For isolation of fungal endophytes, samples were processed within 24 h after harvest.

### Isolation and purification of fungal endophytes

The isolation of fungal endophytes from collected plant parts was according to a standard procedure established previously (Stierle et al. 1993). The fibrous root, rhizome, and leaf of *C. chinensis* plants were used to isolate endophytic fungi. These three kinds of tissue were thoroughly washed in running tap water for 3–6 h followed by drying with sterile filter paper. Then, the cleared tissue was surface-sterilized by sequential immersion in 75% ethanol for 30 s, 1.3 mol/L sodium hypochlorite (3–5% available chlorine) for 2–5 min, and 75% ethanol for 30 s. All tissues were then rinsed three times in sterile water to remove any excess surface sterilants. After sterilization, the samples were dried off with sterile filter paper and cut into small pieces (about 0.5 cm) using a sterile blade. These tissue pieces were placed on potato dextrose agar (PDA) plates with 50 mg/L penicillin at 25 °C to isolate the fungal endophytes. The tissue pieces were observed every day for mycelia growth. Once the fungal mycelia grew out of the tissue pieces, the hyphal tips were transferred using a sharp sterile sharp needle to fresh PDA plates. Morphologically distinct isolates were similarly sub-cultured several times to purify and obtain pure isolates. The pure isolates were numbered and kept in a storage tube at –20 °C for further study.

### Molecular identification

Identification of fungal isolates was based on molecular and morphological analysis. The molecular identification was carried out using the Internal Transcript Spacer regions (ITS1 and ITS2) and the intervening 5.8 S rRNA region sequencing. Each fungal isolate was cultured on a PDA plate at 25 °C for up to 10 days, and the fungal mycelia were collected. The genomic DNA was extracted using the traditional cetyltrimethylammonium bromide (CTAB) method (Saghai-Marooof et al. 1984). The isolated DNA was air-dried, dissolved in 20 µL of sterile Millipore water, and stored at –20 °C for further study. Polymerase chain reaction (PCR) was performed to amplify the ITS region of the fungal isolates using the universal ITS primers, ITS5 (5'-GGAAGTAAAAGTTCG TAAGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'). The PCR reaction mixture (25 µL) contained 1 µL template (50 ng/µL purified DNA sample), 15 µmol of each primer, 12.5 µL 2×Taq PCR Master Mix, and 10.5 µL ddH<sub>2</sub>O. PCR conditions performed were as follows: initial denaturation at 95 °C for 3 min, followed by 35 cycles of 94 °C for 40 s, 52 °C for 50 s, and 72 °C for 1 min, and a final extension at 72 °C for 10 min. The amplified PCR products were checked on 1% agarose gel and then sent to Sangon

Biotech (Shanghai) Co., Ltd for sequencing. All obtained fungal ITS sequences have been deposited in GenBank and analyzed using BLAST search in the National Center of Biotechnology Information (NCBI) database to compare the sequence homology with closely related organisms. Then, the sequences from closely related organisms were downloaded to conduct the phylogenetic analysis using the neighbor-joining (NJ) method. Bootstrap analysis was carried out using 1000 replications with MEGA6.

### Diversity analyses of endophytic fungi

The relative frequency (RF) was used to estimate an endophytic-specific taxon from the sampled plants (Yao et al. 2017). The colonization frequency (CF) (formula:  $CF = \frac{N_{col}}{N_t} \times 100\%$ ,  $N_{col}$  is the number of segments colonized by an individual species and  $N_t$  is the total number of incubated segments) reflect the extent of endophyte infection (Zhang et al. 2021). The diversity of fungal species from *C. chinensis* was evaluated using the species richness index (S), Shannon–Wiener index ( $H'$ ) and Simpson's diversity index (1-D). The species richness index (S) was obtained by counting the number of endophytic fungal species in corresponding plant tissues. The Shannon–Wiener index ( $H'$ ) and Simpson's diversity index (1-D) were evaluated using the Past ver. 3 software (Fan et al. 2020). Sorensen's index of similarity (QS) (formula:  $QS = \frac{2a}{2a+b+c}$ ,  $a$  is the number of common species in both endophytic tissues, while  $b$  and  $c$  are the number of species specific to the compared tissues, respectively) was used to evaluate the similarity of endophytic fungal assemblage among different tissues (Zhang et al. 2021).

### Extraction of secondary metabolites of different endophytic fungi

Submerged cultivation of the fungal endophytes was carried out to produce secondary metabolites. Five agar plugs (5 mm diameters) of grown isolates were transferred to 250 mL of the Erlenmeyer flask containing 100 mL potato dextrose broth, followed by incubation for 5–7 days at 28 °C under constant shaking (180 rpm). The final cultivation step was performed by transferring the pre-inoculum into 500 mL of the Erlenmeyer flask containing rice solid medium supplemented with 2 g peptone and incubating for 6 weeks at room temperature. After cultivation, the secondary metabolites of each endophyte were extracted by ultrasonic with methanol (analytical grade). Then, the extract solution was concentrated under reduced pressure and dissolved in fresh methanol.

### Bacteria and culture methods

Three *S. aureus* strains (ATCC 25923, clinical strains sequence type (ST)-5 and ST-59) were provided by professor Hao Zeng from the National Engineering Research Center of Immunological Products. Bacteria were grown on Mueller–Hinton agar (MHA) plates at 37 °C overnight, followed by picking and inoculating single colonies in Mueller–Hinton broth (MHB) at 37 °C for 12 h with agitation (220 rpm). An appropriate dilution of bacteria was done with PBS, and the bacterial count was adjusted by determining the absorbance spectrophotometrically at 600 nm.

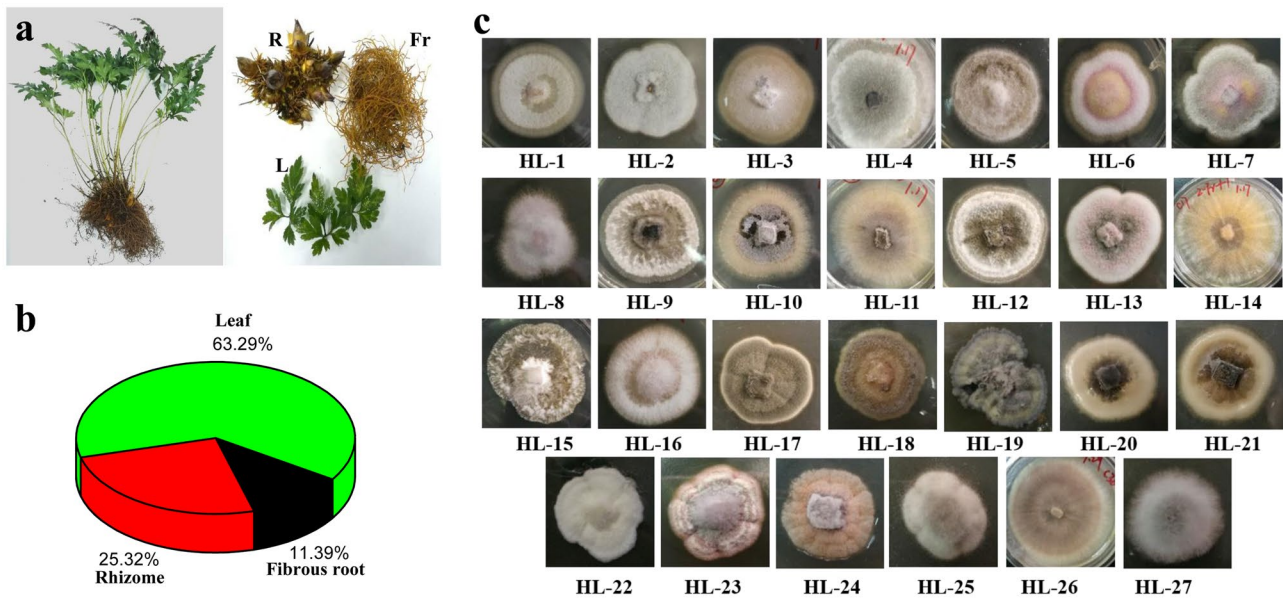
### Antibacterial activity

Crude methanol extracts of each fungal endophyte were evaluated for their antibacterial activity against *S. aureus* ATCC 25923, ST-5, and ST-59. Five milliliter extract solution of each fungal endophyte was dried at 60 °C and redissolved with 50% methanol at the concentration of 50 mg/mL. Sterile filter paper discs with 5 mm diameters were soaked in the test samples and control (methanol) for 2 h followed by natural volatilization and ultraviolet sterilization. Subsequently, the filter papers were placed separately on plates inoculated with an appropriate count of different strains of *S. aureus*. The plates were incubated at 37 °C for 24 h. The antibacterial potential of fungal endophyte extracts was determined following the diameter of the zone of inhibition around the filter paper. Extract samples that showed the zone of inhibition around the filter papers were considered as positive, while those without the zone of inhibition around the filter papers were negative. Experiments were replicated three times and data were presented as means  $\pm$  SEM.

## Results

### Isolation and identification of culturable endophytic fungi from *C. chinensis*

In this study, different healthy tissue of *C. chinensis* (Fig. 1a), including fibrous root (Fr), rhizome (R), and leaf (L), were used to isolate endophytic fungi. We isolated 79 endophytic fungal strains from those tissues of seven *C. chinensis* plants. Among these strains, nine (11.39%) were isolated from fibrous roots, 20 (25.32%) were isolated from the rhizomes, and 50 (63.29%) were isolated from leaves, respectively (Fig. 1b). These results indicated that the leaves contained the most endophytic fungi and the fibrous roots contained the least endophytic fungi.



**Fig. 1** *C. chinensis* and its different tissue **a**; isolation rate of endophytic fungi from different tissue of *C. chinensis* **b**; endophytic fungi isolated from *C. chinensis* **c**

Based on the fungal morphological characteristics and appearance on PDA, all the endophytic fungal strains isolated from *C. chinensis* were assigned to 27 representative morphotypes (Fig. 1c). Subsequently, ITS rDNA sequences of each morphotype were generated and were submitted to NCBI for BLASTn comparison. Table 1 shows the identified endophytic fungal isolates, including GenBank accession number, homolog sequences, sequence identity, and closest accession number. The maximum sequence identity of the ITS rDNA sequences of endophyte compared with those available in GenBank ranged from 98.26 to 100% (Table 1). Endophytic fungi with 100% base similarity of ITS gene sequence between the reference isolates in GenBank are considered to be identified into species. The others with base similarity of ITS gene sequence between the reference isolates at the range of 98–99.9% are considered to be identified to genera. Therefore, 10 of the 27 representative morphotypes were identified to genus, while the other 17 were identified to species (Table 1; Fig. 2). Furthermore, all the endophytic fungal isolates were identified to belong to two phyla (Ascomycota and Basidiomycota), three classes (Agaricomycetes, Dothideomycetes, and Sordariomycetes), six orders, 11 families, 12 genera, and 27 species (Fig. 2).

### The relative frequency (RF) analyses of endophytic fungi from *C. chinensis*

The relative frequency (RF) values of the 27 representative isolates are shown in Table 1. By comparing the RF values, the most frequent species of *C. chinensis* belonged to *C. gloeosporioides* and *F. avenaceum* with RF both of 11.39%

(Table 1). In addition, the most frequent genera of *C. chinensis* associated endophytic fungi belonged to *Colletotrichum* and *Fusarium* with RF of 49.37 and 26.58%, respectively (Fig. 3a). These results indicated that the dominant fungal species of *C. chinensis* were *C. gloeosporioides* and *F. avenaceum*, and the dominant fungal genera of *C. chinensis* were *Colletotrichum* and *Fusarium*, respectively.

On the basis of different tissues, the most frequent endophyte in fibrous root or rhizome belonged to *F. avenaceum*, and the most frequent endophyte in leaf belonged to *C. gloeosporioides* (Table 1). Furthermore, the most frequent genera in fibrous root or rhizome belonged to *Fusarium* with RF of 77.78 or 70.00% respectively, while the most frequent genera in leaf belonged to *Colletotrichum* with RF of 78.00% (Fig. 3b). These results indicated that the dominant endophyte in fibrous root or rhizome was *F. avenaceum*, and that in leaf was *C. gloeosporioides*. In addition, the dominant genus in fibrous root or rhizome was *Fusarium* and that in leaf was *Colletotrichum*.

### Diversity of endophytic fungi from *C. chinensis*

As shown in Table 2, the indices of colonization frequency, species richness (S), Shannon–Wiener ( $H'$ ), and Simpson's diversity index (1-D) are listed to characterize the endophytic community from the three kinds of plant tissue. The colonization frequency of the endophytic fungi in whole plant of *C. chinensis* was 37.86%. Among the different tissue, colonization frequencies of the endophytic fungi were found in the following order: leaf (53.57%) > fibrous root (28.57%) > rhizome (26.79%). The colonization frequency of the endophytic fungi in leaf was significantly higher than that in fibrous root

**Table 1** The ITS sequence identification and the relative frequency of the endophytic fungal strains from *C. chinensis*

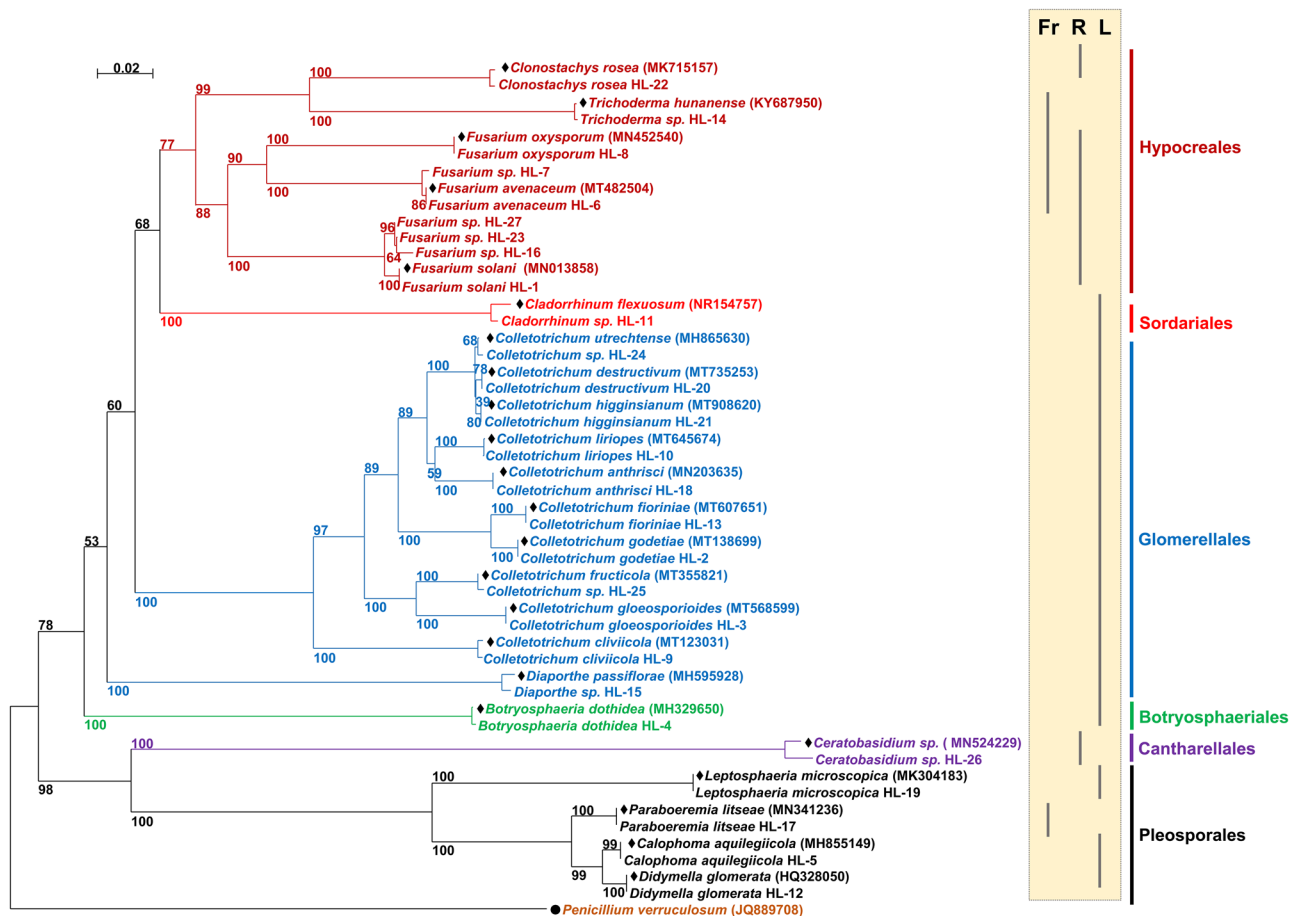
Fungal strain code	GenBank accession number	Homolog sequences	Sequence identity, %	Closest accession number	RF(%)			
					Fr	R	L	Total
HL-1	OK047753	<i>Fusarium solani</i>	100	MN013858	1.27	3.80		5.07
HL-2	OK047754	<i>Colletotrichum godetiae</i>	100	MT138699			7.59	7.59
HL-3	OK090938	<i>Colletotrichum gloeosporioides</i>	100	MT568599			11.39	11.39
HL-4	OK090939	<i>Botryosphaeria dothidea</i>	100	MH329650			2.53	2.53
HL-5	OK090940	<i>Calophoma aquilegiicola</i>	100	MH855149			6.33	6.33
HL-6	OK090944	<i>Fusarium avenaceum</i>	100	MT482504	5.06	6.33		11.39
HL-7	OK090947	<i>Fusarium avenaceum</i>	99.58	MT482504	1.27	2.53		3.8
HL-8	OK104019	<i>Fusarium oxysporum</i>	100	MN452540	1.27			1.27
HL-9	OK104020	<i>Colletotrichum cliiviicola</i>	100	MT123031			3.8	3.8
HL-10	OK090952	<i>Colletotrichum liriopes</i>	100	MT645674			6.33	6.33
HL-11	OK090953	<i>Cladorrhinum flexuosum</i>	98.76	NR154757			1.27	1.27
HL-12	OK090954	<i>Didymella glomerata</i>	100	HQ328050			1.27	1.27
HL-13	OK090995	<i>Colletotrichum fioriniae</i>	100	MT607651			5.06	5.06
HL-14	OK090996	<i>Trichoderma hunanense</i>	99.81	KY687950	1.27			1.27
HL-15	OK090997	<i>Diaporthe passiflorae</i>	99.59	MH595928		1.27		1.27
HL-16	OK090998	<i>Fusarium solani</i>	99.16	MH290450		1.27		1.27
HL-17	OK090999	<i>Paraboeremia litseae</i>	100	MN341236	1.27			1.27
HL-18	OK103605	<i>Colletotrichum anthrisci</i>	100	MN203635			1.27	1.27
HL-19	OK103606	<i>Leptosphaeria microscopica</i>	100	MK304183			2.53	2.53
HL-20	OK103612	<i>Colletotrichum destructivum</i>	100	MT735253			8.86	8.86
HL-21	OK103615	<i>Colletotrichum higginsianum</i>	100	MT908620			2.53	2.53
HL-22	OK103616	<i>Clonostachys rosea</i>	100	MK715157		1.27		1.27
HL-23	OK103617	<i>Fusarium solani</i>	99.79	KJ573076		2.53		2.53
HL-24	OK103620	<i>Colletotrichum higginsianum</i>	99.80	MH638225			1.27	1.27
HL-25	OK103841	<i>Colletotrichum fructicola</i>	99.79	MT355821			1.27	1.27
HL-26	OK103842	<i>Ceratobasidium</i> sp.	98.26	MN524229		5.06		5.06
HL-27	OK103843	<i>Fusarium solani</i>	99.59	KJ573076		1.27		1.27

or rhizome. The difference in the colonization frequency between fibrous roots and rhizomes was not significant.

The species richness (S) of endophytic fungi from *C. chinensis* was 27. Among the different tissue, species richness (S) of the endophytic fungi was found in the following order: leaf (15) > rhizome (9) > fibrous root (6). The Shannon–Wiener Index ( $H'$ ) of endophytic fungi from *C. chinensis* was 2.992. Among the different tissue, the Shannon–Wiener Index ( $H'$ ) of the endophytic fungi was found in the following order: leaf (2.447) > rhizome (2.013) > fibrous root (1.581). The Simpson's diversity index (1-D) of the endophytic fungi from *C. chinensis* was 0.9373. Among the different tissue, Simpson's diversity index (1-D) of the endophytic fungi was found in the following order: leaf (0.8968) > rhizome (0.8450) > fibrous root (0.7407). These results indicated that *C. chinensis* harbored abundant fungal resources, and the diversity of endophytic fungi from leaves was more abundant than that in the other two tissues.

### Tissue specificity of endophytic fungi from *C. chinensis*

The Sorenson similarity analysis for the endophytes among the three kinds of tissue indicated the various species of endophytic fungi from *C. chinensis* with obvious tissue specificity. The Sorenson similarities for the endophytes between fibrous root and rhizome, between fibrous root and leaf, and between rhizome and leaf were 0.40, 0.00, and 0.00, respectively (Table 3). It indicated that the endophytic community in the leaf was quite distinct from that in the rhizome or fibrous root. Similar results were observed in Fig. 2. At the order classification level, endophytic fungi of Sordariales, Glomerellales, and Pleosporales in *C. chinensis* were almost distributed in the leaf except for *Paraboeremia litseae* HL-17 (Pleosporales). Furthermore, endophytic fungi of Hypocreales and Cantharellales were only distributed in rhizome or fibrous root. At the genus classification level,



**Fig. 2** Neighbor-joining phylogenetic tree of 27 representative isolates from *C. chinensis*. The phylogenetic tree is based on ITS gene sequences. The values at each node represent the bootstrap values from 1000 replicates, and the scale bar represents 0.02 substitutions

per nucleotide. *Penicillium verruculosum* (JQ889708) was used as an outgroup. (diamond, the maximum identity ITS rDNA sequences of the endophyte available in GenBank.)

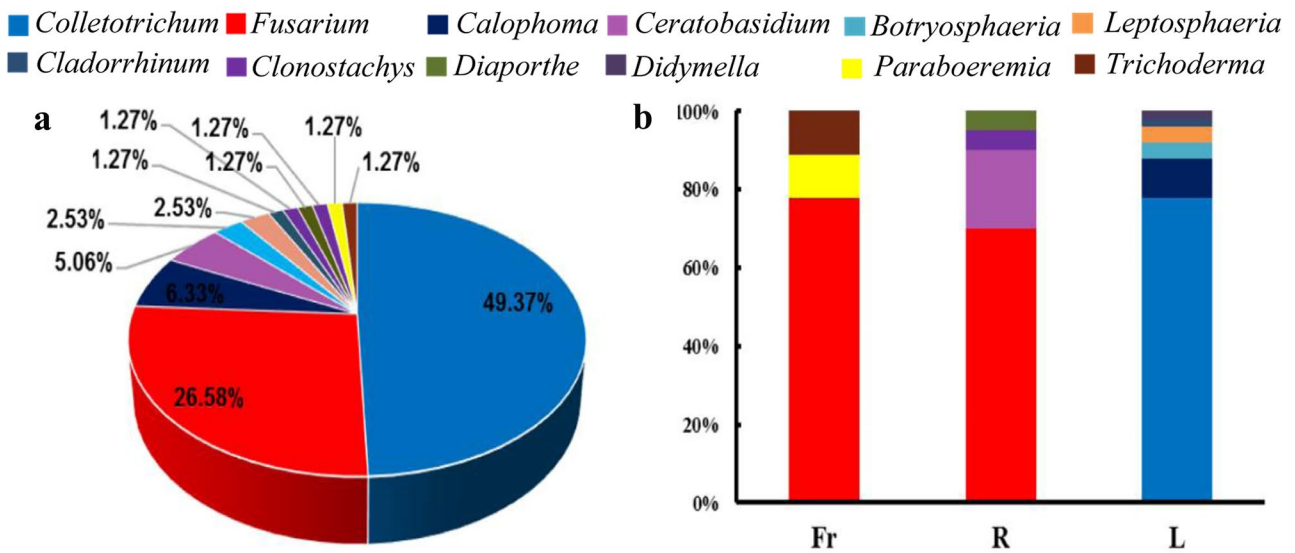
endophytic fungi of *Colletotrichum*, *Calophoma*, *Botryosphaeria*, *Leptosphaeria*, *Cladorrhinum*, and *Didymella* were only distributed in leaf of *C. chinensis*. Furthermore, endophytic fungi of *Clonostachys* and *Diaporthe* were only distributed in rhizome, while that of *Paraboeremia*, and *Trichoderma* were only distributed in fibrous root. Otherwise, endophytic fungi of *Fusarium* were distributed in both fibrous root and rhizome. All these results indicated that the endophytic fungi from *C. chinensis* had an obvious tissue specificity.

### Anti-MRSA activity screening of the methanol extracts from endophytic fungal cultures

To estimate the anti-MRSA activities of culturable endophytic fungi from *C. chinensis*, antimicrobial activities of methanol extracts of 27 representative isolates grown on rice

solid fermentation media were evaluated by the measurement of the diameter of the zone of inhibition against the three *S. aureus* strains (ATCC 25923, clinical strains ST-5 and ST-59). *S. aureus* ATCC 25923, which is no methicillin-resistant, is used as a standard laboratory testing control strain. *S. aureus* ST5 is a hypertransmissible, strong biofilm-forming, methicillin-resistant, and virulent genotype that is frequently encountered in today's operating room environments (Loftus et al. 2018). *S. aureus* ST 59 is an epidemic lineage of community-associated methicillin-resistant *S. aureus* in Asia (Feng et al. 2017).

As shown in Table 4, nine endophytic fungal methanol extracts exhibited inhibitory activities against at least one *S. aureus* strain and the other 18 extracts did not show antimicrobial activities (data not shown). These nine strains belonged to genera of *Fusarium* (4), *Colletotrichum* (3), *Calophoma* (1), and *Paraboeremia* (1). The zone of inhibition of *Colletotrichum* fungi (HL-10, HL-13, and



**Fig. 3** Relative frequency (RF) of endophytic fungi from *C. chinensis* at the level of genus **a** and in different tissue (Fr, fibrous roots; R, rhizomes; L, leaves) of *C. chinensis* at the level of genus **b**

HL-25), *Calophoma* fungus (HL-5), and *Paraboeremia* fungus (HL-17) against *S. aureus* ATCC 25923 were significantly larger than that of *Fusarium* fungi (HL-1, HL-7, HL-23, and HL-27). Furthermore, no significant difference in the diameters of the zone of inhibition found among the *Colletotrichum* fungi (HL-10, HL-13, and HL-25), *Calophoma* fungus (HL-5), and *Paraboeremia* fungus (HL-17). Among the nine strains, three (*P. litseae* HL-17, *Fusarium* sp. HL-23, *Fusarium* sp. HL-27) exhibited obvious inhibition against all the three strains of *S. aureus* (ATCC 25923, ST 5, and ST 59) (Fig. 4) and the other 6 just exhibited obvious inhibition against *S. aureus* ATCC 25923 (Table 4). It is interesting to note that only two strains (HL-23 and HL-27) of the four *Fusarium* fungi exhibited significant inhibition against all the three strains of *S. aureus* and the inhibition of HL-17 against all the three strains of *S. aureus* was much stronger than that of HL-23 or HL-27.

**Table 2** Colonization frequency and diversity indices of endophytic fungi isolated from different tissues of *C. chinensis*

Parameter	<i>C. chinensis</i>			
	Fibrous root	Rhizome	Leaf	Total
Colonization frequency (%)	28.57	26.79	53.57	37.86
Species richness(S)	6	9	15	27
Shannon–Wiener (H')	1.581	2.013	2.447	2.992
Simpson's diversity index (1-D)	0.7407	0.845	0.8968	0.9373

### Discussion

As an important and well-known traditional Chinese medicinal plant, *C. chinensis* has been used to treat various inflammatory disorders and related diseases for a thousand years. As far as we know, there is no report about the endophytic fungal diversity of *C. chinensis*. In our study, 79 endophytic fungi isolated from *C. chinensis* fibrous root, rhizome, and leaf were divided into 27 species, 12 genera, 11 families, six orders, three classes, and two phyla. Two phyla of all endophytic fungi are Ascomycetes and Basidiomycetes. Ascomycetes (74 isolates) are the most common representatives of endophytic fungal communities, while Basidiomycetes constitutes the rest five isolates. This result is consistent with the reports of endophytic fungal contents of most plants (Du et al. 2020). Among the 12 genera, *Colletotrichum* and *Fusarium* were the dominant ones. Thus, the host has a strong affinity towards establishing symbiotic associations with the fungi belonging to the genera *Colletotrichum* and *Fusarium*. Among the 27 species, *C. gloeosporioides* and *F. avenaceum* were the dominant ones. However, *F. avenaceum* has been previously reported as a

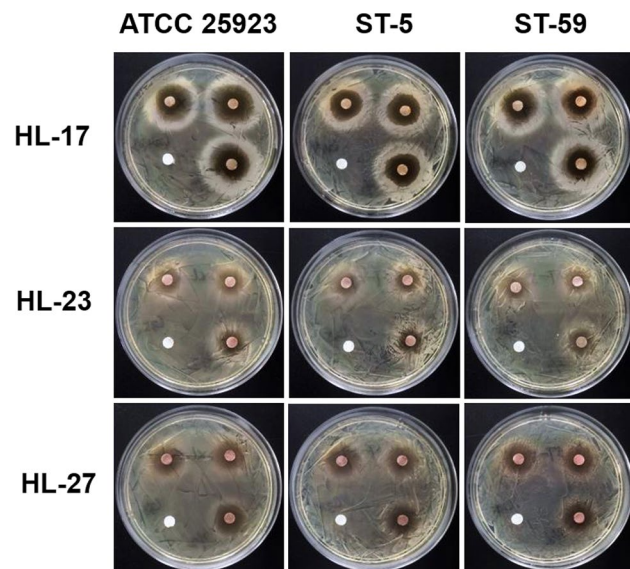
**Table 3** Sorenson's similarity for endophytic fungi from different tissues of *C. chinensis*

Tissues	Fibrous root	Rhizome	Leaf
Fibrous root	1.00	0.40	0.00
Rhizome		1.00	0.00
Leaf			1.00

**Table 4** Anti-MRSA activities of endophytic fungi obtained from *C. chinensis*

Fungal isolate code	The diameter of the zone of inhibition (mm)		
	ATCC 25923	ST-5	ST-59
<i>Fusarium solani</i> HL-1	8.7 ± 1.2	—	—
<i>Calophoma aquilegiicola</i> HL-5	19.7 ± 2.1	—	—
<i>Fusarium</i> sp. HL-7	13.3 ± 0.6	—	—
<i>Colletotrichum liriope</i> HL-10	21.0 ± 1.0	—	—
<i>Colletotrichum fioriniae</i> HL-13	19.3 ± 1.2	—	—
<i>Paraboeremia litseae</i> HL-17	20.3 ± 0.6	19.0 ± 1.0	14.3 ± 0.6
<i>Fusarium</i> sp. HL-23	13.7 ± 0.6	12.3 ± 0.6	11.7 ± 1.2
<i>Colletotrichum</i> sp. HL-25	23.7 ± 3.1	—	—
<i>Fusarium</i> sp. HL-27	15.3 ± 0.6	16.0 ± 1.0	14.3 ± 0.6

pathogen confirmed by re-inoculated back on *C. chinensis* (Mei et al. 2020). Several studies indicate that the endophytes isolated from asymptomatic plant tissues express either mutualistic, commensal, or parasitic lifestyles when re-inoculated back on the original host species (Rodriguez and Redman 2008). Therefore, there is a balance between the fungi and the host plants. When the fungus was inoculated to the host plant, it would be an endophytic fungus if a balanced antagonism is established. Otherwise, it would be a pathogen resulting in disease.

**Fig. 4** Anti-MRSA activities of endophytic fungi *Paraboeremia litseae* HL-17, *Fusarium* sp. HL-23, and *Fusarium* sp. HL-27 were obtained from *C. chinensis*. The white sterile filter paper discs soaked in methanol for 2 h followed by natural volatilization and ultraviolet sterilization were regarded as negative controls

In our study, the analysis of colonization frequency, species richness (S), Shannon–Wiener ( $H'$ ), and Simpson's diversity index (1-D) indices indicated that *C. chinensis* harbored abundant fungal resources. The diversity of endophytic fungi in the above-ground tissues (leaf) of *C. chinensis* was more than that in the under-ground parts (rhizome and/or fibrous root). The result is similar to fungal endophytes from *Glycyrrhiza glabra* (Arora et al. 2019). In the research of *Dillenia indica*, the percent frequency of endophytic fungi was also highest in leaves (Kumar and Prasher 2022b). Furthermore, obvious tissue specificity of endophytic fungi from *C. chinensis* was observed through the Sorenson similarity analysis. The endophytic fungal community structure of leaf was utterly distant from that of rhizome or fibrous root. The difference of micro-environments in the different tissue would be responsible for the different microbiota.

MASA has been considered as the prototype of multiresistant nosocomial pathogens. Methicillin belongs to the  $\beta$ -lactam class of compounds that are hydrolyzed by  $\beta$ -lactamase.  $\beta$ -Lactams are the most widely used broad-spectrum antibiotics, and resistance to  $\beta$ -lactams is a serious threat for infectious disease management, surgery, and organ transplantation. The emergence of MRSA indicates an urgent need for the control of the use of antibiotics and the development of novel therapeutic agents. The main active constituents of *C. chinensis* are protoberberine alkaloids, which can affect the accumulation of amyloid fibers in MRSA biofilm phenol-soluble modulins (PSMs), thus inhibiting the formation of MRSA biofilm and increasing the bactericidal activity of antibiotics. However, the low bioavailability and weak effect of berberine limit its clinical application. Therefore, structural modification and new isomers of berberine are required. Endophytic fungi are a rich source for developing novel host-like biomolecules. Some endophytic fungi isolated from the antimicrobial medicinal plants had shown significant antimicrobial activity (Al Mousa et al. 2021; Deshmukh et al. 2022; Kumar and Prasher 2022a). In our research, methanol extracts of nine endophytic fungi (33% of total screened) showed inhibitory activities against *S. aureus* ATCC 25923. Furthermore, three (*P. litseae* HL-17, *F. sp.* HL-23, *F. sp.* HL-27) of the nine endophytic fungi exhibited obvious inhibition against the two methicillin-resistant *S. aureus* strains (ST-5 and ST-59). Genus of *Fusarium* was previously reported to show antibacterial activity against *S. aureus* (Ratnaweera et al. 2015; Wen et al. 2015). However, new antibacterial compounds were constantly being discovered from the fungus of *Fusarium* (Uz Zaman et al. 2021). Moreover, there is no report about *P. litseae* producing antimicrobial compounds. Thus, three endophytic fungi obtained in this study with antimicrobial activity against the three *S. aureus* strains have the potential to produce natural products of novel structures.



Furthermore, three new polyketides and three new pyrrole alkaloids, which had potential anti-inflammatory activity, were isolated from three endophytic fungi of *C. chinensis* (Wei et al. 2022; Yin et al. 2022a, b) and one endophytic fungus isolated from *C. chinensis* was reported to produce berberine (Zhang et al. 2016). Therefore, it is noteworthy to further investigate the secondary metabolites of the three endophytic fungi (*P. litseae* HL-17, *Fusarium* sp. HL-23, and *Fusarium* sp. HL-27) from *C. chinensis*.

In this study, we report for the first time the diversity and anti-MRSA activity of fungal endophytes from *C. chinensis*. This research extended our knowledge on the distribution of endophytic fungi in *C. chinensis* and endophytic fungi from *C. chinensis* could be considered as a potential source for anti-MRSA agent in future biotechnology applications.

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## Declarations

**Conflict of interest** The authors declare no competing interests.

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