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Full Length Research Paper

Occurrence and diversity of endophytic fungi in *Bletilla ochracea* (Orchidaceae) in Guizhou, China

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Previous studies regarding fungal specificity and diversity of the *Orchidaceae* mainly focus on the mycorrhizal fungi. In contrast, little knowledge of endophytic communities and distributions of non-mycorrhizal fungi in different organs and different sites of orchids are available. In the present study, we investigated the occurrence and species diversity of culturable endophytic fungi from roots and leaves of terrestrial orchid *Bletilla ochracea* from 5 sites in Guizhou. A total of 1026 fungal strains were isolated, and identified to 88 taxa. The 7 species of *Epulorhiza*, *Ceratorhiza* and *Sebacina* (each with > 5% total relative frequency, Basidiomycetes), and 1 species of *Phomopsis* (6.38%, Ascomycetes) were found to be dominant in roots. In comparison, 5 species of *Colletotrichum*, *Guignardia* and *Cercospora* (Ascomycetes) were dominant from leaves. Different species composition was found from different sites for both roots and leaves, and possible reasons are discussed. There was no or very few overlapping species found between roots and leaves in all sampling sites, indicating potential tissue specificity. The occurrence of fungal species from leaves was found to be significantly affected by geographic and environmental factors, and on the other hand, no significant correlation between fungal occurrence and geographic factors was found from roots.

Key words: Ecological distribution, fungal community, identification, mycorrhizal fungi, Shannon-Wiener diversity index.

INTRODUCTION

Plant-fungal symbiotic associations are ubiquitously and anciently distributed in natural plant communities (Alexopoulos et al., 1996). Some endophytes are considered as plant mutualists because they receive nutrition and protection from the host plant while the host plant may benefit from enhanced competitive abilities and increased resistance to herbivores, pathogens, and various abiotic stresses (Newton et al., 2010; Saikkonen et al., 1998, 2010). Endophytic fungi live asymptomatically and internally within host plant tissues; to date, no study has yet shown the existence of a plant species without endophytes (Promputtha et al., 2007). Endophytic fungi can be isolated from leaves, petioles, bark, or stems of trees, shrubs, grasses and ferns (Arnold et al., 2000; Ganley and Newcombe, 2006; Gond et al., 2007; Hoffman and Arnold, 2008; Pandey et al., 2003; Saikkonen et al., 2004). In addition, the species composition and distributions of fungal endophytes are influenced by environmental and geographic factors such as temperature, moisture, altitude, host species and plant tissues (Collado et al., 1999; Fisher et al., 1994; Granath et al., 2007; Guo et al., 2008; Hoffman and Arnold, 2008; Kumaresan and Suryanarayanan, 2002; Photita et al., 2001; Wang and Guo, 2007).

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Table 1. Characters of sampling sites in Guizhou.

Site	Altitude (m)	Geographical locality (lat. N, long. E)	Sampling time
DYXB	990	26°15′ N, 107°33′ E	Jul/2006
GYYL	1120	26°35′ N, 106°48′ E	Jun/2006
QZPS	1310	26°30′ N, 106°27′ E	Aug/2006
QXHS	1315	27°06′ N, 106°00′ E	Jul/2006
SBJP	1635	26°26′ N, 104°44′ E	Jun/2006

DYXB, Xiaba mountain, Duyun; GYYL, Yongle mountain, Guiyang; QZPS, Pianshan mountain, Qingzhen; QXHS, Hongshui mountain, Qianxi; SBJP, Baijipo mountain, Shuicheng.

The *Orchidaceae* is one of the largest plant families, with nearly 25,000 species (Cribb et al., 2003), roughly one tenth of all flowering plants (Jones, 2006). Orchids are fascinating ornamental plant and because of specific mycorrhizal symbiosis during their life cycle, they have become important research materials for fungal diversity and specificity, and coevolution between plants and fungi (Griesbach, 2002; Nontachaiyapoom et al., 2010; Stark et al., 2009; Zettler et al., 2004). However, the study of endophytic fungal communities and diversities in orchid plants mainly focus on the mycorrhizal fungi (Kristiansen et al., 2001; Taylor and Bruns, 1999; Taylor et al., 2003; Selosse et al., 2009), and studies on non-mycorrhizal endophytic fungi are lacking (Dearnaley, 2007; Rasmussen, 2002), especially in leaf tissues. Based on the knowledge of endophytes of other plants (Guo et al., 2001, 2003; Li et al., 2007; Schulz and Boyle, 2005), it is likely that all orchids contain a large community of fungal endophytes which are the important component of fungal biodiversity. Recently, the fungal communities within roots and leaves of the terrestrial orchid *Bletilla ochracea* Schltr. were investigated primarily by the molecular methods, and results indicated that there are abundant endophytic fungi, including mycorrhizal fungi (Tao et al., 2008). Nevertheless, no detailed studies have been conducted on the occurrence of endophytic fungi in roots and leaves of orchid; hence, knowledge of their distributions in different regions in nature is rare.

Therefore, the terrestrial orchid *B. ochracea*, a widely distributed plant in Guizhou, a southwest province in China with high geographic and biological diversity was chosen. The occurrence and distribution of culturable endophytic fungi were investigated by plating segments or discs of roots and leaves. The main purpose of this study was to elucidate the diversity and distribution of endophytic fungi in roots and leaves from different sites, and to discuss whether fungal communities and species distribution are affected by geographic and environmental factors.

MATERIALS AND METHODS

Sampling sites and treatments

In June, July and August of 2006, the 10 *B. ochracea* plants from each site were collected in 5 sites, differing geographic and environmental factors (climate and altitude), in Guizhou province, China (Table 1). Healthy and intact plants with native soil were packed and carefully transported to laboratory within 48 h. The sample plants were treated as follows to remove the microorganism on the plant surface. The 3 symptomless leaves and roots each for one plant were cut from 10 plants for one sampling site, and debris or soil on the surface was removed by careful rinsing under gently running tap water. They were surface-sterilized in a sequence of 75% ethanol for 1 min, 0.1% $HgCl_2$ for 3 min (for leaves) and 3.5 min (for roots), and finally rinsed in five changes of sterile distilled water (Newell, 1976). The rinsing water of the fifth time above was plated on the PDA medium without antibiotics, and these plates were used as control for testing microorganisms left. The surface-sterilized method was optimized by a series of experiments (data not shown) to be suitable for this study.

Isolation and identification of fungi

One hundred and eighty (180) root segments with 5 mm in length and 180 leaf discs with 5 mm in diameter were cut into from the surface-sterilized roots and leaves for each sampling site, and placed on three types of media of potato dextrose agar (PDA) medium, malt extract agar (MEA) medium (Stone et al., 2004) and modified Czapek Dox agar medium (Yamato et al., 2005). Streptomycin sulphate and chloramphenicol were added to a final concentration of 100 and 50 mg/L to inhibit bacterial contamination. Six root segments or leaf discs were plated for one plate, and 10 replicates for each type of medium. Plates were kept in the dark at room temperature (25℃). When colonies appeared, they were sub-cultured into fresh PDA plates and allowed to grow for 14 days or longer before they were subjected to morphological examination. For long-term storage, fungal cultures were maintained in slants of PDA at 4℃. In this study, 1800 tissue segments or discs of roots and leaves from 5 sampling sites were grown in three media.

The fungi were identified mainly based on the morphological characteristics of reproductive structures with the aid of several taxonomic keys (Bailey and Jeger, 1992; Barnett and Hunter, 1999; Carmichael et al., 1980; Domsch et al., 1980; Ellis, 1976; Gerlach and Nirenberg, 1982; Sutton et al., 1980). However, orchid mycorrhizal fungi belonging to *Rhizoctonia*-like group were identified according to culture morphology and microscopic characteristics using differential interference contrast (DIC) illumination for their sclerotial morphology, DAPI fluorescent stain for the nuclear numbers of young hyphal cells and the TEM for the septal pore ultrastructure (Andersen, 1996; Currah and Sherburne, 1992; Moore, 1987). For those poorly sporulating isolates which cannot be identified by morphological characteristics, fungal genomic DNA extraction were conducted using a modified protocol of CTAB (Yang and Liu, 2005), and the internal transcribed spacer (ITS) of ribosomal DNA were amplified and sequenced following the procedure of White et al. (1990). The ITS sequences of similar

taxon retrieved by Basic Local Alignment Search Tool (BLAST) in GenBank/NCBI were used for phylogenetic trees to identify the fungal morphotypes of this study (Tao et al., 2008). The ITS sequences of fungal species in this study were deposited in GenBank under accessions HM751796 - HM751829 (Tables 2 and 3).

Data analysis

The Shannon-Wiener diversity index (*H'*) was employed to evaluate and compare the diversity of fungal communities between different tissues of *B. ochracea* plant, and *H'* was calculated according to the formula:

$$H' = -\sum_{i=1}^{k} pi \times \ln pi$$

Where *k* is the total clone of fungal species, and *pi* is the proportion of individuals that species *i* contributes to the total (Pielou, 1975).

The similarity of fungal communities within plant tissues among different sites was measured by Sorensen similarity index (Cs) which is calculated by formula:

$Cs = 2a/(2a+b+c)$

Where *a* refers to number of overlapping species between 2 sampling sites or communities, *b* or *c* is the number of individual species of site B or site C (Magurran, 2004). Sorensen similarity index (Cs) is applied and expressed with values between 0 (no similarity) and 1 (absolute similarity).

Pielou's evenness index (J) of fungal communities, which measures the fungal evenness of distribution within the plant host, was represented by:

$J = H'/H'max$ (Pielou, 1975)

Where *H´* refers to the Shannon-Wiener diversity index of fungal communities in roots or leaves of one site, and *H´*max means the maximum diversity index among the fungal communities in roots or leaves of 5 sites.

The software SPSS 15.0 was used for the analyses of Pearson's correlation coefficient (SPSS Inc., Chicago, IL, USA). This statistical inference was based on two correlation coefficients of the *r* value ($r > 0.9$) and *P* index (<0.05) to test the null hypothesis. These analyses were used to examine the correlation between fungal occurrence (that is, diversity and total RF, which means the total relative frequency of fungal occurrence in each sampling site) and geographic factors (that is, altitude and locality), and to infer whether these geographic factors influenced the species composition and distribution among the endophytic fungal communities in different sites.

RESULTS

A total of 1026 culturable fungal isolates were obtained from 1800 tissue segments or discs of roots and leaves of *B. ochracea* collected from the 5 sites in Guizhou. These isolates were identified to 88 taxa, including 30 to species level, 43 to genus level and 15 taxa above the family level based on morphological characteristics and molecular analysis of ITS rDNA region of fungal morphotypes. Among them, 775 isolates from leaf tissues were identified to 42 taxa, belonging to 32 species of 14 genera, and 10 to order and above order level; 251 isolates from root tissues were identified to 46 taxa, belonging to 41 species of 19 genera, and 5 to family and above family level.

Dominant species of fungal communities

Within roots from 5 geographic sites, the 7 species of *Epulorhiza*, *Ceratorhiza* and *Sebacina* (each with > 5% F%, Basidiomycetes), and 1 species of *Phomopsis* (6.38% F%, Ascomycetes) were found to be dominant. The species of *Epulorhiza*, *Ceratorhiza* and *Sebacina* comprised 69.74% total relative frequency (F%), and species from genera *Fusarium* and *Phomopsis* comprised 14.36% (Table 2). Correspondingly, the 5 species of *Colletotrichum*, *Guignardia* and *Cercospora* (each with > 5% F%, Ascomycetes) were dominant from leaves. Fourteen species of *Colletotrichum*, *Guignardia* and *Cercospora* genera were dominant with 87.61% to total relative frequency, F% (Table 3). In comparison with species in the roots, all these fungi were taxa of Ascomycetes.

Species composition and distributions

Of all the fungal species isolated from roots, there were 22 taxa of Ascomycetes with 24.28% to total relative frequency (F%), and 21 taxa of Basidiomycetes with 74.12% to total relative frequency (Table 2). Another 3 species of mycelia sterilia were unidentified. The *Epulorhiza* species were the most common (47.42%, F%) of all the isolates from roots, exhibiting in all the 5 sampling sites.

In comparison, there were 33 taxa of Ascomycetes with 96.24% to total relative frequency (F%), and another 9 species were unidentified as mycelia sterilia in leaves from 5 sampling sites (Table 3). The *Colletotrichum* species were the most common (65.40%, F%), exhibiting in all the 5 sampling sites.

On the other hand, ecological distributions of fungal species were different among sampling sites, and occurrence frequency of endophytic fungi were also significantly different. First, the dominant species, for example, *Ceratorhiza* sp.1 and *Periconia macrospinosa* from roots in DYXB site were not discovered from the other 3 sampling sites, and the *Sebacina* species were dominant within roots from GYYL, QXHS, QZPS and SBJP sites, but they were not isolated from roots in DYXB site (Table 2). Secondly, the occurrence frequency of some common species from 5 sampling sites was not

Table 2. Fungal species of endophytes isolated from root tissues and occurrence frequency of five sites.

Taxon[1]	Relative frequency of occurrence (RF[2], %)					F[3] (%)	GenBank accession number
	DYXB-G (N=37)	GYYL-G (N=66)	QXHS-G (N=35)	QZPS-G (N=28)	SBJP-G (N=85)		
Acremonium alternatum	-	1.52	2.86	-	-	0.80	HM751796
Acremonium kiliense	-	-	-	3.57	-	0.40	
Ceratorhiza sp.1	35.14	-	-	-	-	5.18	
Ceratorhiza sp.2	-	3.03	-	-	-	0.80	HM751797
Chaetomium sp.	-	-	-	-	1.18	0.40	HM751798
Colletotrichum dematium	-	-	-	-	1.18	0.40	
Cylindrocarpon olidum	-	1.52	-	-	-	0.40	
Cylindrocarpon spp.	-	-	-	3.57	1.18	0.80	
Cryptosporiopsis ericae	-	-	2.86	-	1.18	0.80	HM751799
Epulorhiza sp.1	21.62				22.35	10.76	
Epulorhiza sp.2	27.03	3.03	17.14	17.86	1.18	9.56	
Epulorhiza sp.3	-	33.33	5.71	-	-	9.56	
Epulorhiza sp.4	-	7.58	-	-		1.99	
Epulorhiza sp.5	-	1.52	-	-	15.29	5.58	
Epulorhiza sp.6	-	-	40.00	-	-	5.58	
Epulorhiza sp.7	-	-	2.86	-	-	0.40	
Epulorhiza sp.8	-	-	-	28.57	-	3.19	
Epulorhiza sp.9	-	-	-		2.35	0.80	
Eutypella scoparia	-	1.52	-	-	-	0.40	HM751800
Fusarium solani	-	4.55	-	-	-	1.20	
Fusarium oxysporum	-	-	5.71	-	-	0.80	
Fusarium proliferatum	-	-	-	3.57	-	0.40	
Fusarium redolens	-	-	-	-	3.53	1.20	
Guignardia mangiferae	-	-	2.86	-	-	0. 40	HM751801
Periconia macrospinosa	10.81	-	2.86	-	-	1.99	
Peziza sp.	-	3.03	-	-	-	0.80	HM751802
Phanerochaete sordida	-	1.52	-	-	-	0.40	HM751803
Phomopsis sp.1	2.70	15.15	-	-		4.38	HM751804
Phomopsis sp.2	-	-	-	-	18.82	6.38	HM751805
Rhexocercosporidium sp.	-	-	-	7.14	1.18	1.20	HM751806
Scolecobasidium microspoum	-	1.52	-	-	-	0.40	
Sebacina sp.1	-	9.09		3.57	4.71	4.38	
Sebacina sp.2	-	3.03	2.86	21.43	1.18	3.98	
Sebacina sp.3	-	1.52	-	-		0.40	
Sebacina sp.4	-	4.55	-	-		1.20	
Sebacina sp.5	-	-	8.57	-	11.77	5.18	
Sebacina sp.6	-	-	2.86	-	-	0.40	
Sebacina sp.7	-	-	-	7.14	-	0.80	
Verticillium chlamydosporium	-	-	-	3.57	-	0.40	
Xylaria sp.1	-	-	-	-	1.18	0.40	HM751807
Tulasnellaceae spp.	-	-	-	-	11.77	3.98	
Mycelia sterilia 1	2.70	-	-	-	-	0.40	

Table 2. Contd.

Mycelia sterilia 6	-	3.03	-	-	-	0.80
Mycelia sterilia 7	-	-	2.86	-	-	0.40
Total RF of each site[4]	14.74	26.30	13.94	11.16	33.87	

[1]Total number of species = 46, [2]Relative frequency of occurrence, RF = number of strains of a specific species from each site/total number of isolates from each site; [3]F (%) = number of strains of a specific species from five sites/total number of strains of all species from five sites; [4]Total RF of each site = total number of isolates of each site/total number of isolates from five sites; N, total number of isolates of each site.

Table 3. Fungal species of endophytes isolated from leaf tissues and ecological distribution from five sites.

Taxon[1]	Relative frequency of occurrence（RF[2], %）					F[3] (%)	GenBank accession number
	DYXB-Y (N=234)	GYYL-Y (N=98)	QXHS-Y (N=177)	QZPS-Y (N=112)	SBJP-Y (N=154)		
Alternaria tenuissima	-	2.04	1.13	-	1.30	0.77	
Arthrinium phaeospermum	-	-	1.70	-	-	0.39	
Arthrinium sp.	0.43	-	2.26	-	-	0.65	HM751808
Botryosphaeria dothidea	0.43	-	0.57	-	-	0.26	HM751809
Cercospora sp.1	0.86	19.39	12.43	-	0.65	5.68	HM751810
Cercospora sp.2	-	-	-	-	3.25	0.65	HM751811
Colletotrichum boninense	5.13	2.04	1.13	27.68	18.83	9.81	
Colletotrichum caudatum	6.41	16.33	2.26	-	-	4.52	
Colletotrichum dematium	10.68	32.65	37.85	28.57	13.64	22.84	
Colletotrichum gloeosporioides	20.09	2.04	15.25	31.25	25.97	19.48	
Colletotrichum graminicola	3.42	-	-	-	-	1.03	
Colletotrichum trifolii	1.71	2.04	6.22	2.68	-	2.58	
Colletotrichum spp.	0.43	4.08	3.39	2.68	16.88	5.16	HM751812 HM751813 HM751814 HM751815 HM751816
Fusarium proliferatum	-	-	-	-	0.65	0.13	
Guignardia mangiferae	33.76	19.39	9.61	7.14	-	15.87	HM751801
Guignardia philoprina	-	-	0.57	-	-	0.13	HM751817
Mycosphaerella sp.	-	-	1.70	-	8.44	2.07	HM751818
Petriella sordida	0.43	-	-	-	-	0.13	HM751819
Phoma glomerata	-	-	0.57	-	-	0.13	HM751820
Phoma sp.1	0.43	-	-	-	0.65	0.26	HM751821
Phomopsis amygdali	-	-	-	-	3.90	0.77	HM751822
Phomopsis spp.	5.13	-	-	-	-	1.55	HM751823 HM751824 HM751825
Podospora spp.	-	-	-	-	1.30	0.26	HM751830 HM751826

Table 3. Contd.

Pseudocercospora sp.	2.99	-	-	-	-	0.90	HM751827
Xylaria sp.2	0.43	-	-	-	-	0.13	HM751828
Pleosporales sp.	0.43	-	-	-	-	0.13	HM751829
Mycelia sterilia 2	5.13	-	-	-	-	1.55	
Mycelia sterilia 3	0.86	-	0.57	-	1.30	0.65	
Mycelia sterilia 4	0.43	-	-	-	-	0.13	
Mycelia sterilia 5	0.43	-	-	-	0.65	0.26	
Mycelia sterilia 8	-	-	2.26	-	-	0.52	
Mycelia sterilia 9	-	-	0.57	-	-	0.13	
Mycelia sterilia 10	-	-	-	-	0.65	0.13	
Mycelia sterilia 11	-	-	-	-	1.30	0.26	
Mycelia sterilia 12	-	-	-	-	0.65	0.13	
Total RF of each site[4]	30.19	12.65	22.84	14.45	19.87		

[1]Total number of species = 42; [2]Relative frequency of occurrence, RF = number of strains of a specific species from each site/total number of isolates from each site; [3]F (%) = number of strains of a specific species from five sites/total number of strains of all species from five sites; [4]Total RF of each site = total number of isolates of each site/total number of isolates from five sites; N, Total number of isolates of each site.

consistent, for example, *Phomopsis* species in Table 2. Thirdly, total RF of each site were different from 5 sites (Table 2).

In addition, the dominant species within leaves from 5 sampling sites were also different in occurrence frequency and distribution. The dominant species, *Cercospora* sp.1 (5.68%, F%), *Guignardia mangiferae* (15.87%) and some species of *Colletotrichum*, *C. caudatum* (4.52%) and *C. trifolii* (2.58%) did not exhibit in all the 5 sampling sites (Table 3). The occurrence frequency of some common species, *Colletotrichum* spp., *Guignardia* spp. and other minority were not consistent at 5 sample sites (Table 3). Furthermore, total RF of each site were also different from 5 sample sites, 30.19 (DYXB), 12.65 (GYYL), 22.84 (QXHS), 14.45 (QZPS) and 19.87% (SBJP), respectively (Table 3).

Fungal species diversity within leaves and roots

For oots, Shannon-Wiener diversity index (*H'*) of endophytic fungi was slightly different among 5 sample sites. In most cases, root support higher diversity than leave, except for DYXB with lowest *H'* within roots, 1.488 (Table 4). However, the Pielou's evenness index (J) between roots and leaves were not consistent, and the fungal evenness within the roots was higher than that in leaves (Table 4). Sorensen similarity index (Cs) of fungal communities among 5 sampling sites was very low, Cs of endophytic fungi among leaves from different sites were much higher than that among roots of sites (Table 5). And between roots and leaves of the same site and different sites, the Cs was near zero or zero (Table 5).

Correlation with environmental characters

Correlation analysis was performed with the software SPSS based on *r* value ($r>0.9$) and *P* index (<0.05), and the Shannon-Wiener diversity index (*H'*) of endophytic fungi in leaves had significant correlations with altitude and latitude, and a certain correlations with longitude in the 5 sampling sites. However, there was no significant correlation in roots between fungal diversity (*H'*) and geographic factors, and between total RF of each site and geographic factors (Table 6), even if there were a bit differences of species richness and composition among sampling sites. These results suggested that the abundance of endophytic fungi in roots was not significantly correlated with altitude, latitude and longitude in the 5 sampling sites, and also indicated that the fungal richness and composition from roots and leaves was not consistent.

DISCUSSION

Why are fungal richness and distribution affected by geographic factors?

From the results of this study, the different sampling sites resulted in different species richness and composition, especially, some dominant species within plants, and the overlapping species were very few. This

Table 4. Species diversity of fungal endophytes within root and leaf tissues from five sites.

Sampling sites	Number of species		Number of fungal strains		Shannon-Wiener index (H')		Pielou's evenness index (J)	
	Roots	Leaves	Roots	Leaves	Roots	Leaves	Roots	Leaves
DYXB	6	23	37	234	1.488	2.171	0.830	0.692
GYYL	18	10	66	98	2.321	1.774	0.803	0.770
QXHS	13	18	35	177	2.019	2.069	0.787	0.716
QZPS	10	6	28	112	1.968	1.459	0.855	0.814
SBJP	18	19	85	154	2.331	2.160	0.807	0.733

Table 5. Sorensen index of fungal communities within root and leaf tissues from five sites.

Sampling sites	Sorensen similarity index (Cs)									
	DYXB		GYYL		QXHS		QZPS		SBJP	
	Roots	Leaves	Roots	Leaves	Roots	Leaves	Roots	Leaves	Roots	Leaves
DYXB	0*		0.167	0.424	0.211	0.439	0.125	0.345	0.167	0.333
GYYL	0*	0*	0*		0.258	0.643	0.214	0.625	0.222	0.414
QXHS	0*	0.053*	0*	0.080*	0.061*		0.174	0.417	0.258	0.432
QZPS	0*	0*	0*	0*	0.095*	0*	0*		0.286	0.240
SBJP	0*	0.047*	0*	0.067*	0*	0.053*	0.065*	0.077*	0.051*	

* means Cs of fungal communities between roots and leaves among the sites.

Table 6. Correlation and probabilities for the relationship between fungal occurrence and geographic factors.

Parameter		Diversity versus altitude	Diversity versus lat. N	Diversity versus long. E	RF *** versus altitude	RF versus lat. N	RF versus long. E
Roots	r *	0.642	0.592	0.191	0.362	0.734	0.286
	P** (2)	0.243	0.327	-0.697	0.526	-0.211	-0.599
Leaves	r	0.884	0.964	0.753	0.652	0.763	0.681
	P (2)	0.091	-0.028	-0.195	-0.227	-0.187	0.254

*, Pearson correlation coefficient; **, two-tail coefficient; ***, total RF of each site.

conclusion is similar to the previous researches of endophytic fungi from non-orchid plants. Taylor et al. (1999) discovered that colonization and isolation rates of fungal endophytes associated with the palm, *Trachycarpus fortunei,* in warm temperate areas of China, were higher than that in tropical areas of Australia and Switzerland. Another studies also indicated that colonization and isolation rates of endophytic fungi in Chinese oil pine (*Pinus tabulaeformis* Carr.) and *Cordemoya integrifolia* (Willd.) Baill were significantly influenced by factors such as moisture regimes and annual temperature (Toofanee and Dulymamode, 2002; Wang and Guo, 2007). Ke et al. (2007) showed that the

species richness in wild plants of *Doritis pulcherrima* (Orchidaceae) in Hainan, China, are significantly different among different habitats, with a much higher richness found from that growing on shrubs than that growing on rocks. In addition, the diversity of endophytic species was more affected by habitat types *Azadirachta indica*, the composition, richness, and distribution of endophytic fungi was interesting, as species composition showed no significant difference among different germplasm sources, and some endophytic fungi were host- and tissue-specific, for example, the dominant *Colletotrichum* species (Shao et al., 2008).

The differences of fungal distribution patterns among geographic regions may be caused by individual environmental or geographical factors of sampling sites. For example, differences of altitude (from 900 to 1600 m), rainfall, humidity and temperature at individual sites may have impacts on the biodiversity and species communities. As endophytic fungi often transfer from one host to another through horizontal transmission (Arnold and Herre, 2003; Bayman et al., 1998), and so the rainfall, humidity and temperature of individual regions are important factors to affect the rate of endophytic colonization and infection to new host (Carroll, 1995; Gourbiere et al., 2001; Verma et al., 2007).

Why do fungal communities and diversity differ significantly between roots and leaves?

From the results of this study, fungal communities from leaves and roots were significantly different to each other, no or very few fungi are found overlapping between roots and leaves of the terrestrial orchid *B. ochracea*. Meanwhile, the number of overlapping species in leaves from different sampling sites are higher than that in roots, although root support higher fungal diversity. It also seems to be the trends that fungal communities are more stable within roots, the below-ground tissues, than that within leaves, the above-ground tissues. These results are consistent with the study of Tao et al. (2008).

Similar results, in which different tissue types of plant, differ remarkably in endophyte communities and diversity have been reported from previous studies on other plant species (Collado et al., 2000; Fisher et al., 1994; Wang and Guo, 2007). The reasons might be: (i) individual taxa might have special capacity for utilizing or surviving within a specific substrate (Carroll and Petrini, 1983); (ii) Fungal communities may be affected by tissue texture and changes in the tissue physiology and chemisty (Arnold et al., 2001); and (iii) hosts or ecological sites, for example, below- or above- ground, have different limited factors such as humidity, chemistry, temperature to impact fungal communities and diversity.

However, different story was reported from the epiphytic orchids, in which the fungal communities within the leaves and roots were surprising similar (Bayman et al., 1997).

Diversity and specificity of endophytic fungi for Orchid plants

In the present study, fungal communities within the leaves of *B. ochracea* showed relatively high diversity. In comparison, fungal diversity in roots was generally much higher than that in the leaves. Meanwhile, the dominant species of genera *Epulorhiza*, *Ceratorhiza* and *Sebacina* in *B. ochracea* roots are the typical mycorrhizal fungi of other orchid plants (Athipunyakom et al., 2004; Currah and Zelmar, 1992; Otero et al., 2002), and they exhibited a relatively high host specificity as shown in McCormick et al. (2004), Otero et al. (2007), Shefferson et al. (2005, 2007) and Taylor et al. (2003). However, Otero et al. (2002, 2004) studied the mycorrhizal associations of some fungi from tropical epiphytic orchids, and found mycorrhizal fungi were comprised of generalists. In addition, the species composition of mycorrhizae may also change during the development of individual plants. In *Gastrodia ellata* and *Mycena osmundicola,* mycorrhizae was present in the protocorm stage but was replaced by *Armillaria mellea* in subsequent stages (Xu and Mu, 1990). McCormick et al. (2006) found that *Goodyera pubescens* protocorms and adults associated with only one individual fungus at a time, whereas, environmental variation or other adverse factors (for example, drought) may induce a switch to a different fungal partner. Nevertheless, fungal communities within the leaves of orchids are generalists as shown in previous studies (Guo et al., 2001, 2003; Li et al., 2007; Schulz and Boyle, 2005).

This study has significance for orchid ecology. Human activities have been the major factor causing the diminishing of wild populations of *B. ochracea*. Up to now, little is known about the impacts of mycorrhizal and non-mycorrhizal fungi on the distribution and survival of orchids in the ecosystem. It is possible that, the availability of endophytic fungi is one of the limiting factors for the establishment of a new plants population. Thus, a better understanding of fungus-host relationship would possibly provide useful information in orchid conservation in the near future. Future research should further elucidate the fungal communities within orchid and characterize the fluctuation of fungal diversity under the individual life period, for example, flowering, fruiting and dormancy, and seasons. In addition, it is necessary to elaborate on the possible symbiotic associations between endophytes and plants, within single organs, among organs of a single plant, and possibly among host species from different field sites.

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REFERENCES

Alexopoulos CJ, Mims CW, Blackwell M (1996). Introductory Mycology. 4th ed. John Wiley and Sons, Inc. New York, U.S.A., p. 869.

Andersen TF (1996). A comparative taxonomic study of *Rhizoctonia* sensu lato employing morphological, ultrastructural and molecular methods. Mycol. Res., 100(9): 1117-1128.

Arnold AE, Herre EA (2003). Canopy cover and leaf age affect colonization by tropical fungal endophytes: Ecological pattern and process in *Theobroma cacao* (Malvaceae). Mycologia, 95(3): 388-398.

Arnold AE, Maynard Z, Gilbert GS (2001). Fungal endophytes in dicotyledonous neotropical tree: patterns of abundance and diversity. Mycol. Res., 105: 1502-1507.

Arnold AE, Maynard Z, Gilbert GS, Coley PD, Kursar TA (2000). Are tropical fungal endophytes hyperdiverse? Eco. Lett., 3: 267-274.

Athipunyakom P, Manoch L, Piluek C (2004). Isolation and identification of mycorrhizal fungi from eleven terrestrial orchids. Kasetsart J. (Nat. Sci.), 38: 216-228.

Bailey JA, Jeger MJ (1992). *Collectotrichum*: Biology, Pathology and Control. CAB International press, Wallingford, UK, pp. 1-46.

Barnett HL, Hunter BB (1999). Illustrated genera of imperfect fungi. 4th ed. APS press, St. Paul, Minnesota, USA, pp. 1-218.

Bayman P, Angulo-Sandoval P, Baez-Ortiz Z, Lodge DJ (1998). Distribution and dispersal of *Xylaria* endophytes in two tree species in Puerto Rico. Mycol. Res., 102: 944-948.

Bayman P, Lebron LL, Tremblay RL, Lodge DJ (1997). Variation in endophytic fungi from roots and leaves of *Lepenthes* (Orchidaceae). New Phytol., 135: 143-149.

Carmichael JW, Kendrick WB, Conners IL, Sigler L (1980). Genera of Hyphomycetes. The University of Alberta Press, Edmonton, Alberta, Canada, pp. 1-386.

Carroll GC (1995). Forest endophytes: patter and process. Can. J. Bot., 73: 1316-1324.

Carroll GC, Petrini O (1983). Patterns of substrate utilization by some endophytes from coniferous foliage. Mycologia, 75: 53-56.

Collado J, Platas G, González I, Peláez F (1999). Geographical and seasonal influences on the distribution of fungal endophytes in *Quercus ilex*. New Phytol., 144: 525-532.

Collado J, Platas G, González I, Peláez F (2000). Host specificity in fungal endophytic populations of *Quercus ilex* and *Quercus faginea* from central Spain. Nova Hedwigia, 71: 421-430.

Cribb PJ, Kell SP, Dixon KW, Barrett RL (2003). Orchid conservation: a global perspective. In: Dixon et al. (eds) Orchid conservation. Natural History Publications, Kota Kinabalu, Sabah, pp. 1-24.

Currah RS, Sherburne R (1992). Septal ultrastructure of some fungal endophytes from boreal orchid mycorrhizas. Mycol. Res., 96: 583-587.

Currah RS, Zelmer C (1992). A key and notes for the genera of fungi mycorrhizal with orchids and a new species in the genus *Epulorhiza*. Rept. Tottori Mycol. Inst., 30: 43-59.

Dearnaley JDW (2007). Further advances in orchid mycorrhizal research. Mycorrhiza, 17: 475-486.

Domsch KH, Gams W, Anderson TH (1980). Compendium of soil fungi. Academic Press, London, 1: 1-859.

Ellis MB (1976). More dematiaceous hyphomycetes. Commonwealth Mycological Institute, Kew, Surrey, England, pp. 1-507.

Fisher PJ, Petrini O, Petrini LE, Sutton BC (1994). Fungal endophytes from the leaves and twigs of *Quercus ilex* L. from England, Majorca and Switzerland. New Phytol., 127: 133-137.

Ganley RJ, Newcombe G (2006). Fungal endophytes in seeds and needles of *Pinus monticola.* Mycol. Res., 110: 318-327.

Gerlach W, Nirenberg H (1982). The genus *Fusarium*-a pictorial atlas. Kommissionsverlag Paul Parey, Berlin-Dahlem, pp. 1-406.

Gond SK, Verma VC, Kumar A, Kumar V, Kharwar RN (2007). Study of endophytic fungal community from different parts of *Aegle marmelos* Correae (Rutaceae) from Varanasi (India). World J. Microb. Biot., 23: 1371-1375.

Gourbière F, van Maanen A, Debouzie D (2001). Associations between three fungi on pine needles and their variation along a climatic gradient. Mycol. Res., 105: 1101-1109.

Granath G, Vicari M, Bazely DR, Ball JP, Puentes A, Rakocevic T (2007). Variation in the abundance of fungal endophytes in fescue grasses along altitudinal and grazing gradients. Ecography, 30: 422-430.

Griesbach RJ (2002). Development of *Phalaenopsis* orchids for the mass-market. In Janick and Whipkey (eds) Trends in new crops and new uses. ASHS, Alexandria, USA. pp. 458-465.

Guo LD, Huang GR, Wang Y (2008). Seasonal and tissue age influences on endophytic fungi of *Pinus tabulaeformis* (Pinaceae) in Dongling Mountain, Beijing. J. Integr. Plant Biol., 50: 997-1003.

Guo LD, Huang GR, Wang Y, He WH, Zheng WH, Hyde KD (2003). Molecular identification of white morphotype strains of endophytic fungi from *Pinus tabulaeformis*. Mycol. Res., 107: 680-688.

Guo LD, Hyde KD, Liew ECY (2001). Detection and identification of endophytic fungi within frond tissues of *Livistona chinensis* based on rDNA sequence. Mol. Phylogenet. Evol., 20: 1-13.

Hoffman MT, Arnold AE (2008). Geographic locality and host identity shape fungal endophyte communities in cupressaceous trees. Mycol. Res., 112: 331-344.

Jones DL (2006). A complete guide to native orchids of Australia including the Island Territories. Reed New Holland, Sydney, pp. 418-419.

Ke HL, Song XQ, Tan ZQ, Liu HX, Luo YB (2007). Endophytic fungi diversity in root of *Doritis pulcherrima* (Orchidaceae) (In Chinese). Biodivers. Sci., 15(5): 456-462.

Kristiansen KA, Taylor DL, Kjøller R, Rasmussen HN, Rosendahl S (2001). Identification of mycorrhizal fungi from single pelotons of *Dactylorhiza majalis* (Orchidaceae) using single-strand conformation polymorphism and mitochondrial ribosomal large subunit DNA sequences. Mol. Ecol., 10(8): 2089-2093.

Kumaresan V, Suryanaryanan TS (2002). Endophyte assemblages in young, mature and senescent leaves of *Rhizophora apiculata*: evidence for the role of endophytes in mangrove littler degradation. Fungal Divers., 9: 81-91.

Li WC, Zhou J, Guo SY, Guo LD (2007). Endophytic fungi associated with lichens in Baihua mountain of Beijing, China. Fungal Divers., 25: 69-80.

Magurran AE (2004). Measuring biological diversity. Blackwell Publishing, Oxford, pp. 131-161.

McCormick MK, Whigham DF, O'Neill J (2004). Mycorrhizal diversity in photosynthetic terrestrial orchids. New Phytol., 163: 425-438.

McCormick MK, Whigham DF, Sloan D, O'Malley K, Hodkinson B (2006). Orchid-fungus fidelity: a marriage meant to last? Ecology, 87: 903-911.

Moore RT (1987). The genera of *Rhizoctonia*-like fungi: *Ascorhitonia*, *Ceratorhiza* gen. nov., *Epulorhiza* gen. nov., *Moniliopsis*, and *Rhizoctonia*. Mycotaxon, 29: 91-99.

Newell ST (1976). Mangrove fungi: The succession in the mycoflora of red mangrove (*Rhizophora mangle* L.). In Jones (eds) Recent Advances in Aquatic Mycology. John Wiley, New York, pp. 51-59.

Newton AC, Fitt BDL, Atkins SD, Walters DR, Daniell TJ (2010). Pathogenesis, parasitism and mutualism in the trophic space of microbe-plant interactions. Trends Microbiol., 18(8): 365-373.

Nontachaiyapoom S, Sasirat S, Manoch L (2010). Isolation and identification of *Rhizoctonia*-like fungi from roots of three orchid genera, *Paphiopedilum, Dendrobium,* and *Cymbidium,* collected in Chiang Rai and Chiang Mai provinces of Thailand. Mycorrhiza, 20(7): 459-471.

Otero JT, Ackerman JD, Bayman P (2002). Diversity and host specificity

of endophytic *Rhizoctonia*-like fungi from tropical orchids. Am. J. Bot., 89: 1852-1858.
Otero JT, Ackerman JD, Bayman P (2004). Differences in mycorrhizal preferences between two tropical orchids. Mol. Ecol., 13: 2393-2404.
Otero JT, Flanagan NS, Herre EA, Ackerman JD, Bayman P (2007). Widespread mycorrhizal specificity correlates to mycorrhizal function in the neotropical, epiphytic orchid *Ionopsis utricularioides* (Orchidaceae). Am. J. Bot., 94: 1944-1950.
Pandey AK, Reddy MS, Suryanarayanan TS (2003). ITS-RFLP and ITS sequence analysis of a foliar endophytic *Phyllosticta* from different tropical trees. Mycol. Res., 107: 439-444.
Photita W, Lumyong S, Lumyong P, Hyde KD (2001). Endophytic fungi of wild banana (*Musa acuminata*) at Doi Suthep Pui National Park, Thailand. Mycol. Res., 105: 1508-1513.
Pielou EC (1975). Ecological Diversity. Wiley-Intescience Publication, New York, p. 165.
Promputtha I, Lumyong S, Dhanasekaran V, Huge E, McKenzie C, Hyde KD, Jeewon R (2007). A phylogenetic evaluation of whether endophytes become saprotrophs at host senescence. Microb. Ecol., 53: 579-590.
Rasmussen HN (2002). Recent developments in the study of orchid mycorrhiza. Plant Soil, 244: 149-163.
Saikkonen K, Faeth SH, Helander M, Sullivan TJ (1998). Fungal endophytes: A Continuum of Interactions with Host Plants. Annu. Rev. Ecol. S., 29: 319-343.
Saikkonen K, Saari S, Helander M (2010). Defensive mutualism between plants and endophytic fungi? Fungal Divers., 41(1): 101-113.
Saikkonen K, Wäli P, Helander M, Faeth SH (2004). Evolution of endophyte-plant Symbioses. TRENDS Plant Sci., 9(6): 275-280.
Schulz B, Boyle C (2005). The endophytic continuum. Mycol. Res., 109(6): 661-687.
Selosse M-A, Dubois M-P, Alvarez N (2009). Do Sebacinales commonly associate with plant roots as endophytes? Mycol. Res., 113(10): 1062-1069.
Shao SC, Wu SH, Chen YW, Wang LD, Yang LY, Li SL, Li ZY (2008). Composition of endophytic fungi in *Azadirachta indica* from Yuanjiang County of Yunnan. Biodivers. Sci. (In Chinese), 16(1): 63-67.
Shefferson RP, Taylor DL, Weiß M, Garnica S, McCormick MK, Adams S, Gray HM, McFarland JW, Kull T, Tali K, Yukawa T, Kawahara T, Miyoshi K, Lee YI (2007). The evolutionary history of mycorrhizal specificity among lady's slipper orchids. Evolution, 61:1380-1390.
Shefferson RP, Weiß M, Kull T, Taylor DL (2005). High specificity generally characterizes mycorrhizal association in rare lady's slipper orchids, genus *Cypripedium*. Mol. Ecol., 14: 613-626.
Stark C, Babik W, Durka W (2009). Fungi from the roots of the common terrestrial orchid *Gymnadenia conopsea*. Mycol. Res., 113(9): 952-959.
Stone JK, Polishook JD, White JF (2004). Endophytic Fungi, In Gregory et al. (eds) Biodiversity of Fungi, Inventory and Monitoring Method, Elsevier Acad. Press, New York,pp. 241-270.
Sutton BC (1980). The *Coelomycetes*. Commonwealth Mycological Institute, Kew, Surrey, England, pp. 1-672.
Tao G, Liu ZY, Hyde KD, Liu XZ, Yu ZN (2008). Whole rDNA analysis reveals novel and endophytic fungi in *Bletilla ochracea* (Orchidaceae). Fungal Divers., 33: 101-122.
Taylor DL, Bruns TD (1999). Population, habitat and genetic correlates of mycorrhizal specialization in the 'cheating' orchids *Corallorhiza maculata* and *C. mertensiana*. Mol. Ecol., 8(10): 1719-1732.
Taylor DL, Bruns TD, Szaro TM, Hodges SA (2003). Divergence in mycorrhizal pecialization within *Hexalectris spicata* (Orchidaceae), a nonphotosynthetic desert orchid. Am. J. Bot., 90: 1168-1179.
Taylor JE, Hyde KD, Jones EBG (1999). Endophytic fungi associated with the temperate palm, *Trachycarpus fortunei*, within and outside its natural geographic range. New Phytol., 142(2): 335-346.
Toofanee SB, Dulymamode R (2002). Fungal endophytes associated with *Cordemoya integrifolia*. Fungal Divers., 11: 169-175.
Verma VC, Gond SK, Kumar A, Kharwar RN, Strobel G (2007). The endophytic mycoflora of bark, leaf and srem tissues of *Azadirachta indica* A. Juss (Neem) from Varanasi (India). Microb. Ecol., 54: 119-125.
Wang Y, Guo LD (2007). A comparative study of endophytic fungi in needles, bark, and xylem of *Pinus tabulaeformis*. Can. J. Bot., 85: 911-917.
White TJ, Bruns TD, Lee SB, Taylor JW (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In Innis et al. (eds) PCR Protocols: a Guide to Methods and Applications for Phylogenetics. Acad. Press, San Diego, C A, pp. 315-322.
Xu JT, Mu C (1990). The relation between growth of *Gastrodia elata* protocorms and fungi. Acta Botanica Sinica, 32: 26-31.
Yamato M, Yagame T, Iwase ASK (2005). Isolation and identification of mycorrhizal fungi associating with an achlorophyllous plant, *Epipogium roseum* (Orchidaceae). Mycoscience, 46: 73-77.
Yang Y, Liu XZ (2005). *Dactylella coccinella* sp. nov., an anamorphic species. Mycotaxon, 91: 127-132.
Zettler LW, Sharma J, Rasmussen F (2004). Mycorrhizal diversity. In Dixon et al. (eds) Orchid Conservation. Kota Kinabalu, Sabah, Malaysia: Natural Hist. Pub., pp 185-203.