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ORIGINAL PAPER

Diversity of root-associated fungal endophytes in *Rhododendron fortunei* in subtropical forests of China

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Abstract To investigate the diversity of root endophytes in *Rhododendron fortunei*, fungal strains were isolated from the hair roots of plants from four habitats in subtropical forests of China. In total, 220 slow-growing fungal isolates were isolated from the hair roots of *R. fortunei.* The isolates were initially grouped into 17 types based on the results of internal transcribed spacer-restriction fragment length polymorphism (ITS-RFLP) analysis. ITS sequences were obtained for representative isolates from each RFLP type and compared phylogenetically with known sequences of ericoid mycorrhizal endophytes and selected ascomycetes or basidiomycetes. Based on phylogenetic analysis of the ITS sequences in GenBank, 15 RFLP types were confirmed as ascomycetes, and two as basidiomycetes; nine of these were shown to be ericoid mycorrhizal endophytes in experimental cultures. The only common endophytes of *R. fortunei* were identified as *Oidiodendron maius* at four sites, although the isolation frequency (3–65%) differed sharply according to habitat. *Phialocephala fortinii* strains were isolated most abundantly from two habitats which related to the more acidic soil and pine mixed forests. A number of less common mycorrhizal RFLP types were isolated from *R. fortunei* at three, two, or one of the sites. Most of these appeared to have strong affinities for some unidentified root endophytes from Ericaceae hosts in Australian forests. We concluded that the endophyte population isolated from *R. fortunei* is composed mainly of ascomycete, as well as a few basidiomycete strains. In addition, one basidiomycete strain was confirmed as a putative ericoid mycorrhizal fungus.

Keywords *Rhododendron fortunei* L. · Fungal diversity · Ericoid endophyte · Ericoid mycorrhizal fungi

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Introduction

Plants in the Ericales are widespread in both the northern and southern hemispheres and are found in geographically and climatically disparate heathland and forest habitats (Read 1996). Although varied, these habitats commonly possess soils with low nutrient content, low pH, poor or free drainage, and they often experience high or low temperatures (Cairney and Meharg 2003). In such habitats, the ability to form ericoid mycorrhizas is regarded as central to the success of ericaceous plants (Read 1996; Cairney and Meharg 2003). Mycorrhizae have an ecologically significant role in the establishment of the host plant in heathlands by facilitating the transfer of nutrition to the host plant and by contributing to the detoxification of the root environment (Read and Kerley 1999).

Hymenoscyphus ericae (Read) Korf & Kernan and several *Oidiodendron* species have long been recognized as typical ericoid mycorrhizal fungal partners (Hambleton and Currah 1997; Strandberg and Johannsson 1999). The diversity of mycorrhizal endophytes present on ericaceous hosts also includes numerous sterile fungi that have been isolated from ericaceous roots, and some of these have formed typical ericoid mycorrhiza in synthesis experiments

(Xiao and Birch 1996; Hambleton and Currah 1997; Mclean et al. 1999; Johansson 2001; Usuki et al. 2003). Molecular analyses have clarified phylogenetic relationships and revealed additional genetic diversity among the ericoid mycorrhizal fungi (Chambers et al. 2000; Sharples et al. 2000; Berch et al. 2002; Allen et al. 2003; Midgley et al. 2004; Bougoure and Cairney 2005b). Most of the unidentified putative mycorrhizal endophytes belong to the ascomycete order Helotiales (Monreal et al. 1999; Sharples et al. 2000; Berch et al. 2002; Midgley et al. 2004; Bougoure and Cairney 2005b). Recently, intensive studies have shown that ericoid mycorrhizal endophytes also include some basidiomycete fungi, such as a Sebacina-like strain in *Gaultheria shallon* Pursh (Berch et al. 2002; Bougoure and Cairney 2005b). However, only a few basidiomycete strains have ever been isolated in pure culture from ericaceous plants, and their mycorrhizal status has not yet been defined (Bougoure and Cairney 2005a, b).

Despite the wide distribution of ericaceous species in various types of forest vegetation in China, there has been only limited investigation of their mycorrhizal associations. *Rhododendron fortunei* Lindl is endemic to mountainous areas between 620 and 2,000 m above sea level in Chinese subtropical forests and usually occur as an important ornamental ericaceous plant in natural scenic sites. But there are some problems with the species in propagation and cultivation in horticultural practices. Zhang et al. (2008) reported the ericoid mycorrhizal coils are common in root epidermal cells of *R. fortunei*, and several isolated mycorrhizal strains also showed positive effects on the seedlings' growth during the in vitro inoculation experiments (Yu et al. 2008). However, there is currently no information regarding the diversity and identity of the fungi that form ericoid mycorrhizal associations with the species, as well as other ericaceous plants in Chinese forests.

The objective of this study was to isolate and identify the mycorrhizal fungi from the root systems of *R. fortunei* at different subtropical forest sites, and all the isolated strains were also inoculated to *R. fortunei* seedlings under in vitro conditions to define their mycorrhizal status. The research will not only provide new ericoid mycorrhizal material for the world resource but also prepare selective mycorrhizal fungal strains for rhododendrons in horticultural practices.

Materials and methods

Collection of root samples

Roots of six *R. fortunei* plants less than 40 cm in height were collected from each of the four subtropical broadleaf or mixed coniferous forest habitats in eastern and central China: Huading Forest Park (HFP; 29°15′ N 121°06′ E), Mufu Forest Park (MFP; 28°59′ N 113°52′ E), Huangshan (HS; 30°10′ N 118°11′ E), Siming Forest Park (SFP; 29°20′ N 121°46′ E) in May 2006 or 2007.

Isolation of the fungi

Fungi were isolated from hair roots by the direct plating method (Stoyke and Currah 1991). Fine roots were removed from rhizomes, soaked in cool tap water, washed gently to remove soil and plant debris, surface sterilized in a 72% ethanol solution for 30 s, transferred to a solution of 10% sodium hypochlorite for 10–15 min, and rinsed four times in sterile distilled water. The sterilized fine roots were cut into 0.3 to 0.5 cm segments, plated on modified Melin–Norkans agar (Xiao and Birch 1992), and incubated in the dark at 25°C for 3 to 5 weeks. A total of 100 root pieces from plants representing each of the four habitats were excised and plated. Root pieces exhibiting fast growing rapidly sporulating fungi were removed, while slower growing fungi were subcultured on 2% malt extract agar. Cultures were maintained in the dark at 25°C for 3 to 4 weeks and then stored at 4°C until further use.

DNA extraction and ITS-RFLP analysis

Genomic DNA was extracted from axenic fungal mycelia using a modified version of the cetyltrimethylammonium bromide method (Gardes and Bruns 1993). Amplification of the internal transcribed spacer (ITS) region was performed using the ITS1 (5′ TCCGTAGGTGAA CCTGCGG 3′) and ITS4 (5′ TCCTCCGCTTATTGA TATGC 3′) primers. Reactions were carried out with a thermocycler Gene Amp polymerase chain reaction (PCR) system (BIORAD DNA Engine). Amplifications were performed in a 50-μl reaction volume containing 50–100 ng of genomic DNA, 50 pmol of each primer, 100 μmol each of dATP, dCTP, dCTP, and dTTP, 1 U of *Taq* polymerase, and 5 μl of PCR buffer. The tubes were incubated at 95°C for 2 min and then subjected to 35 cycles as follows: 94°C for 40 s, 60°C for 40 s, and 72°C for 45 s; a final incubation was carried out for an additional 5 min at 72°C. The PCR products were separated by electrophoresis in 0.8% agarose gels in Tris-acetate–ethylenediaminetetraacetic acid buffer, stained with ethidium bromide, and viewed under UV light.

All ITS products were digested with the restriction endonucleases, *Hinf I*, *Rsa I*, *Msp I*, *BsuR I*, and *Taq I*, according to the manufacturer's recommendations. The restriction fragments were separated by electrophoresis in 2.5% (*w*/*v*) agarose gels and viewed as previously described. The base pair lengths of individual fragments were determined by comparison with a 50-bp ladder. No fragments smaller than 50 bp were scored.

Sequencing of amplified ITS regions

Endophytes were grouped based on ITS-restriction fragment length polymorphism (RFLP) analysis, and the ITS region of at least one endophyte from each group was sequenced. Purified PCR products were cloned with the PMD18-T easy vector system and analyzed by an ABI 3730XI automatic DNA sequencer. Sequencing reactions were performed using the M13R(-48) primer.

Data analysis

Sequences of the endophyte species that most closely matched the new isolates from this study were obtained by Basic Local Alignment Search Tool (BLAST) from the GenBank database. The alignments were performed with ClustalX and then manually adjusted to optimize the aligned sites. The sequences from our 20 isolates and selected fungi from GenBank database were analyzed by neighbor joining using distances from Kimura's two-parameter model with the MEGA3.1 software system. To assess support for nodes, 1,000 bootstrap replications were performed.

Mycorrhizal resynthesis with isolated root-associated fungi

Fungal isolates were tested for their ability to form ericoid mycorrhizae by inoculating *R. fortunei* seedlings. Seeds of *R. fortunei* were sterilized in a 10% solution of sodium hypochlorite for 10–15 min and then placed on water agar for germination. Seedlings were transplanted to the sterilized soil mixture (2:1 (*v*/*v*) peat moss/vermiculite, autoclaved twice for 40 min), and then inoculated with the isolated fungal strains. Four months later, the roots were extracted from the growth chambers and stained with Trypan blue. The colonization status of the root cortical cells was observed under a light microscope.

Results

ITS-RFLP analysis of the cultured isolates

The 220 slow-growing fungal isolates were isolated from the hair roots of *R. fortunei* at the four habitats, with a total of 84, 58, 61, and 17 isolates from the HFP, SFP, MFP, and HS habitats, respectively (Table 1). Genomic DNA was extracted from all isolates and in each case, ITS amplification produced a single band of approximately 550–636 bp. All PCR products were digested with the endonucleases *Hinf I*, *Rsa I*, *Msp I*, *BsuR I*, and *Taq I*. The ITS-RFLP patterns grouped the 220 cultured isolates into 17 RFLP types, with ten, nine, eight, and six RFLP types from HFP, SFP, MFP, and HS, respectively (Table 1; Fig. 1). Of these, 12 of the 17 RFLP types and 96% of the total isolates were isolated from more than two field sites. One RFLP type was common to isolates cultured from all four of the habitats. One type was unique to HFP, SFP, and HS, and three types were unique to MFP. One RFLP type was common to isolates cultured from HFP, SFP, and HS; another to HFP, MFP, and HS; and a third type was common to HFP, SFP, and MFP. Two RFLP types were common to HFP and MFP, four to HFP and SFP, and two to SFP and HS. Together, the cultured isolates formed a total of 17 RFLP types representing the four habitats, and each type had a distinctive ITS-RFLP pattern with at least one restriction endonuclease.

Approximately 75% of the cultured isolates from HFP and 80% from MFP were identified as RFLP type 2, while RFLP type 1 was represented by approximately 57% of the isolates from SFP and 65% of isolates from HS habitats. The remaining RFLP types each comprised a relatively small proportion of the isolates.

Sequence analysis of cultured fungal ITS-RFLP types

At least one isolated fungus representing each RFLP type was selected for ITS sequencing and comparison with the available sequences in the GenBank/European Molecular Biology Laboratory (EMBL)/DNA Data Bank of Japan (DDBJ)/Protein Data Bank (PDB) databases. Putative taxonomic affinities were assigned conservatively to the RFLP types based on BLAST sequence similarity and the identities of the several most closely matched sequences obtained by BLAST searches (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The ITS sequence of RFLP type 1 matched *Oidiodendron maius* and also grouped (100% bootstrap) with this species in the neighbor-joining analysis. The isolates of the group (RFLP type 1) were found in different proportions in the roots of plants from each site: ca. 4% from HFP, ca. 57% from SFP, ca. 3% from MFP, and ca. 65% from HS.

RFLP type 2 isolates matched (99% similarity) a dark septate endophyte, *Phialocephala fortinii* Wang & Wilcox and formed a strongly supported (100%) monophyletic group with that species. The fungal strains of the RFLP type 2 were isolated from HFP and MFP in high abundance (ca. 75% of total isolates from HFP and 80% from MFP), whereas no strains were obtained from SFP and HS.

Eight RFLP types(RFLP types 3–7 and 11–13) comprising significant portions of the isolated strains (ca. 14% of HFP, 33% of SFP, 11% of MFP, and 12% of HS) were most closely related to various unidentified Epacrid root endophytes or ericoid mycorrhizal fungal endophytes from southern hemisphere Ericaceae hosts. A neighbor-joining analysis employing database sequences grouped these RFLP types (98–100% bootstrap) with different Epacrid or Ericoid root endophytes: such as *Epacris microphylla*

Table 1 Putative taxonomic affinities of RFLP types present in cultured isolate assemblages from the root systems of *R. fortunei* plants at four sites as inferred via BLAST searches of ITS sequences in the GenBank/EMBL/DDBJ/PDB databases

RFLP type	Accession code	Mycorrhizal status	Closest species match	Sequence similarity (%)	Number of isolates from each site			
					HFP	SFP	MFP	HS
1	EU888629	MYC	*Oidiodendron maius* (AF062798)	99	3	33	2	11
	EU888631		*Oidiodendron maius* (AF062798)	99				
	EU888632		*Oidiodendron maius* (AF062800)	99				
	EU888634		*Oidiodendron maius* (AF062800)	98				
2	EU888624	Unknown	*Phialocephala fortinii* (AY394921)	99	63		49	
	EU888626		*Phialocephala fortinii* (AY394921)	99				
3	EU639688	MYC	*Epacris microphylla* root associated fungus (AY268217)	98	4	7		1
	EU880594		*Epacris microphylla* root associated fungus (AY268217)	98				
4	EU880592	MYC	Epacrid root endophyte sp. (AY279189)	96	6	2		
5	EU880591	MYC	Epacrid root endophyte sp. (AY279189)	96	2	5		
6	EU880593	MYC	Epacrid root endophyte sp. (AY279189)	95			1	
7	EU888611	Non-MYC	*Epacris microphylla* root associated fungus (AY268215)	97		3		1
8	EU880587	MYC	*Cryptosporiopsis ericae* (AY853167)	99	1		1	1
	EU880588		*Cryptosporiopsis ericae* (AY853167)	99				
9	EU888615	Non-MYC	Uncultured ascomycete (AY969967)	93		1		2
10	EU888618	Non-MYC	Helotiales sp. (EF093150)	96	1	1		
11	EU888616	Non-MYC	Uncultured mycorrhizal (AY656940)	99		2		
12	EU880589	MYC	*Epacris pulchella* root associated fungus (AY627824)	97			5	
13	EU888619	MYC	*Epacris microphylla* root associated fungus (AY268218)	92			1	
14	EU880596	Non-MYC	*Cladosporium* sp. (EF424419)	99	2	4	1	
	EU888620		*Cladosporium* sp. (EF424419)	99				
15	EU888622	Non-MYC	Uncultured soil fungus (DQ421072)	98	1		1	
16	EU888636	MYC	Basidiomycete sp. (AY605706)	99	1			
17	EU888623	Non-MYC	*Schizophyllum commune* (EF155505)	99				1

MYC formed typical ericoid mycorrhizal coils, *Non-MYC* did not form typical mycorrhizal coils, *unknown* could not be confirmed as ericoid mycorrhizal fungi

root-associated fungi, *Epacris pulchella* root-associated fungi, *Rhododendron lochiae* endophytes, et al. Among these RFLP types, type 11 was grouped (99% bootstrap) with a strain of *H. ericae* in neighbor-joining tree.

Three cultured fungal strains isolated from HFP, MFP, and HS were placed in a strongly supported (100% bootstrap) group with *Cryptosporiopsis ericae* as RFLP type 8; the isolates showed high sequence similarity (99% over at least 560 bp) with *C. ericae*.

The remaining RFLP types were designated as probable ascomycete isolates, such as the Helotiales species. One isolate from HFP (RFLP type 16) was probably a basidiomycete as its sequence was identical to a basidiomycete sp. strain from GenBank.

Ericoid mycorrhiza formation

Isolates from nine of the 17 RFLP types formed typical ericoid mycorrhizal coils in epidermal cells of *R. fortunei* and thus were regarded as ericoid mycorrhizal endophytes. Based on this, ca. 20%, 81%, 16%, and 76% of the cultured isolates from *R. fortunei* plants of HFP, SFP, MFP, and HS, respectively, were ericoid mycorrhizal endophytes. According to the ITS sequence comparisons, most ericoid mycorrhizal isolates were putatively classified as *O. maius*, *C. ericae*, or a group of currently unidentified fungal stains whose members are known to form ericoid mycorrhizal associations with Ericaceae plants. The basidiomycetous isolate in RFLP type 16 formed coil-like structures in the epidermal cells.

Discussion

Although the diversity of fungal endophytes in the roots of Ericaceae taxa has been reported previously (Sharples et al. 2000; Usuki et al. 2003; Bougoure and Cairney 2005b), this research represents the first attempt to isolate and infer the

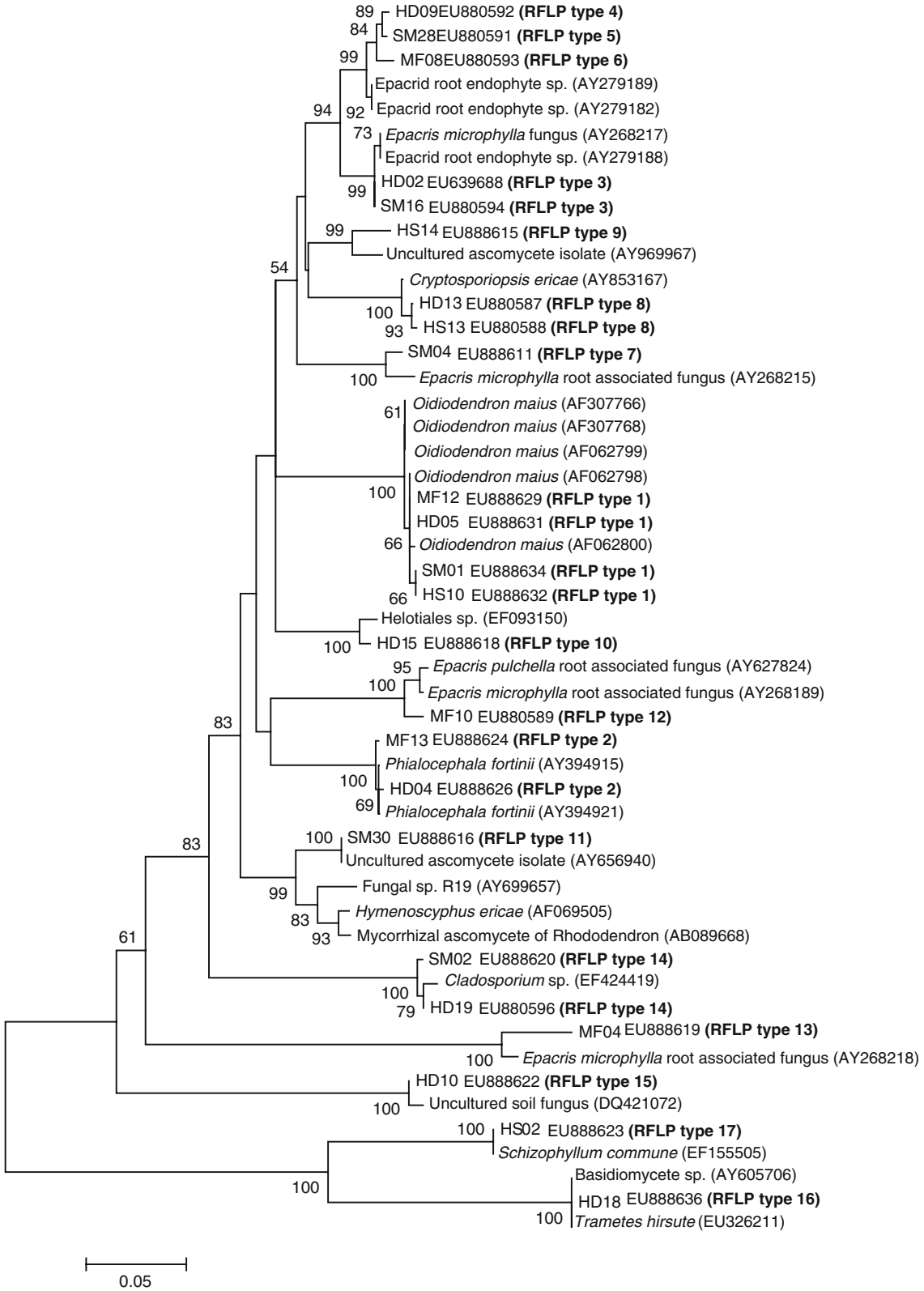

Fig. 1 Neighbor-joining phylogenetic tree based on rDNA ITS sequence data from endophytes of 17 RFLP types isolated from the root systems of *Rhododendron fortunei*, along with known ericoid endophytes and selected fungal species from GenBank with high sequence similarity. Numerical values above the branches indicate bootstrap percentiles from 1000 replicates. Bootstrap numbers over 50% are indicated. Horizontal branch lengths are proportional to the scale of substitutions

taxonomic and genetic diversity of endophytes present in the root system of rhododendrons grown in China. Our investigation showed that the root systems of *R. fortunei* in subtropical forests in China associate with communities of ericoid mycorrhizal endophytes and other root-associated fungi.

The majority of the ericoid mycorrhizal endophytes in *R. fortunei*, regardless of the site origin, were confirmed as *Oidiodendron* sp. Some species in genus *Oidiodendron*, such as *O. maius*, have been frequently recorded as ericoid mycorrhizal endophytes of several taxa in the Ericaceae (Addy et al. 2005) and are especially common in the roots of *Rhododendron* sp.(Usuki et al. 2003; Bougoure and Cairney 2005b). *Oidiodendron* sp. strains were isolated from *R. fortunei* at the four sites, but the isolation frequency of the taxa revealed obvious differences between the four habitats. *Oidiodendron* sp. displayed high abundance at the SFP and HS sites, but very low abundance at the other two sites. Johansson (2001) and Zijlstra et al. (2005), who also isolated ericoid endophytes from root pieces, obtained very low *Oidiodendron* sp. numbers, whereas a high abundance of *Oidiodendron* sp. was found in ericaceous roots from sites in Alberta and Vancouver Island, Canada, and northern Italy (Hambleton and Currah 1997; Monreal et al. 1999). The abundance of *Oidiodendron* sp. isolates appears to be related to particular site conditions (Perotto et al. 2002).

The most common isolates at HFP and MFP were of RFLP type 2. ITS sequence analysis confirmed these isolates as *P. fortinii*, suggesting that this taxon or its sibling species might be the dominant root endophytes of *R. fortunei* at the HFP and MFP sites. However, no *P. fortinii*-like strains were isolated from the SFP and HS sites. The plant communities of the HFP and MFP were pine (*Pinus taiwanensis*) mixed forests, while no coniferous trees surround *R. fortunei* in the habitats at the SFP and HS sites. Moreover, the soil present at HFP and MFP had a lower pH than that at SFP and HS. It seems that the abundance of the *P. fortinii*-like isolates is related to plant communities and edaphic factors (Hambleton and Currah 1997; Addy et al. 2000). In our experiment, some isolates of *P. fortinii*-like formed coil-like mycorrhizal structures with the roots of *R. fortunei* in vitro and positively affected seedlings. These results are consistent with the effects of *P. fortinii* on host plants seeming to range from parasitism to mutualism (Jumpponen and Trappe 1998; Monreal et al. 1999; Jumpponen 2001; Zijlstra et al. 2005). Additional research is needed to elucidate the mycorrhizal status of these isolates.

The 40 isolates (ca. 18% of the total isolates) obtained from the four sites fell into eight RFLP types that grouped with different unidentified root endophytes from Ericaceae plants in Australian forests (Bougoure and Cairney 2005a, b). These isolates, excluding isolates of RFLP types 7 and 11, were confirmed as ericoid mycorrhizal fungi under experimental conditions. ITS sequence analysis showed that the isolates have a high affinity for root endophytes from Ericaceae plants in Australian forests and are probably homologous fungi. The 40 isolates were separate in a neighbor-joining tree, indicating that isolates displaying different RFLP types had a distant relationship and might exist in different fungal taxa. These fungi isolated from ericaceous plants in Australian forests which were applied in our neighbor-joining tree are currently unnamed; most of the endophytes appear to belong to several groups of Helotiales ascomycetes based on rDNA-ITS sequence comparison. Helotiales assemblages are commonly isolated ericoid mycorrhizal endophytes of *R. fortunei* in the subtropical forests of China.

C. ericae assemblages were isolated at a low frequency from the roots of *R. fortunei*. Related taxa of *Cryptosporiopsis* were isolated from some Ericaceae plants, such as *Vaccinium ovalifolium* Mathers, *Vaccinium membranaceum* Dougl. ex Torr. and *G. shallon* Pursh (Sigler et al. 2005). The present research is the first to isolate *C. ericae* assemblages from Rhododendrons and confirm their ericoid mycorrhizal status.

Among all isolates examined in the present study, two were confirmed as basidiomycete sp. Several basidiomycete strains have been isolated from Ericaceae plants, such as *G. shallon* Pursh (Allen et al. 2003), *E. pulchella* (Bougoure and Cairney 2005a), and *R. lochiae* (Bougoure and Cairney 2005b). Similar to Bougoure and Cairney, basidiomycete strains were cultured at a very low isolation rate, with only one isolate obtained from the HFP and HS sites. While basidiomycete hyphae are occasionally observed in the epidermal cells of the hair roots of Australian Ericaceae (Allen et al. 1989), there is no previous evidence that these form ericoid mycorrhizal associations (Cairney and Ashford 2002). In our research, one basidiomycete strain of RFLP type 16 formed coil-like mycorrhizal structures in the roots of *R. fortunei* in vitro and showed positively effects on the seedlings of *R. fortunei*. However, the mycorrhizal infection rate of the strain was quite low in our experimental inoculation test, which might be one reason why basidiomycete strains are usually isolated at a very low frequency. These results confirm for the first time the basidiomycete strain as putative ericoid mycorrhizal fungi. However, the taxonomic status and function of the strain requires further research.

Conclusion

Our research indicated that *R. fortunei* in subtropical forests in China are associated with communities of root endophytes. *Oidiodendron* sp. strains were found to be the most common and major ericoid mycorrhizal fungi in the root

system of *R. fortunei*. *P. fortinii*-like strains were the major root endophytes of *R. fortunei* in pine mixed forests. Basidiomycete strains were also isolated from *R. fortunei* at a very low frequency, and their ericoid mycorrhizal status was confirmed for the first time.

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References

Addy HD, Hambleton S, Currah RS (2000) Distribution and molecular characterization of the root endophyte *Phialocephala fortinii* along an environmental gradient in the boreal forest of Alberta. Mycol Res 104:1213–1221. doi:10.1017/S0953756200002896

Addy HD, Piercey MM, Currah RS (2005) Microfungal endophytes in roots. Can J Bot 83:1–13. doi:10.1139/b04-171

Allen WK, Allaway WG, Cox GC, Valder PG (1989) Ultrastructure of mycorrhizas of Dracophyllum secundum R. Br. (Ericales: Epacridaceae). Aust J Plant Physiol 16:147–153

Allen TR, Miller T, Berch SM, Berbee ML (2003) Culturing and direct DNA extraction find different fungi from the same ericoid mycorrhizal roots. New Phytol 160:255–272. doi:10.1046/j.1469-8137.2003.00885.x

Berch SM, Allen TR, Berbee ML (2002) Molecular detection, community structure and phylogeny of ericoid mycorrhizal fungi. Plant Soil 244:55–66. doi:10.1023/A:1020291516884

Bougoure DS, Cairney JWG (2005a) Assemblages of ericoid mycorrhizal and other root-associated fungi from Epacris pulchella (Ericaceae) as determined by culturing and direct DNA extraction from roots. Environ Microbiol 7:819–827. doi:10.1111/j.1462-2920.2005.00755.x

Bougoure DS, Cairney JWG (2005b) Fungi associated with hair roots of *Rhododendron lochiae* (Ericaceae) in an Australian tropical cloud forest revealed by culturing and culture-independent molecular methods. Environ Microbiol 7:1743–1754. doi:10.1111/j.1462-2920.2005.00919.x

Cairney JWG, Ashford AE (2002) Biology of mycorrhizal associations of epacrids (Ericaceae). Tansley Review No. 135. New Phytol 154:305–326. doi:10.1046/j.1469-8137.2002.00398.x

Cairney JWG, Meharg AA (2003) Ericoid mycorrhiza: a partnership that exploits harsh edaphic conditions. Eur J Soil Sci 54:735–740. doi:10.1046/j.1351-0754.2003.0555.x

Chambers SM, Liu G, Cairney JWG (2000) ITS rDNA sequence comparison of ericoid mycorrhizal endophytes from *Woollsia pungens*. Mycol Res 104:169–175. doi:10.1017/S0953756299001306

Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes—application to the identification of mycorrhizae and rusts. Mol Ecol 2:113–118. doi:10.1111/j.1365-294X.1993.tb00005.x

Hambleton S, Currah RS (1997) Fungal endophytes from the roots of alpine and boreal Ericaceae. Can J Bot 75:1570–1581. doi:10.1139/b97-869

Johansson M (2001) Fungal associations of Danish *Calluna vulgaris* roots with special reference to ericoid mycorrhiza. Plant Soil 231:225–232. doi:10.1023/A:1010310921738

Jumpponen A (2001) Dark septate endophytes are they mycorrhizal? Mycorrhiza 11:207–211. doi:10.1007/s005720100112

Jumpponen A, Trappe JM (1998) Dark septate endophytes: a review of facultative biotrophic root-colonizing fungi. New Phytol 140:295–310. doi:10.1046/j.1469-8137.1998.00265.x

Mclean CB, Cunnington JH, Lawrie AC (1999) Molecular diversity within and between ericoid endophytes from the Ericaceae and Epacridaceae. New Phytol 144:351–358. doi:10.1046/j.1469-8137.1999.00510.x

Midgley DJ, Chambers SM, Cairney JWG (2004) Distribution of ericoid mycorrhizal endophytes and root-associated fungi in neighbouring Ericaceae plants in the field. Plant Soil 259:137–151. doi:10.1023/B:PLSO.0000020947.13655.9f

Monreal M, Berch SM, Berbee M (1999) Molecular diversity of ericoid mycorrhizal fungi. Can J Bot 77:1580–1594. doi:10.1139/cjb-77-11-1580

Perotto S, Girlanda M, Martino E (2002) Ericoid mycorrhizal fungi: some new perspectives on old acquaintances. Plant Soil 244:41–53. doi:10.1023/A:1020289401610

Read DJ (1996) The structure and function of the ericoid mycorrhizal root. Ann Bot (Lond) 77:365–374. doi:10.1006/anbo.1996.0044

Read DJ, Kerley S (1999) The status and function of ericoid mycorrhizal systems. In: Varma A, Hock B (eds) Mycorrhiza, 2nd edn. Springer, Berlin, pp 499–520

Sharples JM, Meharg AA, Chambers SM, Cairney JWG (2000) Symbiotic solution to arsenic contamination. Nature 40:951–952

Sigler L, Allan T, Lim SR, Berch S, Berbee M (2005) Two new Cryptosporiopsis species from roots of ericaceous hosts in western North America. Stud Mycol 53:53–62

Stoyke G, Currah RS (1991) Endophytic fungi from the mycorrhizae of alpine ericoid plant. Can J Bot 69:347–352. doi:10.1139/b91-047

Strandberg M, Johannsson M (1999) Uptake of nutrients in *Calluna vulgaris* seed plants growth with and without mycorrhiza. For Ecol Manage 114:129–135. doi:10.1016/S0378-1127(98)00387-9

Usuki F, Abe JP, Kakishima M (2003) Diversity of ericoid mycorrhizl fungi isolated from hair roots of *Rhododendron obtusum* var. *Kaempferi* in a Japanese red pine forest. Mycoscience 44:97–102. doi:10.1007/s10267-002-0086-8

Xiao G, Berch SM (1992) The ericoid mycorrhizal fungi of *Gaultheria shallon*. Mycologia 84:470–471. doi:10.2307/3760201

Xiao G, Berch SM (1996) Diversity and abundance of ericoid mycorrhizal fungi to form mycorrhizal fungi of *Gaultheria shallon* on forest clearcut. Can J Bot 74:337–346. doi:10.1139/b96-042

Yu F, Zhang CY, Yin LJ, Lai ZX (2008) In vitro inoculation technology of *Rhododendron fortunei* L. with ericoid mycorrhizal fungi and its inoculation effect. J Fujian Agric For Univ 37 (4):360–364 Natural Science Edition

Zhang CY, Hou YM, Dai SL (2008) Observation on microstructure of mycorrhizal root of *Rhododendron fortunei* L. Acta Hortic Sin 35 (11):1641–1646

Zijlstra JD, Hof PV, Baar J, Verkley GJM, Summerbell RC, Paradi I, Braakhekke WG, Berendse F (2005) Diversity of symbiotic root endophytes of the Helotiales in ericaceous plants and the grass, *Deschampsia flexuosa*. Stud Mycol 53:147–162