

## RESEARCH NOTE

# One Unrecorded Endophytic Fungi from Sub-alpine Conifer, *Rhizosphaera pini*

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## ABSTRACT

An endophytic fungus, *Rhizosphaera pini* strain NIE7426, was isolated from the sub-alpine coniferous tree *Abies nephrolepis* in Mt. Nochu of Gangwon Province. It was characterized by macroscopic and microscopic features, as well as the internal transcribed spacer (ITS) 1, 2 and 5.8S sequences. All morphological and molecular features support the first recognition of this taxon in Korea. In addition, this study adds *A. nephrolepis* as a host plant *R. pini*.

**Keywords:** *Abies nephrolepis*, Endophytic fungi, *Rhizosphaera pini*, Sub-alpine conifer

## INTRODUCTION

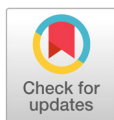
Endophytic fungi are defined as asymptomatic fungi that cause no obvious sign or damage on plants. They live in various tissues of host plant, such as leaves, barks, and stems [1]. Most of them belong to Ascomycota and Basidiomycota [2]. Many previous studies have focused on elucidating their biodiversity, and the number of their species is estimated to be in range of 0.1 to 10 million [3, 4].

*Abies nephrolepis* (Trautv.) Maxim. is a sub-alpine coniferous tree distributed from Russia to Korea [5]. This tree is a related species of *Abies koreana* E. H. Wilson, an endemic species of Korea. Both species are the representative trees of the decline of the sub-alpine forests in Korea over the last decade. Under these circumstances, we are trying to isolate and cultivate endophytic fungi from sub-alpine conifers. As a result, we found one unrecorded fungus in Korea and report it here.

## MATERIALS AND METHODS

### Isolation of foliar endophytic fungi

Needle leaves of *A. nephrolepis* were taken from the Mt. Nochu (N 37°32', E 128°45', 1,342 m) in Gangwon Province of Korea in 2018. The samples were transported in a zipper bag and fungi were isolated within 24 hours. All samples were washed with tap water and cut into 1 cm pieces. Surface sterilization was performed; they were immersed in 96% ethyl alcohol for 1 minute, sodium hypochlorite for 3 minutes, in



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96% ethyl alcohol for 30 seconds and then washed twice with sterilized water. Each sample was placed on potato dextrose agar (PDA, MBcell, Seoul, Korea) and incubated in the dark for 4 weeks at in 25°C to isolate endophytic fungi [6]. PDA and maltose extract agar (MEA, MBcell, Seoul, Korea) media were used for the pure culture of endophytic fungi. Macroscopic and microscopic features of the fungi were measured by light microscopy (DM2500, Leica Microsystems, Germany). Finally, this unrecorded endophytic fungus was deposited in the Korean Collection for Type Cultures (KCTC).

## DNA analysis of foliar endophytic fungi

Genomic DNA was extracted from the fungus using a plant tissues genomic DNA extraction kit (Xi'an Tianlong Science & Technology, Taiwan) as per the manufacturer's instructions. Polymerase Chain Reaction (PCR) was performed using primers ITS1F and ITS4 to amplify the internal transcribed spacer (ITS) 1, 2 and 5.8S ribosomal DNA regions [7]. The condition of PCR is like this. Pre-denaturing for 5 minutes at 94°C with 1 cycle, denaturing for 30 seconds at 94°C, annealing for 30 seconds at 50°C, extending for 1 minute at 72°C in 30 cycles, and then finally stabilizing for 10 minutes at 72°C in 1 cycle. The PCR product was confirmed by electrophoresis using 1.5% agarose gel [8]. DNA sequencing was applied to Solgent (Daejeon, Korea), and the analyzed DNA sequence was then identified based on similarity with the National Center for Biotechnology Information (NCBI, <http://www.ncbi.nlm.nih.gov/>) using the Basic Local Alignment Search Tool (BLAST). A neighbor-joining tree was generated by MEGA 7.0 [9] based on the Kimura-2 parameter distance model with the 1,000 times bootstrap method.

## RESULTS AND DISCUSSION

### *Rhizosphaera pini* (Corda) Maubl.

[MB#202128]

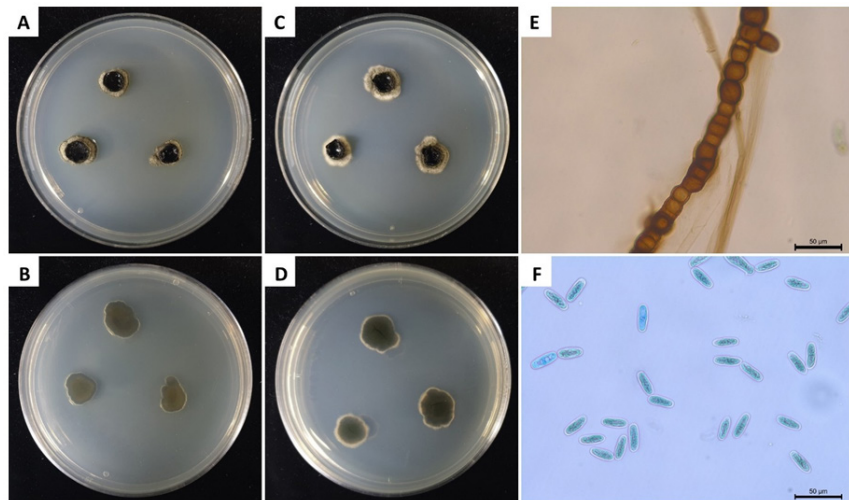
Diameter of colony is 2.1-6.5 mm on PDA and 2.6-6.6 mm on MEA after 7 days. Mycelium is rather dense and green-yellow colour in surface (Munsell color notation: 2.5GY 5/4) [10] with irregular margin, velvety texture, and no exudate. Spore are 9.7~11.3 × 26.7~29.7 μm, elliptical or cylindrical with rounded ends, hyaline and dyed well with lactophenol cotton blue, and have no spetum inside. The DNA sequence (ITS1, ITS2 and 5.8S) had 99% similarity (571/578 bp) with the reference sequence of *Rhizosphaera pini* (AY183365.1) (Fig. 1, 2).

**Specimen examined:** Mt. Nochu of Gangwon Province in Korea, collected September 4, 2018, isolated from the leaves of *A. nephrolepis*, strain NIE7426, KCTC no. 56431, GenBank no. MN037709.

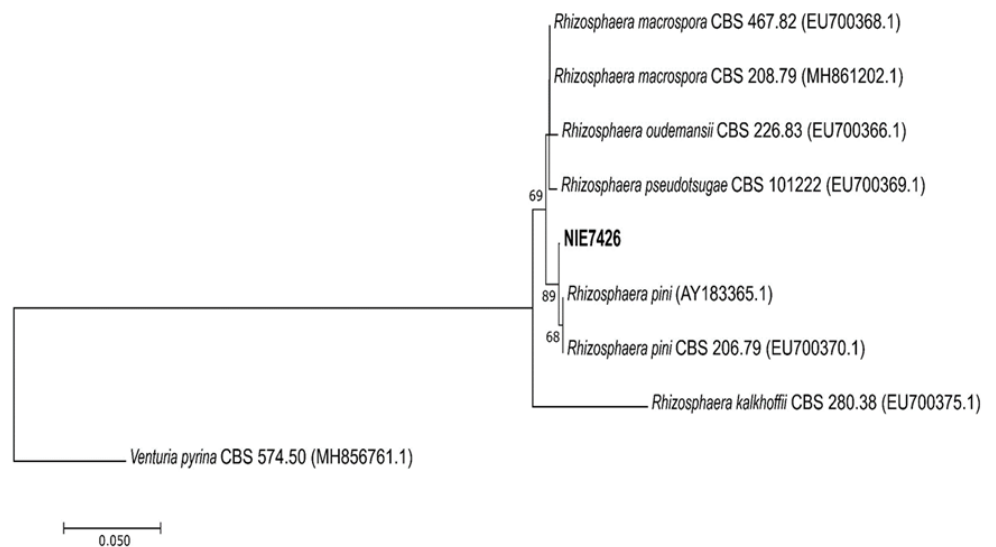
**Note:** Species of the genus *Rhizosphaera* can be distinguished by the size and shape of spores [10]. Until now, the distribution of *R. pini* has been reported in Europe, and North America, but only in Japan in Asia, but the present study confirmed for the first time that this fungus is distributed in Korea as well. Only conifer trees have been reported as the host plants of the genus *Rhizosphaera* [11, 12]. In this study, *A. nephrolepis* was also first reported as a host species.

## ACKNOWLEDGEMENT

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**Fig. 1.** Cultural and morphological characteristics of *Rhizosphaera pini* strain NIE7426 isolated from *Abies nephrolepis*. (A, B) Front and reverse sides of the colony on PDA. (C, D) Front and reverse sides of the colony on MEA. (E, F) Hyphae and spores. Scale bars: E & F = 50  $\mu$ m



**Fig. 2.** Phylogenetic tree of *Rhizosphaera* species, including *Rhizosphaera pini* strain NIE7426 isolated from *Abies nephrolepis* and the reference isolates of NCBI GenBank, based on ITS rDNA sequences. *Venturia pyrina* was used as an outgroup, and bootstrap values are shown at the branches (1,000 replicates).

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