

***Pezicula ericae*, a new record of a mycorrhizal fungus from *Pinus taiwanensis* in Taiwan**

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ABSTRACT

A *Pezicula* species was isolated from the roots of *Pinus taiwanensis*. According to the morphology, cultural characteristics, and phylogenetic analysis of the internal transcribed spacer (ITS1-5.8S-ITS2) sequence of the rRNA gene, the strain was identified as *Pezicula ericae*. It is a new record in Taiwan. Morphological and cultural characteristics are illustrated and described.

Key words: Ascomycota, Leotiomycetes

Introduction

Endophytes can colonize any part of host plant and perform variable life strategies in symbiosis including facultative saprophyte to parasite to exploitative to mutualism (Schulz and Boyle 2006). Species of *Pezicula* are frequently reported from the dead or living branches and roots in the temperate regions worldwide, and occur as endophytes or saprobes displaying no disease symptoms (Chen et al. 2016, Toju and Sato 2018). Endophytic *Pezicula* species are also suggested to associate with Ericaceae plants forming ericoid mycorrhiza (Berch, et al. 2002), to facilitate the uptake of nutrients from soils rich in organic matter and phenolic compounds in harsh habitats for host plants (Lin, et al. 2011).

The genus *Pezicula* (Dermateaceae, Helotiales) (anamorphic *Cryptosporiopsis*, Helotiales) was first reported in 1865 (Chen et al. 2016), until now there are 138 record of species according to MycoBank database (Robert et al. 2005). In Taiwan, one species of *Pezicula* isolated from bark of *Dendropanax pelucidopuncata* has been reported (Liu and Chen, 1977). In this paper, *Pezicula ericae*, isolated from *Pinus taiwanensis* roots, is newly recorded in Taiwan and illustrated.

Materials and Methods

Collection and isolation of mycorrhizal fungi

About 80 ectomycorrhizal segments of *Pinus taiwanensis* were collected for the isolation of ectomycorrhizal fungi. The ectomycorrhiza was col-

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lected from the pure forest of the *Pinus taiwanensis* in Renai Township, Nantou County, Taiwan (24° 7'N, 121° 16'E). Roots were washed to remove soil by ultrasonic vibration and were cut into sections of about 1 cm long with a sterile scalpel. The root segments were then subjected to the following steps: submerged in 35 % hydrogen peroxide for 3 minutes; washed in sterile water to remove the residual hydrogen peroxide; dried by paper towel; placed on Modified Melin-Norkrans (MMN) agar medium which was incubated in the dark at 22 °C. After the mycelia were extended, pure mycelia were transferred into fresh medium for subculture. Living culture was deposited in the Bioresource and Collection Center, Hsin-chu (BCRC).

DNA isolation and molecular analysis

The ectomycorrhizal fungi DNA was extracted from approximately 0.1 g of mycelia using DNeasy® Plant Mini Kit following manufacturer's instructions. Primer pair ITS1F/ITS4 was used for amplification of the ITS rDNA (Gardes and Bruns, 1993; White et al., 1990). Amplified DNA was assessed with 1% agarose gel electrophoresis and ClearVision DNA Stain (Protech Technology Enterprise, Taiwan) visualized under UV light. Sequencing of DNA was done by

Genomics (Taipei, Taiwan). The DNA sequence was edited using Bioedit (Bioedit, USA). The ITS rDNA sequence was submitted to the BLAST function Biotechnology Information (NCBI) database (<http://www.ncbi.nlm.nih.gov>). The ITS sequences of strains from *Pezicula* species were retrieved from the NCBI database and used for preparing an alignment with our ITS sequence with the default options of MEGA X. A phylogenetic tree was constructed by the Neighbor joining tree method using the Kimura-2 parameter and Gamma distribution as best model with MEGA X software based on 1000 bootstrap replications. *Pezicula carpinea* was selected as outgroup.

Morphological study

For evaluating the culture morphology, cultures were grown on oatmeal agar (Difco™, OA) and incubated at 24–25 °C in the dark. Microscopic characteristics were observed using fungal material mounted in 5–10 % (w/v) aqueous KOH solution.

Results

A total of nine fungal species were isolated from the 80 1-cm-long root segments of *Pinus taiwanensis*. Among these, only *P. ericae* is ectomycor-

Table 1. The fungal species that we isolated from *Pinus taiwanensis* roots and BLAST results from GenBank.

Code	Accession number	Species	Identity (%)	Query coverage (%)
D1	KP866127.1	<i>Phialocephala</i> sp.	99	99
K2	KX440115.1	<i>Pezicula ericae</i>	98	99
M1	JX885462.1	<i>Fusarium oxysporum</i>	100	97
M6	KX609685.1	<i>Pestalotiopsis trachicarpicola</i>	99	97
E1	AB511815.1	<i>Pestalotiopsis</i> sp.	99	97
E2	JX885462.1	<i>Fusarium oxysporum</i>	100	96
L2	KY750473.1	<i>Ascomycota</i> sp.	96	99
P1	AB457007.1	<i>Talaromyces radicus</i>	99	96
FS1	HQ608121.1	<i>Trichoderma lixii</i>	99	99

rhizal, and the others are likely pathogenic, saprophytic, or endophytic fungi (Table 1). The probability of obtaining *P. ericae* is about 11%.

Taxonomy

Pezicula ericae Johnston, in Johnston, Seifert, Stone, Rossman & Marvanová, IMA Fungus. 5: 104. 2014. Fig. 1

Mycelium mostly septate long lines, thick-walled, rounded ends and almost smooth; hyphae sparingly branched, septa often appearing slightly pale to darkened, rounded apex and narrow truncate. Conidiophores simple, mostly acromonium-like, mainly composed of conidiogenous cells, erect. Conidiogenous cells dark brown-colored, cylindrical, unbranched, arising laterally and terminally from vegetative hyphae, $18\text{--}22 \times 3\text{--}5 \mu\text{m}$ at base. Conidia produced phialidically on the apex of conidiogenous cells, pale yellow, long-cylindrical, slightly curved, $22\text{--}25 \times 6\text{--}8 \mu\text{m}$.

Cultural characteristics. Colonies on MEA variable in color. The initial growth of mycelium is changed from fawn brown to chartreuse, and hyphae exhibit bifurcation growth. Colonies on OA reaching 67 mm diam after 21 days, granular due to profuse sporulation and fasciculate bundles of conidiophores. Reverse chartreuse to dark brown (Fig. 1).

Sequences of the ITS region. BLAST search results suggested that the identification of our isolate was *P. ericae*. (GenBank KX440115.1). Phylogenetic analysis with root-derived sequences and selected sequences from GenBank revealed

that the species is *P. ericae* (Fig. 2).

Specimen examined. TAIWAN. Nantou County, Ren-ai Township, isolated from root of *Pinus taiwanensis*, 21 May 2016, Song, Y.-P.; living culture deposited in the Bioresource and Collection Center, Hsin-chu (BCRC).

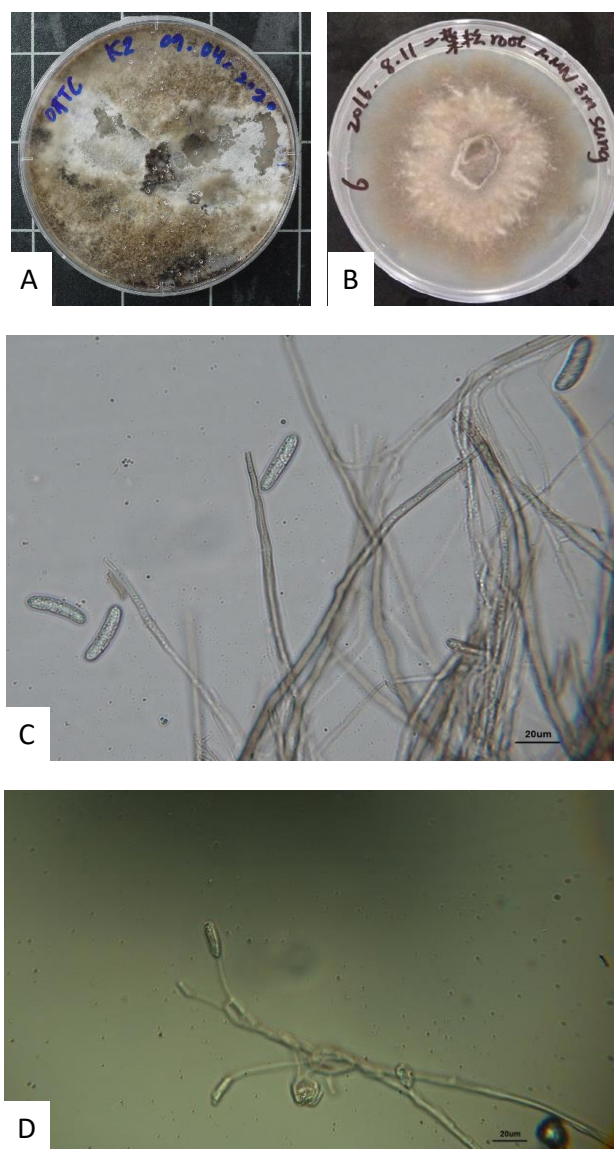


Fig. 1. Morphological characteristics of *Pezicula ericae*. A. 28-day-old culture on OA. B. 21-day-old culture on MMN viewed from the top. C. Conidiophore and conidia. D. Conidia attached on the apex of a conidiophore. Scale bar: 20 μm .

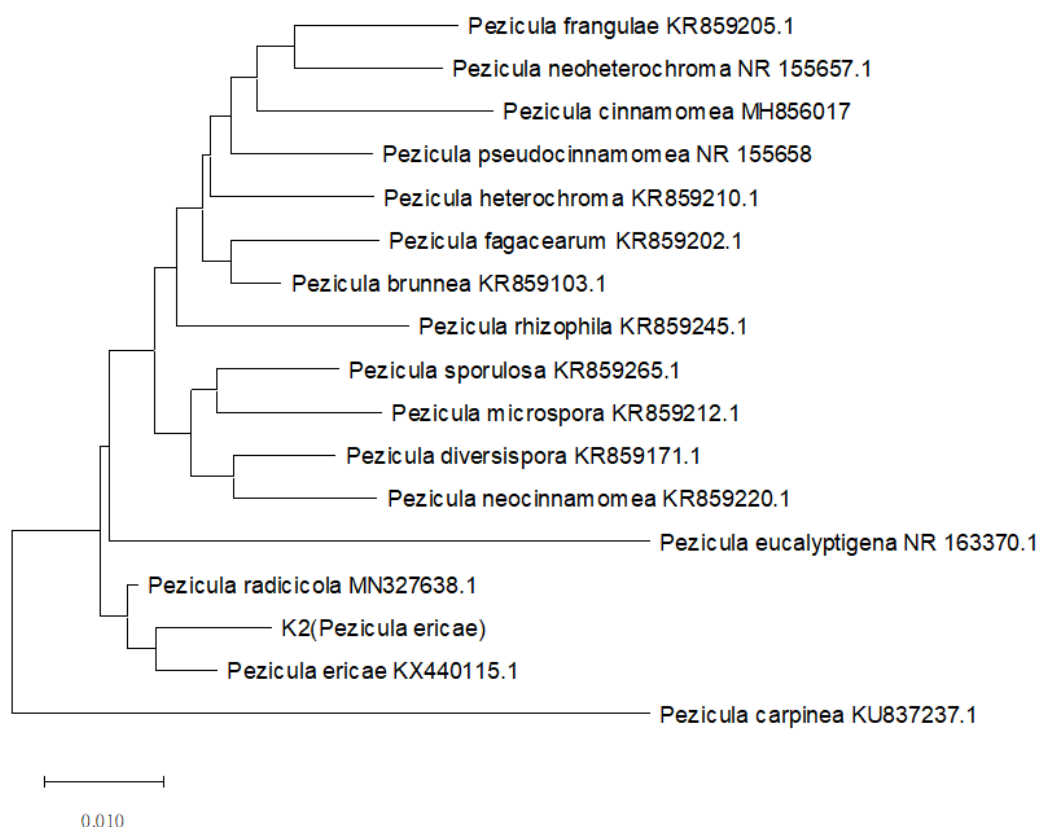


Fig. 2. Phylogenetic tree derived from ITS sequences of *Pezicula* species based on the Neighbor joining tree analysis with 1000 bootstrap replications. The outgroup taxon was *Pezicula carpinea*.

Distribution and habitats. Above-ground parts of woody plant, roots of dying or living trees (Verkley 1999, Abeln et al. 2000, de Jong et al. 2001, Kowalski & Bartnik 1995, Verkley et al. 2003).

Discussion

The ITS sequence of our isolated fungal strain was blasted to *P. ericae* (KX440115.1) with 98.9% identity and 98 % coverage in NCBI GenBank. *P. ericae* had been frequently reported from Europe and North America (Chen et al. 2016), and we isolated the fungi from living roots of *Pinus taiwanensis* for the first time in Taiwan. The fungus was isolated on MMN, but sporulation was only induced on oatmeal agar (OA) overlaid with a sterile filter paper as Sigler et al.

(2005) suggested. Specifically, we observed white and fawn brown areas with masses of conidia and dark mycelium produced on the filter paper. Moreover, we inoculated the fungus on *Pinus taiwanensis* seedling roots and mycorrhizal structures including mantel and Hartig net were observed using a scanning electron microscope after cultivation for nearly 3 months (Fig. 3). The fungus has been also reported to produce hyphal coils in ericoid plant epidermal cells (Currah et al. 1993; Smith and Read 2008). This is the first time that *P. ericae* was observed to have formation of mycorrhizal structures with *Pinus taiwanensis*.

The morphology of our strain is much like that described for *Cryptosporiopsis ericae* ($\equiv$ *P. ericae*) by Sigler et al. (2005) in the shape and size

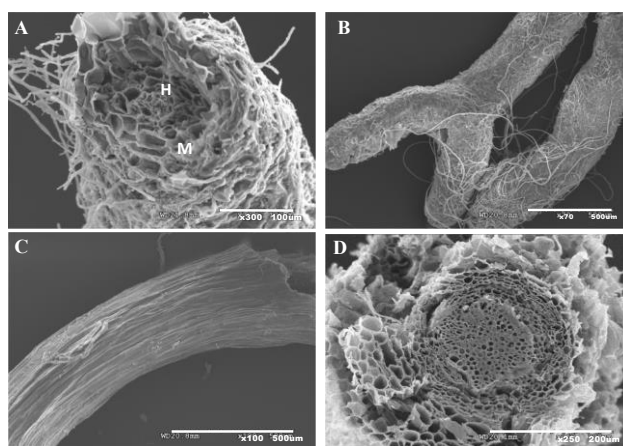


Fig. 3. Roots of *Pinus taiwanensis*. A, B. Mycorrhizal association with *Pezicula ericae*; M and H in A denote mantle and Hartig net, respectively. C, D. Non-mycorrhizal root.

of conidiogenous cells and conidia. However, unlike Sigler et al. (2005), we did not observe conidiomata for our Taiwan isolate.

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臺灣二葉松共生菌根菌的新紀錄種 *Pezicula ericae*

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摘 要

分離自臺灣二葉松根部之 *Pezicula* 物種，根據形態特徵、培養特性、根部菌根構造及 rDNA 基因之內部轉錄區間 (ITS1-5.8S-ITS2) 序列的親緣關係分析，該菌株被鑑定為 *Pezicula ericae*，為臺灣新紀錄種。該菌株的形態特徵及培養特性於文中提供圖片及描述說明。

關鍵詞：子囊菌門、錘舌菌綱