

Cultural endophytic fungi associated with *Dendrobium officinale*: identification, diversity estimation and their antimicrobial potential

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Endophytic fungi associated with Chinese medicinal plant *Dendrobium officinale* have been revealed using the culture-dependent approach and the diversity and antimicrobial activity have been examined in this study. A total of 105 endophytic isolates representing 16 fungal morphotypes were recovered from the roots, stems and leaves of *D. officinale*. Identification by ITS-rDNA sequencing showed 12 distinct operational taxonomic units, and most of them were classified into species level. Shannon's and Simpson's indices were calculated to quantify fungal diversity. The results demonstrated that endophytic fungi associated with *D. officinale* were moderately diverse, and fungal species among three tissues was also different which exhibited tissue-specificity. The composition of endophyte assemblages from the roots was most abundant and diverse, followed by the stems, while the leaves possessed relatively low isolation rate. *Fusarium oxysporum* (21.0%) and *Xylaria cubensis* (20.0%) were the most dominant species, while *Colletotrichum* sp. and *Pestalotiopsis* sp. were also frequently isolated. Three species (*F. oxysporum*, *Aspergillus niger* and *Aureobasidium pullulan*) showed broad-spectrum antimicrobial activity, and *F. oxysporum* had acute inhibition to pathogens tested. Our systematic study could enrich the current knowledge on diversity of endophytic fungi. The fungal isolates from *D. officinale* may be used for exploration of novel bioactive compounds and have potential as biocontrol agents.

Keywords: Antimicrobial activity, *Dendrobium officinale*, diversity, endophytic fungi.

DENDROBIUM OFFICINALE Kimura et Migo is a rare and seriously endangered perennial herb belonging to the family Orchidaceae, which is mainly endemic in the tropics or subtropics of south China. Being one of the precious traditional Chinese medicines in the pharmacopeia, *D. officinale* has long been clinically used as a Yin tonic for promoting body fluid production, benefiting the stomach, clearing away unhealthy heat, moistening the lung,

nourishing the kidneys and in the treatment of dry throat, dry eye and blurred vision symptoms¹. The major bioactive constituents in the stems of *D. officinale* are polysaccharides, dendrobine-type alkaloid, dendrophenol, amino acids and phenanthrene derivatives, which display multiple pharmacological actions of antioxidant, immunomodulatory, antitumorigen and hypoglycemic effects¹. However, *D. officinale* grows slowly in the wild, and it does not grow well or grows poorly without the presence of microbes¹⁻³. Meanwhile, overharvesting as well as habitat destruction and loss have driven the current extinction crisis.

Endophytic fungi constitute valuable and unique microbial communities ubiquitous in plants, and have generally been regarded as plant mutualists for their beneficial effects on the hosts. It is estimated that the global endophytic fungi account for at least 1 million fungal species in nature⁴, whereas only a small fraction of microorganisms has been identified. The great biodiversity and abundance of fungal endophytes occurring in plant tissues demonstrate their functional diversity and ecological importance for the hosts^{5,6}. Fungal endophytes play an indispensable role in plant growth promotion and development by nutrient absorption or phytohormone regulation. They may also provide protection to their host plants by producing several of secondary metabolites to resist and suppress plant pathogens⁷⁻⁹. In recent years, an immense variety of natural compounds with bactericidal and fungicidal activity of endophytic origin have been reported¹⁰⁻¹². Therefore, screening of endophytic fungi for antimicrobial properties would be a promising way to combat the increasing problem of drug-resistant human pathogens. Endophytes are potential sources to exploit new and effective antimicrobial agents for practical application in modern medicine and agriculture¹³.

The purpose of this study was to investigate the diversity of culturable endophytic fungi associated with *D. officinale* in China, and compare the differences in taxonomic composition, species diversity and abundance, as well as the distribution and tissue specificity of fungal endophytes in different *Dendrobium* plants.

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The antimicrobial activity of endophyte extracts was evaluated by bioassay. To the best of our knowledge, there are no previous studies on biodiversity and bioactivity of endophytic mycobiota in *D. officinale*, conducted using an integrated approach of isolation, characterization, ITS-based identification and ecological indices.

Materials and methods

Isolation and identification of endophytic fungi

D. officinale was collected from a planting base in Yunnan Province, China in April or May, and then the plant materials were put into sterile plastic bags and stored at 4°C. The procedure of surface sterilization and isolation of endophytes from the roots, stems and leaves of *D. officinale* was conducted according to Xing *et al.*¹⁴. The washed surfaces of plant tissues were disinfected successively by soaking in 70% ethanol for 1 min, 0.1% mercuric chloride for 5 min, and then rinsed thrice in sterile distilled water. Small pieces (1 cm) of plant samples were placed on potato dextrose agar (PDA) plates and incubated at 28°C for 3–5 days. Single colonies recovered from each tissue fragment were selected and transferred to new PDA plates for purity and identification.

The isolates were first identified based on the morphological characteristics of the colony culture and spores. For the molecular identification, fungal genomic DNA was extracted from the mycelia using peqGOLD Fungal DNA Mini Kit (Peqlab, Erlangen, Germany). The internal transcribed spacer (ITS) region of rDNA genes was amplified using universal eukaryotic primers¹⁴: ITS1 (5'-TCC GTA GGT GAA CCT GCG G-3') and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3'). Standard PCR reactions were performed following the routine protocol and PCR products were directly sequenced. The full-length ITS sequences were compared with those available in GenBank by BLAST and were aligned in Clustal X 2.1 (ref. 15). A phylogenetic tree was constructed by the neighbor-joining algorithm using MEGA version 5.2 (refs 16 and 17).

The nucleotide sequences of endophytic isolates have been deposited in GenBank under the accession numbers shown in Table 1.

Estimation and quantification of fungal diversity

Fungal diversity and richness in different tissues were measured and quantified using a variety of indices^{14,18}, including species richness and evenness, Shannon's diversity and Simpson's diversity, dominance and Fisher's alpha diversity.

(i) Isolation rate = Total number of isolates yielded by a given sample divided by the total number of segments in that sample^{15,18}.

(ii) Percentage recovery (r)

$$= \frac{\text{Number of isolates of one species}}{\text{Total number of isolates}}$$

(iii) Species richness (S) = Total number of the taxa (ITS genotype) present within each sample.

(iv) Simpson's dominance ($D = \sum p_i^2$), Simpson's diversity ($1 - D$) and Shannon's diversity ($H' = -\sum p_i \ln p_i$) were calculated for each sample, where $p_i = n_i/N$, is the relative abundance of the endophytic fungal species, n is the number of isolates of one species and N is the total species number of isolates^{15,19}.

(v) Evenness was calculated as follows: $E = H'/\ln S$.

(vi) Fisher's alpha (α) log-series index was calculated as²⁰

$$\alpha = \frac{N(1-x)}{x},$$

where x was calculated by

$$\frac{S}{N} = \frac{(1-x)}{x} \ln \frac{1}{(1-x)}.$$

Preparation of crude fungal extracts

Each of the fungal endophytes was cultured on PDA medium at 28°C separately, with shaking at 160 rpm for 7 day. The collected mycelia were re-suspended in sterile water and put in an ice-water bath under ultrasonication for 20 min. The suspension was then centrifuged at 7024 g for 10 min, and the supernatant was collected as mycelial extracts. The supernatant of culture broth was evaporated and concentrated to 30% of the initial volume. The residue/mycelial extracts was extracted three times with ethyl acetate and kept for 48 h in refrigerator. The combined ethyl acetate extracts collected organic phases were rotary evaporated to dryness under reduced pressure and then vacuum freeze-dried. The powdered sample was re-dissolved in 5 ml of distilled water to different concentrations.

In vitro antimicrobial activity of endophyte extracts

Four pathogenic bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC25923, *Sarcina lutea* ATCC 9341) and two yeasts (*Candida albicans* ATCC 90028 and *Saccharomyces cerevisiae* ATCC Y139) were used as indicator strains, and *in vitro* antimicrobial tests were carried out by using Oxford cup method. Briefly, overnight culture (LB broth) of bacterial pathogens or yeast (10 CFU/ml) pre-culture in yeast extract peptone dextrose (YPD)

Table 1. Identification, abundance and percentage recovery of endophytic fungi isolated from different tissues of *D. officinale*

Endophytic fungal taxon	Accession number	Similarity to closest genus (%)	Number of isolates from plant tissues			Total abundance (percentage recovery)
			Roots	Stems	Leaves	
<i>Fusarium oxysporum</i>	KX179640	<i>Fusarium</i> (100%)	12	10	0	22 (21.0)
<i>Xylaria cubensis</i>	KX179639	<i>Xylaria</i> (99.6%)	9	9	3	21 (20.0)
<i>Colletotrichum</i> sp.	KX179641	<i>Colletotrichum</i> (93%)	7	4	1	12 (11.4)
<i>Pestalotiopsis</i> sp.	KX179642	<i>Pestalotiopsis</i> (98.8%)	3	4	0	7 (6.7)
<i>Alternaria</i> sp.	KX179643	<i>Alternaria</i> (94.7%)	2	2	2	6 (5.7)
<i>Aspergillus niger</i>	KX179644	<i>Aspergillus</i> (100%)	0	2	4	6 (5.7)
<i>Aspergillus flavus</i>	KU095081	<i>Aspergillus</i> (99.7%)	3	3	0	6 (5.7)
<i>Trichoderma atroviride</i>	KX708707	<i>Trichoderma</i> (99.3%)	2	1	2	5 (4.8)
<i>Cochliobolus</i> sp.	KX179646	<i>Cochliobolus</i> (97.8%)	2	1	0	3 (2.9)
<i>Cystobasidium slooffiae</i>	KU095080	<i>Cystobasidium</i> (97.9%)	2	0	0	2 (1.9)
<i>Aureobasidium pullulans</i>	KU170627	<i>Aureobasidium</i> (99.6%)	2	0	0	2 (1.9)
<i>Epicoccum sorghinum</i>	KX179647	<i>Epicoccum</i> (99.6%)	1	1	0	2 (1.9)
Unidentified			4	4	3	11 (10.5)
Total abundance			49	41	15	105
Overall isolation rates			0.98	0.82	0.30	0.70

medium for 48 h was spread onto beef extract–peptone agar plates. Ethyl acetate extracts of endophyte cultures were sterilized using 0.2 µm membrane filter. Sterilized Oxford cups (6 mm diameter) impregnated with an aliquot of 100 µl of each extract were placed on the solid plates. The diameter of the inhibition zone was measured after 24 h (for bacteria) or 72 h (for yeast) of incubation at optimal temperature. Gentamicin and amphotericin (300 µg/ml) were included as positive control, while sterile distilled water and ethyl acetate were used as negative control respectively. All assays were performed in triplicate.

Different concentrations of extract (15.7, 31.3, 62.5, 125, 250, 500 and 1000 µg/ml) delivered into the wells were used to determine minimal inhibitory concentration (MIC) according to Wang *et al.*²¹.

Results

Identification and composition of endophyte assemblage

Endophytic fungal communities residing in plant tissues of *D. officinale* were studied using conventional techniques. In total, 105 culturable endophytic fungi composed of 16 representative morphotypes (see [Supplementary Material online; Figure S1](#)) were isolated from 150 tissue segments of healthy roots, stems and leaves of 10 *D. officinale* samples. Molecular identification of fungi was performed on the basis of a comparative analysis of ITS rDNA sequences and their similarity to reference sequences²². A phylogenetic tree of endophytes and their relatively proximate species was built by neighbor-joining method (Figure 1). The results revealed 12 distinguishing operational taxonomic units (OTUs). Among them, eight different fungal morphotypic groups were

taxonomically assigned to species, and the other four classified at the genus level (Table 1). The PCR-amplified products of the ITS region of 11 isolates were not detected and thus could not be identified. The allocation of isolates to the 12 OTUs was discrepant, which consisted of four common taxa highly represented in the community profiles and eight unusual taxa containing just a few members. Within the genera isolated, *Fusarium oxysporum* accounting for 21.0% of the total isolates was the dominant species in the whole fungal endophytic community; while *Xylaria cubensis* was the second most prevalent species with 20% recovery. *Colletotrichum* sp. and *Pestalotiopsis* sp. were represented by 11.4% and 6.7% of the isolates respectively. In contrast, fungal abundance of other identified genera was significantly lower, each only between 1.9% and 5.7% relative frequency of occurrence.

Tissue recurrence and specificity of endophytic fungi

All 16 endophyte genera were harboured within at least one category of tissue with relative colonization density >1.9%. Abundance and percentage recovery of endophytes varied among different plants tissues (Table 1), suggesting a certain degree of tissue recurrence. A greater diversity (d) and number (n) of endophytic fungal isolates were recovered from roots ($d = 15$, $n = 49$, $r = 98\%$), followed stems ($d = 14$, $n = 41$, $r = 82\%$). However, the difference between the two was not statistically significant. The minimum species richness and endophytic colonization frequency were found in leaves ($d = 8$, $n = 15$, $r = 30\%$). Moreover, there existed certain differences in the respective fungal species composition of endophyte communities of roots, stems and leaves, which

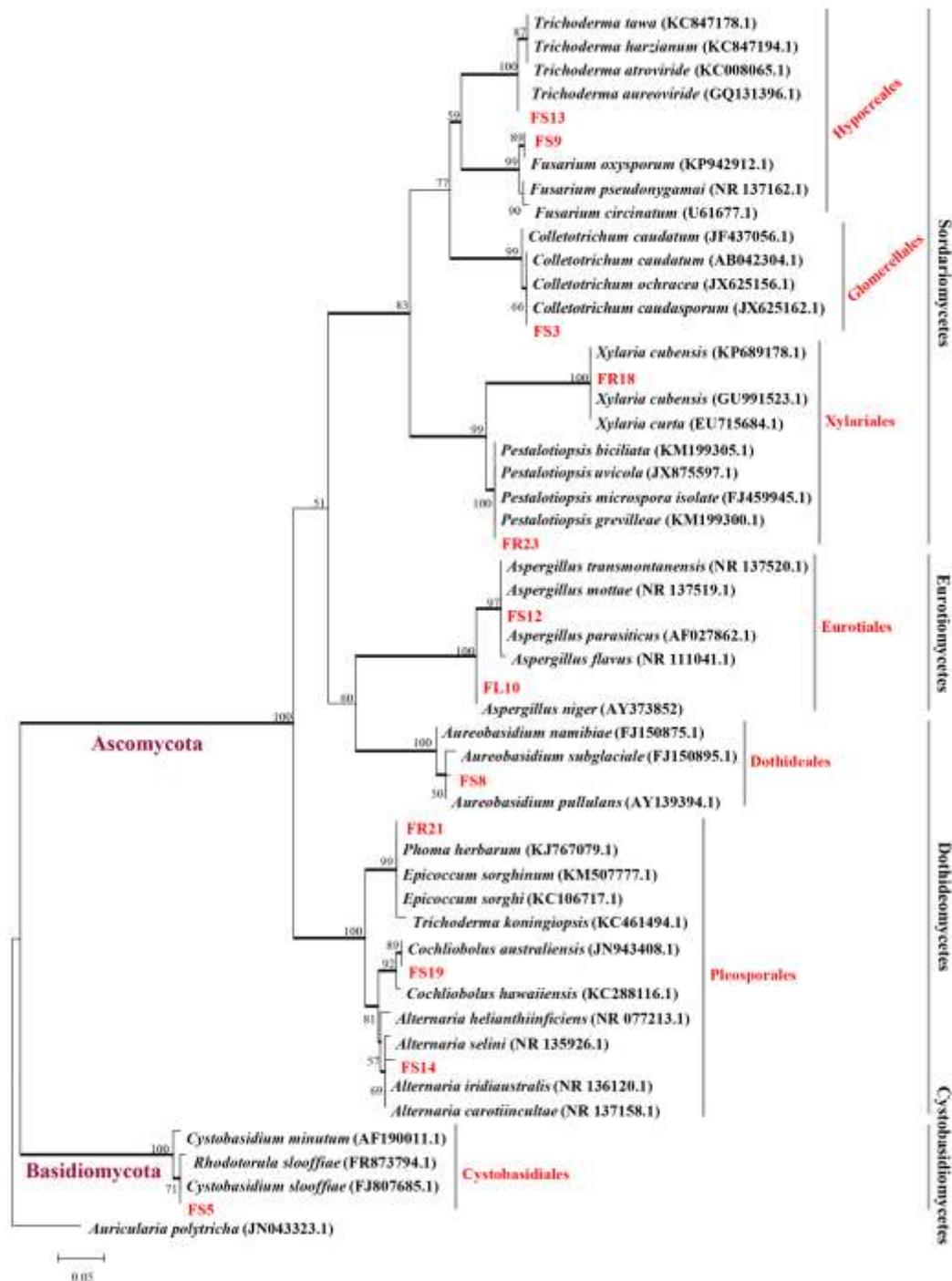


Figure 1. Neighbor-joining phylogenetic tree based on ITS-rDNA gene sequences of endophytic fungi associated with *Dendrobium officinale*. Bootstrap percentages (>50) after 1000 replicates are shown.

reflected tissue specificity (Figure 2). Endophytic fungi belonging to four taxa (*X. cubensis*, *Colletotrichum* sp., *Alternaria* sp. and *Trichoderma atroviride*) symbiotic with plant hosts and were universally present in all the three plant tissues. Preferential occurrence of *Fusarium oxysporum*, *Pestalotiopsis* sp., *Aspergillus flavus*, *Cochliobolus* sp. and *Epicoccum sorghinum* endophytes was

observed in both roots and stems; and *Aspergillus niger* endophytes were particularly obtained from stems and leaves with medium abundance, but were absent in roots. On the other hand, members of *C. slooffiae* and *Aureobasidium pullulans* were exclusively colonized in the roots. Both the species accumulation curves of roots and leaves nearly reached saturation point (Figure 3), which

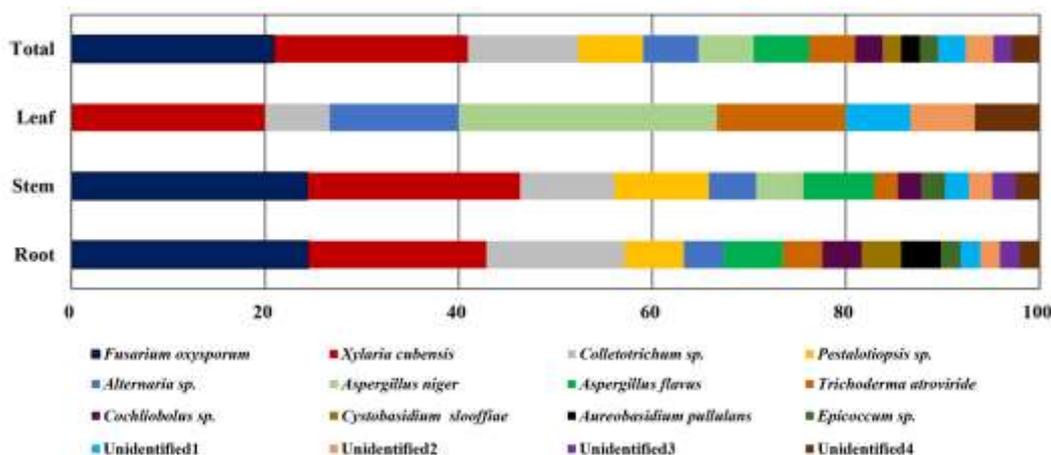


Figure 2. Frequency distribution of 12 endophytic fungal operational taxonomic units in total plant and different plant tissues of *D. officinale*.

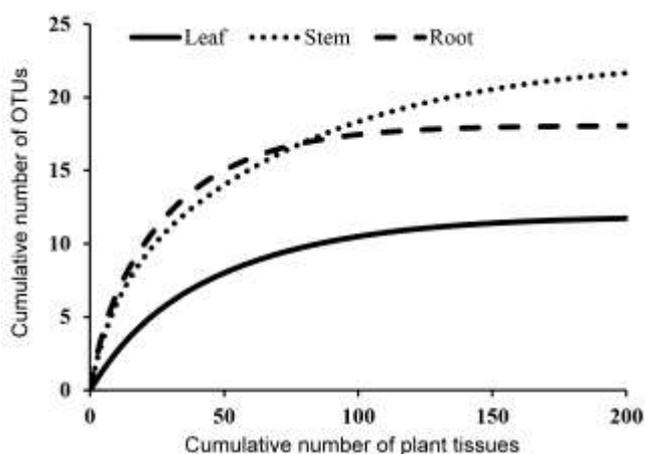


Figure 3. Rarefaction curves for fungal endophytes recovered from different tissue of *D. officinale*.

indicated that sufficient sampling work had been conducted to estimate fungal diversity in the two tissues. On the contrary, the accumulation curve for stems showed a non-asymptotic profile, suggesting that endophytes were not fully recovered and more tissue samples should be used to obtain new fungal taxa.

Diversity estimation of endophytic fungi

The biodiversity of endophytic fungi in *D. officinale* was quantitatively assessed in terms of Shannon’s index (H'), Simpson's dominance (D), Simpson’s diversity ($1-D$) and Fisher’s alpha diversity (α) and species evenness (Table 2). The total endophyte species richness of *D. officinale* was 16 with a high evenness of 0.87, suggesting that the fungal endophyte population was almost equally distributed among all taxa. Species evenness for the stem communities (0.85) was less uniform relative to the other plant parts. The values of Fisher’s alpha diversity, Shan-

non’s and Simpson’s indices for the overall fungal community were 4.85, 2.42 and 0.88 respectively. All the indices indicated that the fungal endophytic community of *D. officinale* had a moderate level of diversity. Detailed calculation of diversity indices of different plant tissues showed the highest diversity in root-associated mycoflora ($\alpha = 7.37$; $H' = 2.33$; $1-D = 0.87$) and lowest in leaf samples ($\alpha = 4.85$; $H' = 2.42$; $1-D = 0.88$).

In vitro antimicrobial activity of endophytic fungi

Culture extracts of 64 endophytic fungal strains (60.95%) were screened for their *in vitro* antimicrobial activity against six human pathogens, including two Gram-negative and two Gram-positive bacteria, and two yeasts. The bioassay results revealed that most of the isolates possessed moderate to strong antibiotic effects on several or all the tested pathogenic microbes (Table 3). The inhibition zone diameter and MIC values ranged from 10.3 to 26.1 mm, 31.3 to 500 $\mu\text{g/ml}$ for bacterial pathogens, and from 11.2 to 19.6 mm, 125 to 500 $\mu\text{g/ml}$ for yeast strains respectively. Among the active extracts, three species, *F. oxysporum*, *A. niger* and *A. pullulan* were proven to have broad-spectrum antimicrobial capability. The most striking findings were that *F. oxysporum* FS9 had the highest antimicrobial activity for six distinct types of clinically important pathogens, and Gram-positive bacteria *Staphylococcus aureus* and *Sarcina lutea* were particularly inhibited by *Trichoderma atroviride* and *Fusarium oxysporum* with maximal diameter of 23.3 mm and 26.1 mm respectively.

Discussion

Endophyte communities offer a special opportunity to discover new microorganisms from the internal niche of plants. Endophytic fungi, as mutualistic plant symbionts,

Table 2. Diversity indices of endophytic fungi for different tissues of *D. officinale*

Origin	Species richness	Evenness	Fisher's α	Shannon's index (H')	Simpson's index (D)	Simpson's diversity ($1-D$)
Roots	15	0.86	7.37	2.33	0.13	0.87
Stems	14	0.85	7.50	2.25	0.14	0.86
Leaves	8	0.93	6.96	1.93	0.16	0.84
Total	16	0.87	4.85	2.42	0.12	0.88

Table 3. *In vitro* antimicrobial activity of broth extracts from endophytic fungi against various pathogens

Endophytic fungal isolate/control	Diameter of inhibition zone (mm) MIC ($\mu\text{g/ml}$)					
	<i>Escherichia coli</i> ATCC 25922	<i>Pseudomonas aeruginosa</i> ATCC27853	<i>Staphylococcus aureus</i> ATCC 25923	<i>Sarcina lutea</i> ATCC 9341	<i>Saccharomyces cerevisiae</i> ATCC Y139	<i>Candida albicans</i> ATCC 90028
<i>Fusarium oxysporum</i>	21.9 \pm 3.2 (62.5)	15.6 \pm 2.2 (250)	22.1 \pm 2.0 (62.5)	26.1 \pm 1.4 (31.3)	18.1 \pm 3.4 (125)	19.6 \pm 1.6 (125)
<i>Aspergillus niger</i>	23.3 \pm 2.1 (62.5)	14.2 \pm 2.5 (250)	21.8 \pm 1.9 (62.5)	16.3 \pm 2.6 (250)	19.1 \pm 3.3 (125)	12.5 \pm 1.5 (500)
<i>Aureobasidium pullulans</i>	15.6 \pm 1.8 (250)	17.1 \pm 1.4 (125)	12.4 \pm 2.3 (500)	18.3 \pm 2.6 (125)	15.1 \pm 1.1 (250)	18.2 \pm 2.1 (125)
<i>Trichoderma atroviride</i>	20.1 \pm 2.6 (62.5)	22.3 \pm 3.3 (62.5)	23.3 \pm 1.2 (62.5)	24.7 \pm 1.6 (31.3)	ND	11.2 \pm 1.8 (500)
<i>Aspergillus flavus</i>	19.3 \pm 1.3 (125)	16.8 \pm 2.2 (125)	17.2 \pm 1.8 (125)	14.4 \pm 2.1 (250)	ND	ND
<i>Cystobasidium slooffiae</i>	16.2 \pm 1.9 (250)	ND	18.1 \pm 1.7 (125)	15.5 \pm 2.8 (500)	ND	17.5 \pm 2.0 (125)
<i>Alternaria</i> sp.	14.2 \pm 1.5 (250)	ND	13.6 \pm 0.9 (500)	ND	ND	17.2 \pm 1.4 (125)
<i>Xylaria cubensis</i>	ND	ND	15.4 \pm 1.2 (250)	10.3 \pm 1.8 (500)	ND	ND
Gentamicin	35 \pm 1.9 (0.001)	38 \pm 2.4 (0.0005)	33 \pm 1.8 (0.001)	32 \pm 1.6 (0.002)	NT	NT
Amphotericin	NT	NT	NT	NT	29 \pm 1.3 (0.0004)	27 \pm 1.6 (0.008)

All assays were performed in triplicate. The results represent mean of three experiments \pm SD. ND, Not detected; NT, not tested.

are abundant in orchids. However, there is little information about the diversity of endophytic mycoflora associated with *D. officinale*. A combination of morphotyping and ITS sequencing resulted in the recognition of endophytic fungi in *D. officinale*. A further comparative analysis was conducted among endophytes from different species of *Dendrobium* plants (see [Supplementary Material online, Table S1](#)). The present study corroborates previous works in the same field showing that there exists a rich endophyte assemblage in *Dendrobium*^{14,23-27}. The data revealed that the endophytic fungi population of *D. officinale* harboured a group of cosmopolitan species such as *F. oxysporum*, *X. cubensis*, *Colletotrichum* sp., *Alternaria* sp. and *T. atroviride*, though their population density was not the highest in the present study. On the other hand, the composition and dominant species of endophytes have been demonstrated to be dependent on the host and different endophytic fungi preferred in different *Dendrobium* species^{23,25,28}. Although *Pestalotiopsis* sp. was also frequently observed in the present study, it was not constantly recorded in the other *Dendrobium* species, with respect to *Epicoecum*, it had a relatively low occurrence both in the present and previous studies. It is noteworthy that *C. slooffiae*, *Cochliobolus* sp., *Aspergillus* sp. and *A. pullulans* were obtained for the first time as single-host endophytes and rare incidental species from the plant of *Dendrobium*. In addition, we did not find any evidence of *Acremonium* sp. and *Phoma* sp. in entire plants of *D. officinale*, which seems to be common for most species of *Dendrobium*.

A slightly uneven distribution of endophyte abundance was observed among fungal species in *D. officinale*. The endophytic fungal assemblage in *D. officinale* was a species complex comprising a few frequent and several infrequent genera. The most abundant fungal genus of *Fusarium* in the present study has always been recognized as one of the highly predominant endophytic inhabitants of other living plants²⁹⁻³⁰, and commonly occurs as a major representative in all species of *Dendrobium* studied. Its extensive host range implies that *Fusarium* is a non-host-specific systemic endophyte; it is more likely to be a chance encounter and acts as an opportunistic plant symbiont³¹. It is somewhat surprising that none of endophytes of *Fusarium* was isolated from leaves of *D. officinale*. Endophytic *Xylaria* sp. have been documented in a variety of herbs and woody plants and appear to be especially common and sometimes dominant in most tropical hosts³²⁻³⁴. The dominant endophytes in seven *Dendrobium* species from tropical regions of southwestern China were found to be *Xylaria* genus. The highest *Xylaria* species diversity and isolation rate of endophytes were achieved in *D. nobile* and *D. chryso-toxum* respectively^{24,25}. A similar scenario was presented with *Colletotrichum* species which commonly isolated from plants in the tropics³⁵. However, high frequency of *Xylaria* sp. was not reported in *D. loddigesii* in a previous study²⁶.

Recent relevant literature on *Dendrobium* endophytes support the idea that fungal endophytes might exhibit tissue specificity. However, the degree of specificity varies

depending on the *Dendrobium* species²³. The occurrence of some isolated taxa was preferentially localized in certain tissue types with an affinity. Yuan *et al.*²⁷ found that endophytic fungal strains of *F. solani* appeared exclusively in the roots of *D. nobile*. Chen *et al.*²³ reported that *Colletotrichum* was absent in the roots, but abundant in the stems and leaves. It was found that the isolation rates of endophytic fungi differed in three tissues of *D. officinale* in the present study. The endophyte distribution was a bit uneven across various host parts. Overall, the roots harboured maximum species richness and highest number of endophytes. Diversity and abundance decreased successively for the stems and especially for the leaves, which provides evidence in favour of tissue heterogeneity. Our results were consistent with similar conclusions in *Dendrobium* species. by Chen *et al.*^{23,26}, but not in agreement with those of Yuan *et al.*²⁷ that the leaves of *D. nobile* were colonized by a greater number of common endophytes, while cauline-associated endophytic fungi were more species-rich. The majority of endophytes are believed to originate from the rhizosphere soil, and our results show that the roots may probably be good habitats or have more chances for horizontal transmission and invasion of endophytic fungi.

Endophytic fungi have been described as beneficial mutualists in the protection of host plants against pathogenic microorganisms. We detected pronounced antimicrobial activity of crude extracts from endophytic fungi of *D. officinale* against an array of pathogenic bacteria and fungi. Similarly, Chen *et al.*²⁶ and Xing *et al.*¹⁴ also reported that endophytic fungi *Fusarium*, *Phoma* and *Epicoecum* in *D. loddigesii*, *D. devonianum* and *D. thyrsiflorum* respectively displayed strong antagonism, but mainly towards one kind of bacterial pathogen (see [Supplementary material online: Table S1](#)). However, the reason for activity being more often discovered in some fungal genera perhaps cannot be due to their outstanding antimicrobial properties, but might be a consequence of the high frequency of occurrence of these endophytic fungi in plants¹². The endophytic fungi in the present study possessed wider antibiotic coverage and showed promising potency among a variety of strains. Some of these endophytes exhibited superior-growth inhibitory activity against Gram-positive bacteria. The present study shows that endophytic fungi can be used as possible candidates for antimicrobial and biocontrol agents in the future.

Conclusion

The endophytic fungi of medical plants are hyperdiverse as often claimed, and hence phylogenetically diverse fungal endophytes are expected to be obtained from unusual habitats inside plants. Although traditional culture-dependent method and limited amounts of specimen may

prevent a large-scale acquisition of the endophytic isolates because more potentially nonculturable taxa have not yet been recovered, diversity statistics of high frequency, abundance as well as moderate diversity and evenness of fungal endophytes was observed in *D. officinale*. Furthermore, endophytic fungi with biosynthesis capability are scientifically proven to be versatile and productive. Cultural fungal endophytes in this context can served as a suitable baseline in the search for various novel antimicrobial substances against drugable targets. However, endophytic fungal diversity and frequency of colonization have been inevitably underestimated by culture-dependent methods. Molecular techniques, such as amplified ribosomal DNA restriction analysis, denaturing gradient gel electrophoresis, terminal restriction fragment length polymorphism analysis, etc. should be applied in future studies with the advantage to comprehensively investigate the diversity of endophytic mycobiota in *Dendrobium* plants.

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