Characteristics of root-cultivable endophytic fungi from *Rhododendron ovatum* Planch

Lei-Chen Lin¹ · Yu-Sin Ye¹ · Wan-Rou Lin²

Received: 21 September 2017 / Accepted: 23 August 2018 / Published online: 3 December 2018
© Sociedade Brasileira de Microbiologia 2018

Abstract

Ericoid mycorrhiza can improve the competitiveness of their host plants at the ecosystem level. The ability of ericoid mycorrhizal fungi to thrive under harsh environmental conditions suggests that they are capable of decomposing plant organic matter. This study aims to characterize 2 strains of root-cultivable endophytic fungi, RooDK1 and RooDK6, from *Rhododendron ovatum* Planch using colony and hyphal morphology, molecular analysis, observations of mycorrhiza, and investigations of adaptation to different sources of organic matter. Nitrogen utilization was also investigated by assessing protease production and growth on different nitrogen sources. Morphological studies indicated that both species are ericoid mycorrhizal fungi; our molecular studies confirmed RooDK1 as *Oidiodendron maius* and classified RooDK6 as *Pezicula ericae*. We observed that only RooDK1 can assist in host plant survival by degrading organic matter. This species also secretes protease and has the highest nitrate reductase activity of these 2 endophytes. Thus, RooDK1 has a greater ability to help the host plants thrive in a harsh habitat.

Keywords Ericoid mycorrhiza · Ericoid mycorrhizal fungi · Nitrogen utilization · Nitrate reductase activity · Organic matter · Protease

Introduction

Ericaceae species are distributed worldwide, spanning Europe and North America in the northern hemisphere to Australia in the southern hemisphere. Their habitats contain tundra, boreal forests, tropical mountain cloud forests, dry sclerophyll forests, sphagnum wetlands, dry sand plains, and mor-humus heathlands [1, 2]. Characteristics of these environments include extreme acidity, poor nutrient availability, and the accumulation of organic matter [3]. Heathlands also contain polyphenol complexes [4, 5]. These environmental factors play an important role in the selection of this highly conserved group of mycobionts [6]. Common microorganisms are unable to

decompose organic matter in environments with such high fungitoxicity [7]. Fungal symbionts are critically positioned to exert control over the exchange of carbon and nutrients between sources and sinks in these biomes. Therefore, it is important to gain a better understanding of their functional capabilities [8].

Host plants can gain more nutrients from soils under stress conditions if associated with ericoid mycorrhiza fungi (ERMFs) [9–12]. These fungi improve the competitiveness of host plants at the ecosystem level [13]. ERMFs absorb nutrients from soils rich in phenolic compounds and organic matter and transport them to host plants, thereby supporting their growth in harsh habitats [4, 14]. *Oidiodendron maius* Barron [15–17], a well-known ERMF symbiont of ericaceous plants, significantly degrades organic matter [11]. The RooDK1 endophyte *O. maius*, first identified in Taiwan, was isolated from *Rhododendron ovatum* [18], along with RooDK6 [19]. The RooDK1 endophyte was identified as *Oidiodendron maius* using traditional morphology [18]. For both RooDK1 and RooDK6, the molecular characterization and classification as ERMFs remain to be determined. This study aims to classify these two endophytes based on rDNA internal transcribed spacer (ITS) sequencing and morphological studies, to determine whether they are ERMFs, and to investigate whether they assist

Responsible Editor: Derlene Attili de Angelis

✉ Lei-Chen Lin
linerm@mail.ncyu.edu.tw

¹ Department of Forestry and Natural Resources, National Chiayi University, Chiayi 60004, Taiwan

² Bioresource Collection and Research Center (BCRC), Food Industry Research and Development Institute (FIRDI), No. 331, Shih-Pin Road, Hsinchu 30062, Taiwan

in the survival of host plants growing on different types of organic matter and nitrogen sources.

Materials and methods

Strains

A total of 23 endophytes were isolated from the roots of *Rhododendron ovatum* Planch located in the Da-Ken area in central Taiwan (longitude, 120° 44' 0.7" E; latitude, 24° 10' 39.1" N; altitude, 1008 m). Of these endophytes, only two (RooDK1 and RooDK6) passed the screening and selection [19]. Specimens of RooDK1 and RooDK6 were deposited in the Forest Mycobiont Laboratory of National Chiayi University, and their ITS genomic sequences are in GenBank (RooDK1, MF611694; RooDK6, MF611695).

Colony morphology and growth

Colonies of these two endophytes were transferred to 2% malt extract agar media (20 g/L; agar, 15 g/L) and potato dextrose agar media (39 g/L) and incubated in a growth chamber at 23 °C. The growth rate of colonies was determined after 14 days of growth, and colony morphology was observed daily [3, 20, 21].

Hyphae morphology

Hyphal morphology was determined using slide cultures. After 7 days of culture on slides, 1% lactophenol containing cotton blue was applied to stain the hyphae, and observations were made using a light microscope; photos were taken for records [22].

DNA extraction, sequencing, and phylogenetic analysis

DNA was extracted using standard methods [21]. Genomic DNA was extracted using Puregene Proteinase K. Total fungal DNA was used as a template for amplification using primers ITS1-F and TW13. PCR products were sequenced commercially (Genomics BioSci and Tech Company, New Taipei, Taiwan). Sequences were assembled, and related sequences were identified using BLAST searches. Phylogenetic relationships were analyzed using Molecular Evolutionary Genetics Analysis (MEGA). Bootstrapping was performed using maximum likelihood analysis.

Inoculation with endophytes

The method of pure resynthesis was used [14, 16]. After surface cleaning, the seeds of *R. ovatum* were sterilized with 35%

hydrogen peroxide solution (H₂O₂) for 1 min and rinsed 3 times with sterilized distilled water, then transferred to test tubes containing 1% agar for germination. Germinated seedlings were transplanted to modified Mitchell and Read medium (NH₄Cl, 32 mg/L; CaCl₂ 7H₂O, 43.5 mg/L; MgSO₄ 7H₂O, 10 mg/L; KCl, 5.5 mg/L; FeCl₃, 3.75 mg/L; sucrose, 2 g/L; KH₂PO₄, 210 mg/L; pyridoxine, 100 µg/L; thiamine, 100 µg/L; agar, 10 g/L). After 7 days of incubation, the aseptic seedlings were inoculated with these 2 endophytes and grown in a growth chamber (23 °C, 16 h/day, 5000 lx).

Observation of root morphology

The roots of inoculated seedlings were sampled and cleaned with water in a supersonic oscillator after 2 months of incubation [23]. A stereomicroscope was used to observe the morphology of root associations [24], and the roots were stained with aniline blue for staining root studies [25].

Adaption of seedlings to growth on organic matter

Organic matter was prepared using a modified method of Piercey et al. [11] and Lin et al. [14]. Organic matter adaptation was evaluated using the method of Lin et al. [14]. The adaptation ability of these two endophytes was determined by placing a 0.5-mm³ plug of endophyte mycelium onto 2 g (dry weight) of organic matter moistened with 10 mL d-H₂O in a glass tube (25 × 150 mm). When the seedlings had 2 cotyledons, they were transplanted into the sterilized glass tube with organic matter and grown in a growth chamber (2 °C and 16 h of light at a maximum illumination of 5000 lx). Three replicates were prepared for each endophyte, and 3 uninoculated tubes served as controls.

Nitrogen utilization under different nitrogen sources

Nitrogen utilization was assessed by growth in axenic liquid cultures (25 mL), with the addition of (NH₄)₂HPO₄, Ca(NO₃)₂, glutamine, and bovine serum albumin (BSA), and MMN to the base medium, with a final nitrogen concentration of 106 mg/L, a C:N ratio of 39:1, and pH adjusted to 5.5 [26]. Following incubation for 17 days at 23 °C in the dark, the mycelia were harvested, dried overnight at 80 °C, and the biomass determined [27].

Protease production

To determine the level of protease production by each of these 2 endophytes, a 3-mm diameter piece of mycelium was inoculated onto a skim-milk agar plate (skim-milk, 10 g/L; agar, 10 g/L) and maintained at 23 °C in a growth chamber. Observations were made after 5 days of culture. If the medium turned

transparent, it was determined that the endophyte had secreted protease [28, 29].

Nitrate reductase assay

Nitrate reductase activity in fungal filtrates was assayed according to the procedure modified by Jaworski [30], Ho and Trappe [31], and Hamed et al. [32]. These 2 endophytes were cultured in MMN medium with different nitrogen sources [26]. After 17 days of incubation, the mycelia were separated from the medium by filtration and washed extensively with sterile distilled water to prevent any contamination by the medium. Filtered mycelia then were mixed with 5 mL of assay solution (0.1 M KNO_3 [1 mL], 100% 2-propanol [0.25 mL], distilled water [1.25 mL], and 0.2 M KH_2PO_4 [1.25 mL]; pH 7.5) and incubated at 25 °C in the absence of light for 24 h. The reaction was stopped by adding the 1 mL of a solution of 1% sulfanilic acid in 3 M HCl. To this mixture was added 1 mL of 0.02% N-(1-naphthyl ethylene) diamine. 2HCl to serve as an indicator of the nitrite formation in the solution. The absorbance of the resultant pink solutions at 540 nm was measured using a spectrophotometer (U-2000, Hitachi, Tokyo, Japan). The standard curve was determined using KNO_2 .

Statistical analyses

Statistical analysis was performed using the software Statistical Package for the Social Science (SPSS 12.0) (Illinois, USA) for windows. All data are presented as the mean of 3 separate experiments $\pm$ standard error ($n = 3$). Differences between

endophytes were analyzed using Turkey's multiple range test. $p \leq 0.05$ was considered significant.

Results

Strain morphology

After 14 days, colonies of RooDK1 cultured on 2% MEA at 23 °C were white in color (Fig. 1a), and their average growth rate at 23 °C was 0.86 ± 0.01 mm/day; colonies of RooDK1 cultured on PDA at 23 °C were a light-yellow color (Fig. 1b), and the average growth rate of the colony at 23 °C was 0.98 ± 0.01 mm/day. After treating the slide cultures for 7 days, conidiophores ($160\text{--}250 \times 2.48\text{--}3.7$ μm) and conidia ($2.5\text{--}4.5 \times 1.0\text{--}2.5$ μm) were visible under light microscopy (Fig. 1c and d).

After 14 days, colonies of RooDK6 cultured on 2% MEA at 2 °C were brown in color (Fig. 2a), and the average growth rate of the colony at 23 °C was 1.69 ± 0.01 mm/day; colonies of RooDK6 cultured on PDA at 23 °C were brown with white edges (Fig. 2b), and the average growth rate of the colony at 23 °C was 2.57 ± 0.04 mm/day. After treating the slide cultures for 7 days, moniliform hyphae (Fig. 2c) and dark septate hyphae (Fig. 2d) were visible under light microscopy.

Molecular phylogenetic analysis

The genetic diversity of these ERMFs was successfully demonstrated based on the phylogenetic relationships determined using rDNA ITS [33]. Taxonomic affinities were assigned to

Fig. 1 Morphology of the RooDK1 endophyte. **a** Colony morphology on 2% MEA. **b** Colony morphology on 2% PDA. **c** and **d** Conidiophore (arrow) and conidia (arrowhead)

Fig. 2 Morphology of the RooDK6 endophyte. **a** Colony morphology on 2% MEA. **b** Colony morphology on PDA. **c** Moniliform hyphae (arrow). **d** Dark septate hyphae (arrowhead)

RooDK1 and RooDK6 based on BLAST sequence similarity analysis (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) including several closely matched sequences. The RooDK1 ITS sequence was similar to those of *Oidiodendron maius* and clustered with *Oidiodendron maius* with strong bootstrap

values (99%) (Fig. 3), suggesting that the RooDK1 endophyte belonged to *Oidiodendron maius*.

RooDK6 ITS sequences closely match those of *Pezicula ericae* (EF413591) [34] and *P. ericae* (HQ889712); therefore, these organisms are grouped in maximum likelihood analysis

Fig. 3 Maximum likelihood phylogenetic tree based on rDNA ITS sequence data from RooDK1 and RooDK6 endophytes isolated from the root systems of *Rhododendron ovatum* with *Oidiodendron* spp. and *Pezicula* spp. and selected fungal species from GenBank. Sequences in italic type mean sequences from ex-type or type isolate. Numerical values above the branches indicate bootstrap percentiles from 1000 replicates. Horizontal branch lengths are proportional to the scale of base pair substitutions

Fig. 4 Morphology of RooDK1-inoculated seedlings after culture on MMR medium for 70 days. **a** Seedlings inoculated with RooDK1. **b** Conidiophore and conidia (arrow) cover the surface. **c** Root associations (arrows). **d** Hyphal coils (arrows)

(83% bootstrap) (Fig. 3). These results suggest that RooDK6 is *P. ericae*.

Observations of mycorrhiza

Sterilized seeds of *R. ovatum* germinated after 14 days, with most germination noted after 30 days [35]. RooDK1-inoculated seedlings exhibited vigorous growth

after 2 months of cultivation (Fig. 4a). Under a stereomicroscope, viewing of root associations revealed conidia and conidiophores (Fig. 4b) and a covering of hyphae (Fig. 4c). Hyphal complexes were observed in the cortical cells of root associations of resynthesized seedlings under light microscopy (Fig. 4d).

After cultivating for 2 months, RooDK6-inoculated seedlings exhibited vigorous growth (Fig. 5a). Using a

Fig. 5 Morphology of RooDK6-inoculated seedlings after culture on the MMR medium for 70 days. **a** Seedlings inoculated with RooDK6. **b** Hyphae (arrow) cover the surface. **c** Root associations (arrow). **d** Hyphal coils (arrow)

Fig. 6 Morphology of *Rhododendron ovatum* seedlings after culture on organic matter for 21 days. **a** Seedlings inoculated with RooDK1. **b** Seedlings inoculated with RooDK6. **c** Controls

stereomicroscope, we observed that the root associations were covered by hyphae (Fig. 5b) and were swollen (Fig. 5c). Hyphal complex structures were observed in cortical cells of root associations using light microscopy (Fig. 5d).

Ability to use organic matter

After 5 days of incubation, Roo6-inoculated seedlings and controls began to appear withered, while RooDK1-inoculated seedlings exhibited vigorous growth. On the 21st day of incubation, the RooDK1-inoculated seedling still exhibited vigorous growth, while all RooDK6-inoculated seedlings and controls appeared withered (Fig. 6). The RooDK1-inoculated seedlings had a significantly greater fresh weight (1.47 ± 0.15 mg) than did RooDK6-inoculated and control

seedlings (0.90 ± 0.06 mg and 0.77 ± 0.12 mg, respectively; $p < 0.05$) (Fig. 7).

Nitrogen utilization from different nitrogen sources

The nitrogen utilization of these 2 endophytes was assessed by their growth on a variety of nitrogen sources (Table 1). After incubation for 17 days, the RooDK1 endophyte yielded the highest dry weight of glutamine (51.53 ± 4.72 mg) as compared to $(\text{NH}_4)_2\text{HPO}_4$ (30.15 ± 5.43 mg) and BSA (27.63 ± 0.73 mg). A low dry weight of $\text{Ca}(\text{NO}_3)_2$ (8.48 ± 0.89 mg) was observed. The RooDK6 endophyte exhibited a similar trend. The highest dry weight was for glutamine (39.50 ± 1.75 mg), as compared to that of BSA (31.10 ± 0.86 mg) and $(\text{NH}_4)_2\text{HPO}_4$ (25.68 ± 1.02 mg). The lowest dry weight (18.18 ± 2.23 mg) was noted for $\text{Ca}(\text{NO}_3)_2$.

Fig. 7 Biomass after culture with inoculated or control seedlings for 21 days. Values with the same letter did not differ significantly ($p = 0.05$)

Table 1 Comparison of biomass between mycelia inoculated with either endophyte after incubation in media differing in nitrogen source

Nitrogen source	Dry weight (mg)	
	RooDK1	RooDK6
(NH ₄) ₂ HPO ₄	30.15 ± 5.43 ^b	25.68 ± 1.02 ^b
Ca(NO ₃) ₂	8.48 ± 0.89 ^c	18.18 ± 2.23 ^c
BSA	27.63 ± 0.73 ^b	31.10 ± 0.86 ^b
glutamine	51.53 ± 4.72 ^a	39.50 ± 1.75 ^a

All values represent the mean ± standard deviation of 3 replicate cultures
Values in the same column with different letters differ significantly ($p \leq 0.05$)

Protease production

The secretion of proteases from these 2 endophytes was confirmed by their ability to react with the skim-milk agar plates. Skim-milk agar plates bearing the RooDK1 endophytes turned transparent after 5 days (Fig. 8a), while those with RooDK6 showed no change (Fig. 8b).

Nitrate reductase assay

After 17 days of incubation, the nitrate reductase activity of RooDK1 differed significantly depending on which nitrogen source was used ($p < 0.05$) (Table 2). RooDK1 grown on medium with glutamine showed the greatest nitrate reductase activity ($80.25 \pm 8.25 \mu\text{g NO}_2^-/\text{h.g}$) compared with that grown on (NH₄)₂HPO₄ ($34.08 \pm 6.27 \mu\text{g NO}_2^-/\text{h.g}$), Ca(NO₃)₂ ($2.46 \pm 0.21 \mu\text{g NO}_2^-/\text{h.g}$), or BSA ($1.06 \pm 0.10 \mu\text{gNO}_2^-/\text{h.g}$). The nitrate reductase activity of RooDK6 did not differ significantly between samples grown on different nitrogen sources ($p > 0.05$) (Table 2).

Discussion

Our morphological and molecular analysis revealed that the RooDK1 endophyte produces conidia and conidiophores on mycelium as anamorphs (Fig. 1c and d). Results of the rDNA ITS analysis (Fig. 3) indicate that the identity of RooDK1 is

Oidiodendron maius, which is consistent with the findings of Wang and Lin [18]. We observed that RooDK6 produces only moniliform hyphae (Fig. 2c) and stained septates (Fig. 2d) on mycelium. rDNA ITS analysis (Fig. 3) indicated that the identity of RooDK6 is *Pezicula ericae* which is a newly recorded species in the fungal flora of Taiwan. *O. maius* is more frequently detected in the field [36], and this is the first time it has been isolated in Taiwan [18].

Numerous fungi have been isolated from the roots of Ericaceae and Epacridaceae. These isolates have not been identified at the species level because they do not form telemorphs or conidia [37, 38]. However, ITSs of rDNA recently were used to successfully identify phylogenetic relationships and elucidate the genetic diversity of ERMFs [33, 39]. Clearly, the contributions of ITS analyses to ERMF research are significant.

ERM are Endomycorrhizae that have hyphal complexes or hyphal coils in root epidermal cells that are visible microscopically in stained roots [37, 40]. Sterilized seeds germinated after 14 days, with most germination noted after 30 days [35]. After 2 months of incubation, all mycelia inoculated with either endophyte exhibited vigorous growth, and hyphal complexes were observed in the cortical cells of root associations in resynthesized seedlings (Figs. 4d and 5d). The results suggest that these 2 endophytes can form ERM with *R. ovatum* and that they are ERMFs.

Major nutrients in the Ericaceae habitat, including nitrogen, are bound in organic complexes and thus cannot be used by plants. However, many studies have demonstrated that ERMFs can help their host plants to use this organic matter for growth [11, 14]. Organic matter from Ericaceae species is extremely acidic, has a high C/N ratio, and over 30% of its content is phenolic compounds [5, 6, 14]. ERMFs such as *Oidiodendron maius* [11] and *Helotiales* sp. Rf32 and Rf28 have been shown to use nutrients from these phenol-rich environments [14]. Furthermore, we observed that resynthesized seedlings not only have the features of ERMFs but are also capable of decomposing more organic matter than the control seedlings to provide nutrients needed for the growth of the endophytes themselves and the host plants [14]. The results of this study demonstrate that while both RooDK1 and RooDK6 are ERMF, only

Fig. 8 Representative protein medium after incubation with 2 endophytes for 5 days. The change in medium from opaque to transparent indicates protease production of. **a** RooDK1 endophyte. **b** RooDK6 endophyte

Table 2 Comparison of nitrate reductase activity in mycelia inoculated with either endophyte incubated in media differing in nitrogen sources

Nitrogen source	Nitrate reductase activity ($\mu\text{g NO}_2^-/\text{h.g}$)	
	RooDK1	RooDK6
$(\text{NH}_4)_2\text{HPO}_4$	34.08 ± 6.27^b	1.74 ± 0.39^a
$\text{Ca}(\text{NO}_3)_2$	2.46 ± 0.21^c	2.09 ± 0.27^a
BSA	1.06 ± 0.10^c	1.49 ± 0.06^a
Glutamine	80.25 ± 8.25^a	1.29 ± 0.21^a

All values represent the mean $\pm$ standard deviation of 3 replicate cultures
Values in the same column with different letters differ significantly ($p \leq 0.05$)

RooDK1 has the ability to survive on organic matter (Fig. 6). Thus, not every ERMF can make nutrients from organic matter available for host plant growth.

We observed that the type of nitrogen source available had a significant effect on the fungus biomass. The use of glutamine as a nitrogen source resulted in the greatest biomass in both endophytes (Table 1). This result differs from that of other studies. For example, *O. maius* from field salal (*Gaultheria shallon*) roots yielded the greatest biomass when grown on $(\text{NH}_4)_2\text{HPO}_4$ [41]; three strains of *Oidiodendron* sp. from *R. fortune* roots yielded the greatest biomass when grown on $(\text{NH}_4)_2\text{HPO}_4$ or $\text{Ca}(\text{NO}_3)_2$ [42, 43]. Therefore, our results demonstrate that ERMFs isolated from different habitats have different characteristics, as has also been reported by Rice and Currah [22].

We also observed that nitrate reductase activity (NRA) differed between RooDK1 and RooDK6. The highest NRA in RooDK1 was observed using glutamine as the nitrogen source (Table 2).

Taken together, our findings indicate that RooDK1 has the ability to secrete protease and yields the greatest biomass and highest NRA in glutamine. The RooDK1 endophyte is capable of decomposing organic matter to provide nutrients needed for the growth of the host plant.

Conclusions

This study characterizes 2 root-cultivable endophytic fungi, RooDK1 and RooDK6, from *Rhododendron ovatum*. Based on molecular analysis, RooDK1 is classified as *Oidiodendron maius*, while RooDK6 is classified as *Pezizula ericae*. RooDK6 is a newly recorded species in the fungal flora of Taiwan, and both of these endophytes are ERMFs. RooDK1 can assist in host plant survival by degrading organic matter, secretes protease, and has the highest NRA of these 2 endophytes. Thus, RooDK1 has a greater ability to help the host plants thrive in a harsh habitat.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

References

- Luteyn JL (2002) Diversity, adaptation, and endemism in neotropical Ericaceae: biogeographical patterns in the Vaccinieae. *Bot Rev* 68:55–87
- Perotto S, Girlanda M, Martino E (2002) Ericoid mycorrhizal fungi: some new perspectives on old acquaintances. *Plant Soil* 244:41–53
- Johansson M (2001) Fungal associations of Danish *Calluna vulgaris* roots with special reference to ericoid mycorrhiza. *Plant Soil* 231:225–232
- Jalal MAF, Read DJ (1983) The organic acid composition of *Calluna* heathland soil with special reference to phyto- and fungitoxicity. I. Isolation and identification of organic acids. *Plant Soil* 70:257–272
- Jalal MAF, Read DJ, Haslam E (1982) Phenolic composition and its seasonal variation in *Calluna vulgaris*. *Phytochemistry* 21:1397–1401
- Read DJ, Perez-Moreno J (2003) Mycorrhizas and nutrient cycling in ecosystems- a journey towards relevance? *New Phytol* 157:475–492
- Leake JR, Read DJ (1989) The effects of phenolic compounds on nitrogen mobilization by ericoid mycorrhizal systems. *Agric Ecosyst Environ* 29:225–236
- Read DJ, Leake JR, Perez-Moreno J (2004) Mycorrhizal fungi as drivers of ecosystem processes in heathland and boreal forest biomes. *Can J Bot* 82:1243–1263
- Schmid E, Oberwinkler F, Gomez LD (1995) Light and electron microscopy of a host-fungus interaction in the roots of some epiphytic ferns from Costa Rica. *Can J Bot* 73:991–996
- Rice AV, Currah RS (2001) Physiological and morphological variation in *Oidiodendron maius*. *Mycotaxon* 79:383–396
- Piercey MM, Thormann MN, Currah RS (2002) Saprobic characteristics of three fungal taxa from ericacean roots and their association with the roots of *Rhododendron groenlandicum* and *Picea mariana* in culture. *Mycorrhiza* 12:175–180
- Gibson BR, Mitchell DT (2005) Influence of pH on copper and zinc sensitivity of ericoid mycobionts in vitro. *Mycorrhiza* 15: 231–234
- Michelsen A, Schmidt IK, Jonasson S, Quarmby C, Sleep D (1996) Leaf ^{15}N abundance of subarctic plants provides field evidence that ericoid, ectomycorrhizal and non- and arbuscular mycorrhizal species access different sources of soil nitrogen. *Oecologia* 105:53–63
- Lin LC, Lee MJ, Chen JL (2011) Decomposition of organic matter by the ericoid mycorrhizal endophytes of Formosan rhododendron (*Rhododendron formosanum* Hemsl.). *Mycorrhiza* 21:331–339
- Couture M, Fortin JA, Dalpé Y (1983) *Oidiodendron griseurn* Robak: an endophyte of ericoid mycorrhiza in *Vaccinium* spp. *New Phytol* 95:375–380
- Dalpé Y (1986) Axenic synthesis of ericoid mycorrhiza in *Vaccinium angustifolium* Ait. by *Oidiodendron* species. *New Phytol* 103:391–396
- Douglas GC, Heslin MC, Reid C (1989) Isolation of *Oidiodendron maius* from *Rhododendron* and ultrastructural characterization of synthesized mycorrhizas. *Can J Bot* 67:2206–2212
- Wang YZ, Lin LC (2014) A note on *Oidiodendron maius* in Taiwan. *CAR* 27:1–3
- Ye YS (2015) The characteristics of endophytes isolated from *Rhododendron ovatum* Planch. var. *ovatum* and the resynthesis effect with its host plant. [Master's thesis], Department of Forestry

- and Natural Resources, National Chiayi Univ., Chiayi, Taiwan. [In Chinese]
20. McLean CB, Cunningham JH, Lawrie AC (1999) Molecular diversity within and between ericoid endophytes from the Ericaceae and Epacridaceae. *New Phytol* 144:351–358
 21. Sigler L, Allan T, Lim SR, Berch S, Berbee M (2005) 2 new *Cryptosporiopsis* species from roots of ericaceous hosts in western north America. *Stud Mycol* 53:53–62
 22. Rice AV, Currah RS (2006) *Oidiodendron maius*: saprobe in sphagnum peat, mutualist in ericaceous roots? Microbial root endophytes. Springer, Berlin, pp 227–246
 23. Upson R, Read DJ, Newsham KK (2007) Microscopy analyses of field-collected *Cephalospora varians*. *New Phytol* 176:460–471
 24. Usuki F, Narisawa K (2005) Formation of structures resembling ericoid mycorrhizas by the root endophytic fungus *Heteroconium chaetospora* within roots of *Rhododendron obtusum* var. *kaempferi*. *Mycorrhiza* 15:61–64
 25. McGonigle TP, Miller MH, Evans DG, Fairchild GL, Swan JA (1990) A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. *New Phytol* 115:495–501
 26. Anderson IC, Chambers SM, Cairney JWG (1999) Intra- and inter-specific variation in patterns of organic and inorganic nitrogen utilization by three Australian *Pisolithus* species. *Mycol Res* 103:1579–1587
 27. Cairney JWG, Sawyer NA, Sharples JM, Meharg AA (2000) Intraspecific variation in nitrogen source utilisation by isolates of the ericoid mycorrhizal fungus *Hymenoscyphus ericae* (Read) Korf and Kernan. *Soil Biol Biochem* 32:1319–1322
 28. Wu SP (2005) Characteristics, pedogenesis and classification of podzolic soil in Tai-Ping mountain of Ilan. [Ph. D. dissertation], Taipei, Taiwan, Graduate institute of Agricultural chemistry, National Taiwan University. [In Chinese]
 29. Nygren CM, Edqvist J, Elfstrand M, Heller G, Taylor AF (2007) Detection of extracellular protease activity in different species and genera of ectomycorrhizal fungi. *Mycorrhiza* 17:241–248
 30. Jaworski EG (1971) Nitrate reductase assay in intact plant tissues. *Biochem Biophys Res Commun* 43:1274–1279
 31. Ho I, Trappe JM (1975) Nitrate reducing capacity of 2 vesicular-arbuscular mycorrhizal fungi. *Mycologia* 67:886–888
 32. Hamed S, Shojasadati SA, Shokrollahzadeh S, Hashemi-Najafabadi S (2013) Extracellular biosynthesis of silver nanoparticles using a novel and nonpathogenic fungus, *Neurospora intermedia*: controlled synthesis and antibacterial activity. *World J Microbiol Biotechnol* 30:693–704
 33. Usuki F, Abe PJ, Kakishima M (2003) Diversity of ericoid mycorrhizal fungi isolated from hair roots of *Rhododendron obtusum* var. *kaempferi* in a Japanese red pine forest. *Mycoscience* 44:97–102
 34. Johnston PR, Seifert KA, Stone JK, Rossman AY, Marvanová L (2014) Recommendations on generic names competing for use in Leotiomycetes (Ascomycota). *IMA Fungus* 5(1):91–120
 35. Lin LC, Wang CS (2017) Influence of light intensity and photoperiod on the seed germination of four *Rhododendron* species in Taiwan. *Pak J Biol Sci* 20:253–259
 36. Baba T, Hirose D, Sasaki N, Watanabe N, Kobayashi N, Kurashige Y, Karimi F, Ban T (2016) Mycorrhizal formation and diversity of endophytic fungi in hair roots of *Vaccinium oldhamii* Miq. in Japan. *Microbes Environ* 31:186–189
 37. Xiao G, Berch SM (1996) Diversity and abundance of ericoid mycorrhizal fungi of *Gaultheria shallon* on forest clearcuts. *Can J Bot* 74:337–346
 38. Hambleton S, Currah RS (1997) Fungal endophytes from the roots of alpine and boreal Ericaceae. *Can J Bot* 75:1570–1581
 39. Sharples JM, Chambers SM, Meharg AA, Cairney JWG (2000) Genetic diversity of root-associated fungal endophytes from *Calluna vulgaris* at contrasting field sites. *New Phytol* 148:153–162
 40. Peterson RL, Massicotte HB, Melville LH (2004) Mycorrhizas: anatomy and cell biology. NRC Reserch Press, Ottawa
 41. Xiao G, Berch SM (1999) Organic nitrogen use by salal ericoid mycorrhizal fungi from northern Vancouver Island and impacts on growth in vitro of *Gaultheria shallon*. *Mycorrhiza* 9:145–149
 42. Yin LJ, Zhang CY, Yang B (2010) Characteristics of nitrogen absorbed by ericoid mycorrhizal fungi and impact on growth of *Rhododendron fortune*. *Sci Agric Sin* 43:868–872 [In Chinese]
 43. Sun QL, Zhang CY, Dai SL (2014) Effects of different nitrogen sources on the nitrogen uptake and nitrate reductase activities of ericoid mycorrhizal fungi. *J Nanjing For Univ* 38:175–178 [In Chinese]