

RESEARCH NOTE

First Report of Endophytic Fungi, *Aequabiliella effusa*, Isolated from Twigs of *Larix gmelinii* var. *olgensis* in Korea

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ABSTRACT

We isolated endophytic fungal strains from twigs of *Larix gmelinii* var. *olgensis*. These strains were identified based on their morphological characteristics and phylogenetic analyses including internal transcribed spacers and large subunit rDNA. We confirmed the presence of a previously unrecorded fungal species in Korea, *Aequabiliella effusa*. This study presents the morphological characteristics and phylogenetic analysis of *A. effusa*.

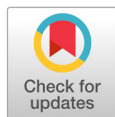
Keywords: *Aequabiliella effusa*, Endophytic fungi, Phylogenetic analysis

Endophytic fungi form symbiotic relationships within plant tissues, causing no negative symptoms or diseases [1]. Approximately 84% of endophytic fungi belong to the phylum Ascomycota and 10% belong to the phylum Basidiomycota [2]. Although the specific mechanisms by which these fungi confer benefits to plants remain largely unknown, their documented roles include providing protection against pathogens and herbivores and improving resistance to abiotic stresses, such as drought, high salinity, and temperature variations. This highlights their potential application in sustainable agriculture [3]. The diverse secondary metabolites and enzymes produced by endophytic fungi have high industrial value, particularly in the field of natural pharmaceutical development and discovery [4-6]. Despite their importance, the diversity of endophytic fungi remains underexplored in many regions. This knowledge gap hinders our understanding of the full spectrum of fungal diversity and their potential applications. Therefore, studying previously unrecorded endophytic fungal species is vital to expand our understanding of their roles in ecosystems and their potential use in various industries.

In Korea, studies on endophytic fungi are becoming increasingly comprehensive, with an expanding focus on their taxonomy and ecological interactions [7]. This study aimed to describe the morphological features and perform a phylogenetic analysis of the previously unrecorded endophytic fungus *Aequabiliella effusa* in Korea, which was discovered during the isolation of endophytes from *Larix gmelinii* var. *olgensis*.

In June 2023, the twigs of *L. gmelinii* var. *olgensis* were obtained from Danyang, Chungcheongbuk-do, Korea. Healthy plant tissue samples without any symptoms of disease were collected and transported to the laboratory within 24 h in polyethylene bags for fungal isolation.

The samples were washed with distilled water to remove debris and subjected to surface sterilization



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with 30% H₂O₂ for 90 s. A secondary surface sterilization was performed using 70% ethanol for 30 s. After sterilization, the samples were cut into 1.5 cm pieces and placed on potato dextrose agar (PDA; Difco Lab., Detroit, USA) for cultivation. Following the observation of fungal growth in the interior of the plant samples, subculturing was conducted to isolate pure fungal strains.

For the observation of colony morphology and fungal microstructures, the fungal cultures were incubated on potato dextrose agar and malt extract agar (MEA; Kisan bio, Seoul, Korea) at 25°C in the dark for 7 days. The microstructures were observed under a light microscope (Axio Imager A2, Carl ZWISS, Oberkochen, Germany).

DNA was extracted from the mycelia using the HiGene Genomic DNA Prep Kit (BioFACT, Daejeon, Korea) according to the manufacturer's instructions. For phylogenetic analysis, two DNA regions were amplified using Polymerase Chain Reaction (PCR): the internal transcribed spacer (ITS) using ITS1F/ITS4 primers [8,9] and the large subunit ribosomal DNA (LSU) region using LROR/LR16 primers [10].

The PCR conditions for the ITS and LSU regions started with an initial DNA denaturation at 95°C for 2 minutes, followed by 35 cycles of 95°C for 20 seconds, 50°C (for ITS) or 44°C (for LSU) for 40 seconds, and 72°C for 1 minutes, with a final DNA extension step at 72°C for 5 minutes.

The PCR products were electrophoresed on 1.5% agarose gels for 20 min to verify DNA amplification by assessing the size of the DNA fragments. Sanger sequencing was performed by SolGent Co., Ltd. (Daejeon, Korea). The DNA sequences of the isolates were compared with those in the GenBank database of the National Center for Biotechnology Information (NCBI) using the Basic Local Alignment Search Tool (BLAST). The DNA sequences of each isolate were concatenated, and a neighbor-joining phylogenetic tree was constructed using MEGA7 software with 1,000 bootstrap replications [11]. The strains were deposited at the National Institute of Biological Resources (NIBR) and the DNA sequences were registered in the NCBI database.

Phylogenetic analysis was performed using DNA sequences from the ITS and LSU regions. The ITS sequence of strain KNUE 23P121 showed 99.44% similarity with *A. effusa* CBS 120883 (NR_132005), and the LSU sequence was 99.4% identical to *A. effusa* CBS 120883 (NR_056966). Strain KNUE 23P121 formed a cluster with *A. effusa* CBS 120883 in a neighbor-joining phylogenetic tree constructed from the combined ITS and LSU sequences (Fig. 1).

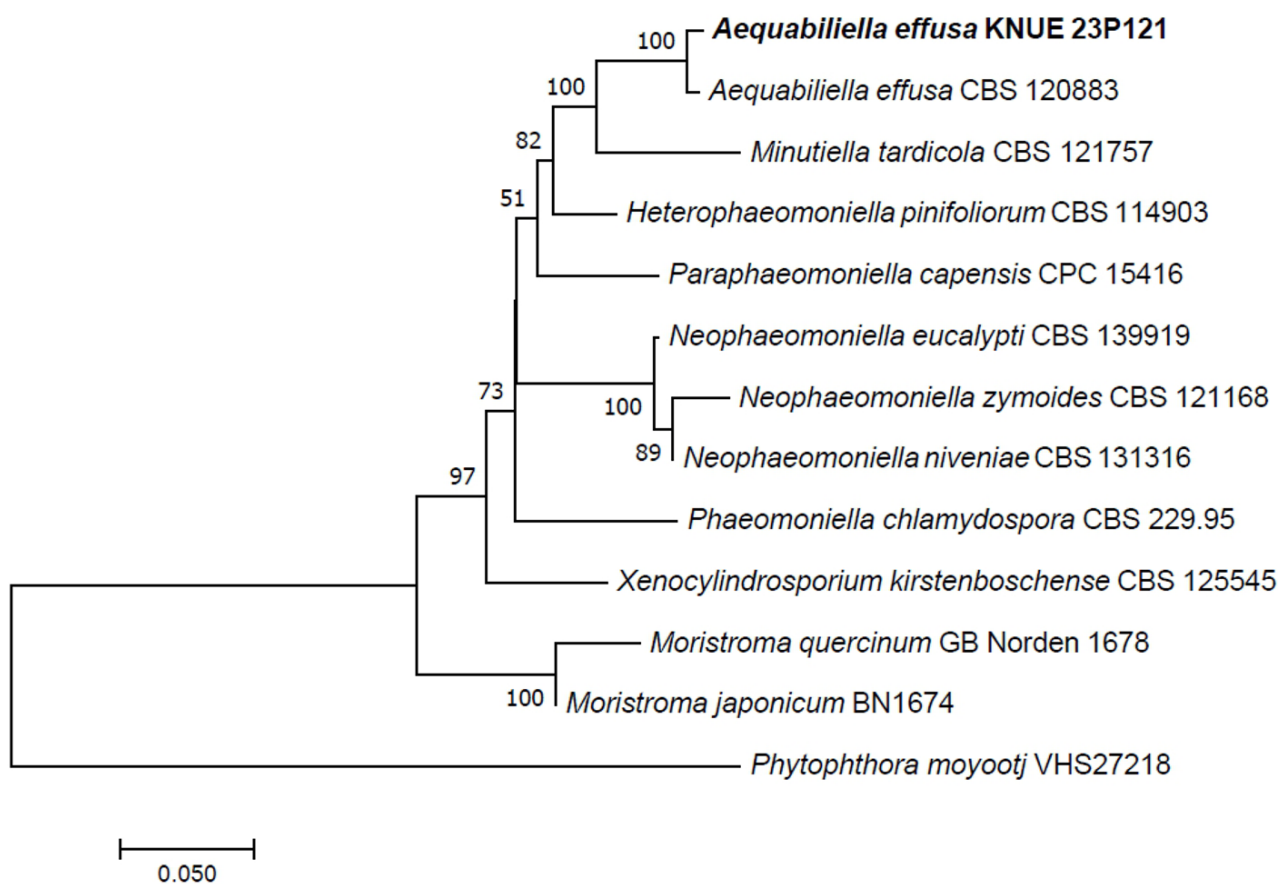


Fig. 1. Neighbor-joining phylogenetic tree of *Aequabiliella effusa* KNUE 23P121 based on concatenated sequences of internal transcribed spacer (ITS) and large subunit ribosomal DNA (LSU) regions. *Phytophthora moyootj* was used as an outgroup. The numbers on the nodes represent bootstrap values greater than 50% (1,000 replicates).

Aequabiliella effusa (Damm & Crous) Crous, Persoonia 34: 225 (2015) [MB#812476]

On PDA, the colony morphology appeared circular, with flat elevation and entire margin, displaying moist and mucilaginous texture. The colony diameter was 19.5 mm, with an ivory color (Hex code: E1E4D1) and beige color (Hex code: E3E2C6) on the reverse side (Fig. 2A). On MEA, the colony morphology remained circular with flat elevation, entire margin and moist texture. The colony diameter was 19 mm, with both the surface and reverse sides presenting an ivory color (Hex code: DEE4D7) (Fig. 2B). The mycelia were hyaline with septate and smooth cell walls. The conidia were cylindrical, with both ends rounded and slightly curved. The conidial walls were smooth without septa. The dimensions of the conidia ranged from (2.42-) 2.97-3.26 (-4.00) \times (1.10-) 1.26-1.38 (-1.66) μm (Fig. 2C and D).

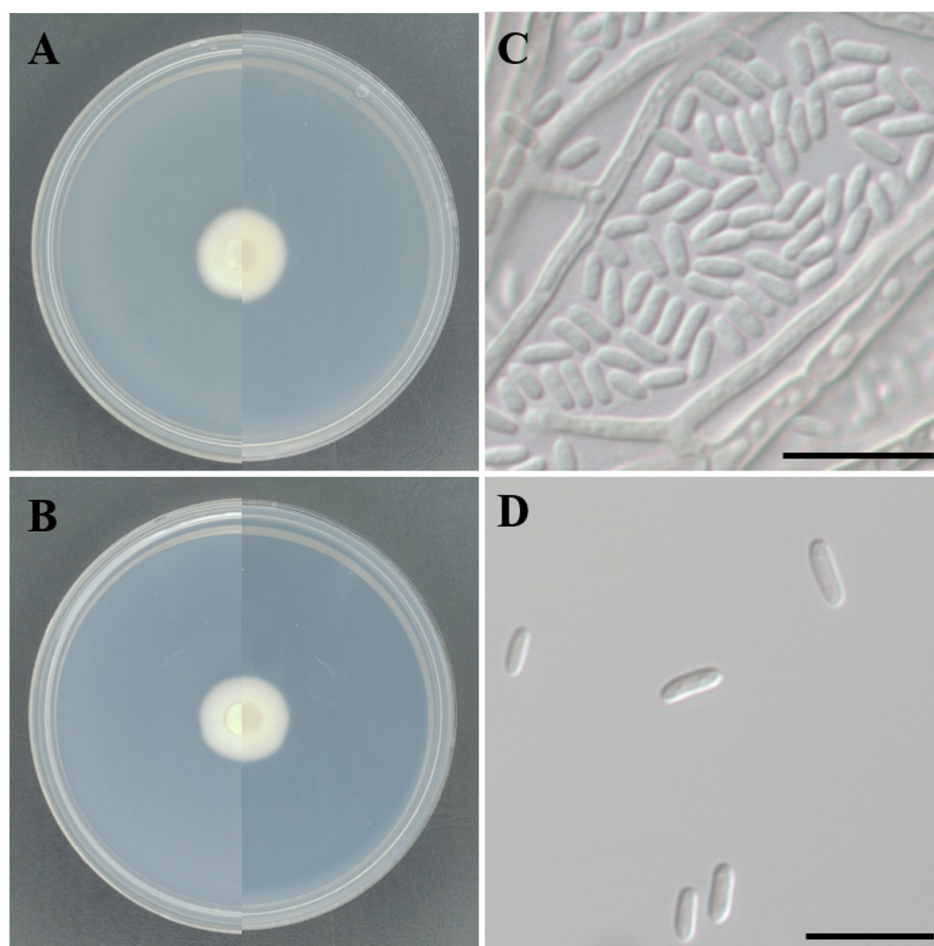


Fig. 2. Morphology of *Aequabiliella effusa* KNUE 23P121. A: A colony on potato dextrose agar (PDA), left side is front side and right side is reverse side after 7 days at 25°C, B: A colony on malt extract agar (MEA), left side is front side and right side is reverse side after 7 days at 25°C, C, D: Conidia. Scale bars: 10 µm.

Specimen examined: Danyang, Chungcheongbuk-do, Korea, 37°00'02.9"N 128°16'15.6"E, June 2, 2023, *Aequabiliella effusa*, isolated from twig of *Larix gmelinii* var. *olgensis*, KNUE 23P121, and NIBRFGC000510708; GenBank No. PP217727 (ITS) and PP196669 (LSU).

Notes: *Aequabilella effusa*, initially reported as *Phaeomoniella effusa* in 2010, was reclassified in 2015 [12,13]. The first isolation was from the necrotic parts of peach trees and was later reported in branches of *Pinus sylvestris* in Spain [12,14]. The colonies of KNUE 23P121 showed slight differences in color compared to the original description; KNUE 23P121 was ivory and beige, whereas CBS 120883 was herbage-green and olivaceous. However, the shape and size of the conidia are consistent with those in the original description (Table 1).

In the present study, endophytic fungal strains isolated from the twigs of *L. gmelinii* var. *olgensis* were isolated and identified based on their morphological and molecular characteristics. The strain KNUE 23P121 was identified as *A. effusa*. To the best of our knowledge, these fungal species were not reported previously in Korea. In this study, we aimed to expand our knowledge of fungal diversity in Korea.

Table 1. Morphological characteristics of strains KNUE 23P121 and CBS 120883 of *Aequabiliella effusa*

Characteristics	<i>Aequabiliella effusa</i> KNUE 23P121	<i>Aequabiliella effusa</i> CBS 120883 [14]
Colony	PDA, MEA, 25°C, 7 days	PDA, MEA, 25°C, 2 weeks
Color	PDA, front ivory, reverse beige MEA, ivory	PDA, herbage-green, dark herbage-green to olivaceous, white at the margin and sometimes in the centre MEA, olivaceous-gray to pale olivaceous-grey
Size	PDA, 19.5 mm diam MEA, 19 mm diam	PDA, no observation MEA, 32 mm diam
Shape	PDA, circular, flat, entire margin, moist MEA, circular, flat, entire margin, moist	PDA, flat, moist, with sparse aerial mycelium in the centre and undulate to lobate margin MEA, flat, moist, with radial growth rings, very little villose, olivaceous-grey aerial mycelium, with entire margin
Conidia	No septate, cylindrical, sometimes slightly curved, both ends obtuse, smooth-walled, (2.42-) 2.97-3.26 (-4.00) × (1.10-) 1.26-1.38 (-1.66) µm	Hyaline, 1-celled, cylindrical to obovate, sometimes slightly curved, both ends obtuse, smooth-walled, containing small droplets, (2-) 2.5-3.5 (-4.5) × 1-1.5 µm mean±SD=3.0±0.6×1.2±0.15 µm

PDA: potato dextrose agar; MEA: malt extract agar.

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