한 국 균 학 회 지 The Korean Journal of Mycology Research Note

# First Record of Endophytic *Paraconiothyrium brasiliense* Isolated from Chinese Maple Leaves in Korea

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**ABSTRACT :** The fungal endophyte EML-CM25 was isolated in Korea from surface sterilized Chinese maple leaf tissues. Internal transcribed spacer sequence analysis indicated that the isolate showed 99% sequence similarity with *Paraconiothyrium brasiliense*, a finding that was confirmed by morphological analyses. The fungal colonies did not express aerial hyphae. Conidiomata formation was observed in the fungus cultured on potato dextrose agar at 25°C for 7 days. Visible scattered black dots consisting of pycnidia were present throughout the colony. This is the first record of *P. brasiliense* in Korea.

**KEYWORDS : Chinese maple, Endophytic fungi, ITS, Morphology, *Paraconiothyrium brasiliense***

The all-inclusive, widely accepted definition of "endophyte", as given by Bacon and White [1], is "microbes that colonize living internal tissues of plants without causing any immediate, overt negative effects". Plants provide numerous and diverse niches for endophytic organisms; the biological associations between these organisms and their respective hosts could be closer than those developed by epiphytes or soil related organisms. The Chinese maple tree, with its bright colors, is a great addition to almost any yard, and is a favorite among people who wish to add color and variety to their gardens. This tree is commonly seen in all regions of Korea. Therefore, this study attempted the isolation of endophytic fungi from this plant. A number of endophytic fungi were isolated and characterized. Among these, the EML-CM25 isolate was identified as a new record in Korea through the analysis of molecular and morphological data.

Kor. J. Mycol. 2014 December, **42**(4): 349-352
http://dx.doi.org/10.4489/KJM.2014.42.4.349
pISSN 0253-651X • eISSN 2383-5249


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**Received** November 28, 2014
**Revised** December 16, 2014
**Accepted** December 17, 2014



Coelomycetes, such as *Paraconiothyrium* or *Coniothyrium* or *Microsphaeropsis*, which are widely distributed at different temperatures around the world, have been commonly isolated in recent studies [2,3]. These express biological control activity, and produce antibiotics [2,4,5]. So far, only a few *Paraconiothyrium* fungal species have been reported in Korea. In this study, we have isolated the fungal endophyte *Paraconiothyrium* from Chinese maple leaf tissues.

Samples for this study were collected from different sites in Gwangju metropolitan city, in the Republic of Korea. Live, symptomless leaf samples were collected and stored in sterile polyethylene bags. The samples were cleaned under running tap water to remove debris, air-dried, and processed within 5 h post-collection. The tissues were cut into small (1 cm length and 0.5 cm width) pieces. The surface-sterilized segments were plated on potato dextrose agar (PDA) (Difco; BD diagnostics, Franklin Lakes, NJ, USA) and rose bengal chloramphenicol agar (DRBC; BD Diagnostics) supplemented with streptomycin sulfate (0.4 mg/mL, Sigma-Aldrich, St. Louis, MO, USA) to restrict bacterial growth, and incubated at 25°C for 5 days. The individual hyphal tips of the developing fungal colonies were collected, plated on PDA, incubated for 5-10 days, and analyzed for culture purity. Pure cultures and *Paraconiothyrium*-like fungi were transferred to PDA-slants and eppendorf tubes with 20% glycerol stock solution. Selected isolates were assigned strain numbers, which were deposited in the Environmental Microbiology Research Lab (EML) Herbarium,

Chonnam National University, Gwangju, Korea. The EML-CM25 isolate was subjected to molecular and morphological data analysis.

Genomic DNA was extracted from the mycelia developed on PDA plates using the HiGene™ Genomic DNA Prep Kit (BIOFACT, Daejeon, Korea). The internal transcribed spacer (ITS) region was amplified with the primer pair ITS1 and ITS4 [6], using the Accupower® PCR premix (Bioneer, Daejeon, Korea) and the PCR conditions were as follows: an initial denaturation step of 94°C for 5 min followed by 35 cycles of 94°C for 1 min, 55°C for 1min, 72°C for 1 min. A final elongation step of 72°C was performed for 10 min. PCR products were purified using the Accuprep® PCR Purification Kit (Bioneer, Daejeon, Korea) according to the manufacturer instructions. The samples were sequenced in an ABI 3700 Automated DNA sequencer (Applied Biosystems Inc., Foster City, CA, USA). The resulting sequences were initially aligned to relevant sequences in the GenBank database using the Clustal X program [7]. Maximum parsimony was carried out using the MEGA4 [8] software and a phylogenetic tree was constructed (Fig. 1) that was evaluated with 1,000 bootstrap replications. The ITS sequence of the EML-CM25 isolate showed 99.00% sequence similarity with the type strain *Paraconiothyrium brasiliense* CBS 254.88$^{T}$ in a basic local alignment search tool (BLAST) analysis. The phylogenetic analysis revealed the sequence of EML-CM25 isolate was identical to the type strain and other reliable *P. brasiliense* strains as supported by a bootstrap value of 64% (Fig. 1). The results confirmed that the isolate EML-CM25 was the species of *Paraconiothyrium brasiliense*. The sequence of the isolate deposited in GenBank and assigned accession number of KP 222879.

*Paraconiothyrium brasiliense* Verkley 2004 (Fig. 2)

**Description:** The isolate was cultured on PDA and malt extract agar (MEA) to assess it's cultural and morphological characteristics. The colonies were characterized after a 7-day incubation period at 25°C. The color of the colony cultured on PDA was transparent white. Multiple visible scattered black dots with a ring like structure were observed after incubation for 2 weeks. The colony obtained on MEA was colorless to buff, with the surface almost covered by a dense mat of wooly-floccose aerial mycelium that remained pure white except in the center. Numerous complex conidiomata were observed after 5 to 6 days, along with many pycnidia with some extruding

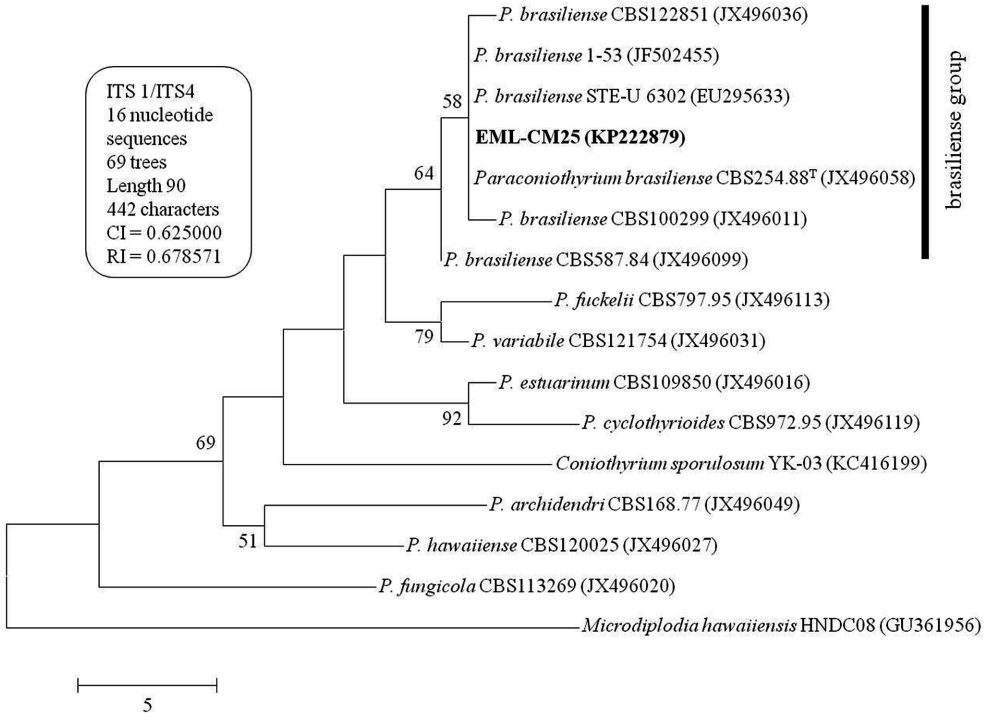


**Fig. 1.** A parsimonious tree inferred from the ITS sequence region. The numbers above the branches indicate bootstrap values for 1,000 bootstrap replicates. Only bootstrap values higher than 50 are shown. The scale bar indicates the number of nucleotide substitutions. GenBank accession numbers are provided within parentheses.

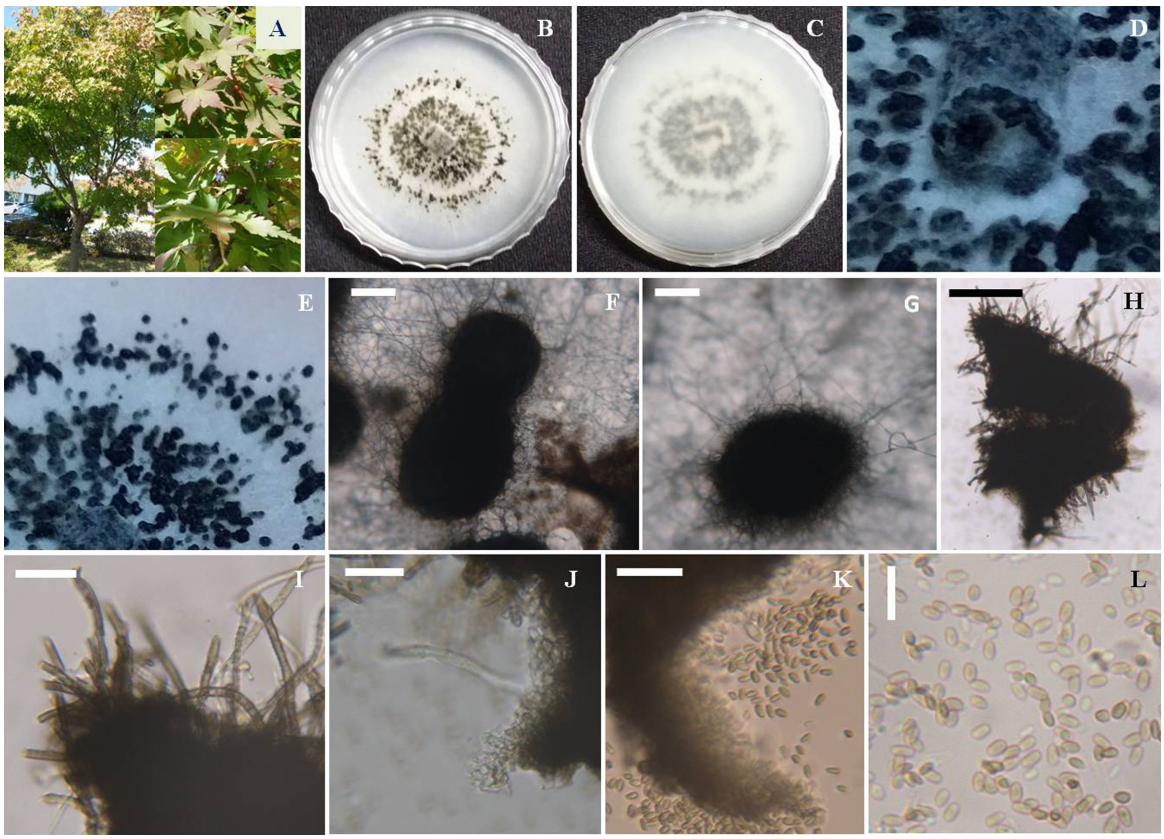


**Fig. 2.** Morphological characteristics of the isolate EML-CM25**.** A; Asymptomatic leaf samples were collected from the Chinese maple leaves. B, C; Colony characteristics on potato dextrose agar (B, obverse; C, reverse). D-H; Colony texture, pycnidia with some extruding cirri of conidia, and conidiomata on PDA after two weeks of incubation at 25°C. I; Pycnidial wall with mycelial appendages; J, K; Dark layered pycnidial wall, conidiogenous cells and conidia; L; Conidia. Scale bars = 200 μm (F, G), 50 μm (H), 20 μm (I-K), and 10 μm (L).

cirri of conidia; a number of these conidiomata grew to a large size within two weeks. The conidiomata were dark brown to black in color and 0.5-1.5 mm in diameter. Ostioles, which are openings created by the dissolution of upper cells and typical of such fungal species, were absent. The walls of conidiomata had a thickly texture; they were relatively thinner on the inside, and darker and thicker on the outside. The conidiomata were composed of a number of complex pycnidia surrounded by smooth-walled light brown to brown hyphal appendages (Fig. 2H and 2I). Conidiogenous cells were formed from the inner cells of the conidiomatal wall (Fig. 2J and 2K). Conidiogeneous cells were discrete, or assembled into protruding masses. Conidial morphology on PDA was examined under a compound light microscope (Olympus BX50; Olympus, Tokyo, Japan). Single-celled conidia were mainly ellipsoid to small-cylindrical, and rounded at the ends. However, in some cases, they were obpyriform (narrowing towards the base). The conidial mass was dark brown to blackish in color. The size of the conidia on PDA was 2.5-5.5 × 1.5-3.0 μm. Based on the morphological characteristics, the endophytic fungus was identified as *Paraconiothyrium brasiliense* [2].

**Isolates examined:** The endophytes of Chinese maple leaves were isolated; the isolates EML-CM25 and EML-CM06 were subjected to morphological analysis.

**Host and distribution:** These strains were isolated from *Coffea arabica* fruits in Brazil [2], and as endophytes from *Ginkgo biloba*, *Pinus glauca* leaves (Canada), and *Alliaria petiolata* (USA). This fungus was also isolated from a marine fish in China, surface water from wetlands in Japan, discolored wood of a living *Platanus acerifolia* tree in Italy, and South African peach, nectarine, and plum trees [3]. In this study, endophytic *Paraconiothyrium brasiliense* was isolated from Chinese maple leaves in Korea.

**Note:** The morphological characteristics did not present significant differences when compared to the strain reported by Verkely et al. [2]. This identification was well-

supported by phylogenetic analysis. This is the first detailed report on endophytic *Paraconiothyrium brasiliense* in Korea.

## Acknowledgements

This work was supported by a grant from the National Institute of Biological Resources (NIBR), funded by the Ministry of Environment (MOE) of the Republic of Korea.

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