RESEARCH NOTE

First Report of *Chlorocillium lepidopterorum* as a Pathogen of Cultivated *Cordyceps militaris* in Korea

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ABSTRACT

Cordyceps militaris is widely cultivated in East Asia as an edible and medicinal mushroom. Although fungal infections affecting *C. militaris* have been reported in several countries, no such outbreaks have been documented in South Korea. In the present study, we investigated an occurrence of fungal infection of *C. militaris* at a farm in Pyeongtaek, South Korea. Infected fruiting bodies were initially covered with white cottony mycelia that later developed into yellowish-green colonies. Two fungal strains were isolated from the infected fruiting bodies and identified as *Chlorocillium lepidopterorum* based on morphological characteristics and phylogenetic analysis using a multigene dataset (the large subunit of nuclear ribosomal RNA gene, internal transcribed spacer regions, translation elongation factor 1-alpha, and RNA polymerase II second largest subunit). Pathogenicity of the strains was confirmed by artificial inoculation. The symptoms of the strains were consistent with those observed in naturally infected fruiting bodies on farms. This is the first report of *C. militaris* infection caused by *C. lepidopterorum* in South Korea.

Keywords: *Chlorocillium lepidopterorum*, *Cordyceps militaris*, Mushroom diseases, Unrecorded species

pISSN : 0253-651X
eISSN : 2383-5249

Kor. J. Mycol. 2025 June, 53(2):121-125
https://doi.org/10.4489/kjm.2025.53.2.7

Received: May 30, 2025
Revised: June 11, 2025
Accepted: June 11, 2025

Cordyceps militaris (L.) Fr., belonging to the order Hypocreales, is well known as an important edible and medicinal mushroom in China, Korea, and Japan [1]. *Cordyceps militaris* has been used as a health supplement and crude drug in East Asia due to various bioactive compounds such as cordycepin, polysaccharides, and ergosterol [2,3]. *Cordyceps militaris* has traditionally been harvested from the wild, making it extremely rare and difficult to obtain. However, it is currently cultivated in China, Japan, and Korea [3].

Fungal diseases have been reported during commercial production of *C. militaris*. The primary causative agents are, to date, *Calcarisporium cordycipiticola* in China [4] and *Lecanicillium coprophilum* in Vietnam [5]. Although *C. militaris* is continuously cultivated in Korea, no serious fungal disease outbreaks have been reported. However, recently, fungi that form white cottony colonies have been frequently observed

on the fruiting bodies of *C. militaris*, leading to substantial losses in both quality and yield. Accordingly, we investigated and characterized the infectious mycoparasite affecting *C. militaris* in South Korea.

Samples were collected from the infected fruiting bodies of *C. militaris* grown on sterilized oats at the farmOLIN, Pyeongtaek, on March 16, 2023, and April 23, 2023. The fruiting bodies initially displayed white mycelia, which gradually developed into yellowish-green cottony mycelial colonies (Figs. 1A and B). Infected fruiting bodies were transferred to potato dextrose agar (PDA; Difco, Becton Dickinson) plate and incubated at 25°C for 7 days. Fungal colonies grown from the inoculated samples were transferred to fresh PDA plates. The two isolated strains were preserved in a metabolically inactive state (20% glycerol at –80°C) at the Korea National University of Agriculture and Fisheries.

Fig. 1. Fungal pathogens on fruiting bodies of *Cordyceps militaris*. (A) Initial formation of white mycelium on the fruiting bodies. (B) Progression to yellowish-green mycelial colonies on the fruiting bodies. (C) Symptoms of artificial inoculation with *C. lepidopterorum* MF340.

Genomic DNA was extracted from the isolated strains using an AccuPrep Genomic DNA Extraction Kit (Bioneer, Daejeon, Korea). The PCR amplification was performed using the following primers: LROR and LR5 [6] for the large subunit of nuclear ribosomal RNA gene (LSU), ITS1F [7], and ITS4 [8] for the internal transcribed spacer (ITS) regions, 983F and 2218R [9] for translation elongation factor 1-alpha (*tef*-1α), and fRPB2-5F and fRPB2-7cR [10] for the RNA polymerase II second largest subunit (*RPB*2). The amplified PCR products were sequenced using the corresponding PCR primers at Bioneer (Daejeon, Korea). The obtained sequences were manually curated with MEGA5 [11] and submitted to GenBank (accession numbers: PV110822-PV110823 for LSU, PV110820-PV110821 for ITS, PV134892– PV134893 for *tef*-1α, and PV134894–PV134895 for *RPB*2). Multiple sequence alignments were performed using MAFFT v7 [12] with default parameters. Sequence similarity analysis for each strain was conducted across the three loci using MEGA5 [11]. A maximum likelihood phylogenetic tree was generated using RAxML [13] through the CIPRES web portal [14], employing the GTR + GAMMA substitution model with 1,000 bootstrap replicates to evaluate branch support. On the basis of the concatenated datasets (LSU, ITS, *tef*-1α and *RPB*2), MF340 (NIBRFGC000510859) and MF354 grouped with *C. lepidopterorum* SD05361 (type strain) and *C. lepidopterorum* SD05362 (bootstrap support = 93%; sequence similarity for LSU = 100%, ITS = 100%, and *tef*-1α = 99.9–100%) (Fig. 1). *Pseudometarhizium* has been reported as a new genus within Clavicipitaceae, two species have been identified—*Pseudometarhizium araneogenum* and *Pseudometarhizium lepidopterorum*—based on morphological, phylogenetic, and ecological characteristics.

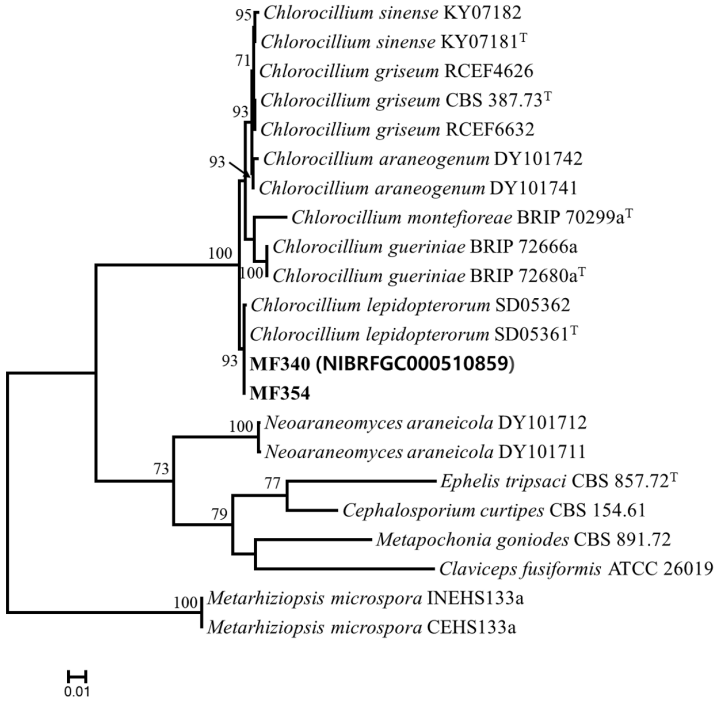

Fig. 2. Maximum likelihood phylogenetic tree based on the concatenated datasets (LSU, ITS, *tef*-1α, and *RPB*2) used to identify *Chlorocillium* strains from infected fruiting bodies of *Cordyceps militaris*. Bootstrap scores of >70 are presented at the nodes. The scale bar indicates the number of nucleotide substitutions per site. "T" indicates the ex-type strains. Strains reported in the current study are represented in bold.

P. araneogenum and *P. lepidopterorum* have been isolated from spiders and insects, respectively [15]. Recent phylogenetic analyses using multigene datasets (LSU, ITS, *tef*-1α, and *RPB*2) have indicated that the genus *Pseudometarhizium* is synonymous with *Chlorocillium*; thus, the two previously recognized species have been reclassified under *Chlorocillium* [16]. We follow this nomenclature in the present study and report, for the first time, *Chlorocillium lepidopterorum* infection of *C. militaris*.

In the pathogenicity test of *C. lepidopterorum* MF340, three healthy fruiting bodies of *C. militaris* from the farmOLIN were sprayed with 5 mL of conidial suspensions (1×10^5 conidia/mL) and then incubated at 25°C. Initially, white mycelia were observed on the fruiting bodies, and these mycelia eventually formed yellow-green mycelial colonies throughout the fruiting bodies (Fig. 1C). The fungus in the inoculated cultures was reisolated and identified as *C. lepidopterorum* based on sequence analysis.

The colony and microscopic features of *C. lepidopterorum* was observed on PDA after incubation for 14 d at 25°C. Color names and alphanumeric codes followed the Methuen Handbook of Color [17]. The microscopic features were examined under a light microscope (DM2000, Leica, Germany).

Chlorocillium lepidopterorum **(W.H. Chen, Y.F. Han, J.D. Liang & Z.Q. Liang) W.H. Chen, Y.F. Han & J.D. Liang, 2024**

Description: Colony diam, 14 d, in mm: PDA 20°C 15-16; PDA 25°C 16-18; PDA 30°C no growth.

Colony characteristics: white to orange-white (5A2), velvety to floccose, and reverse color greyish orange

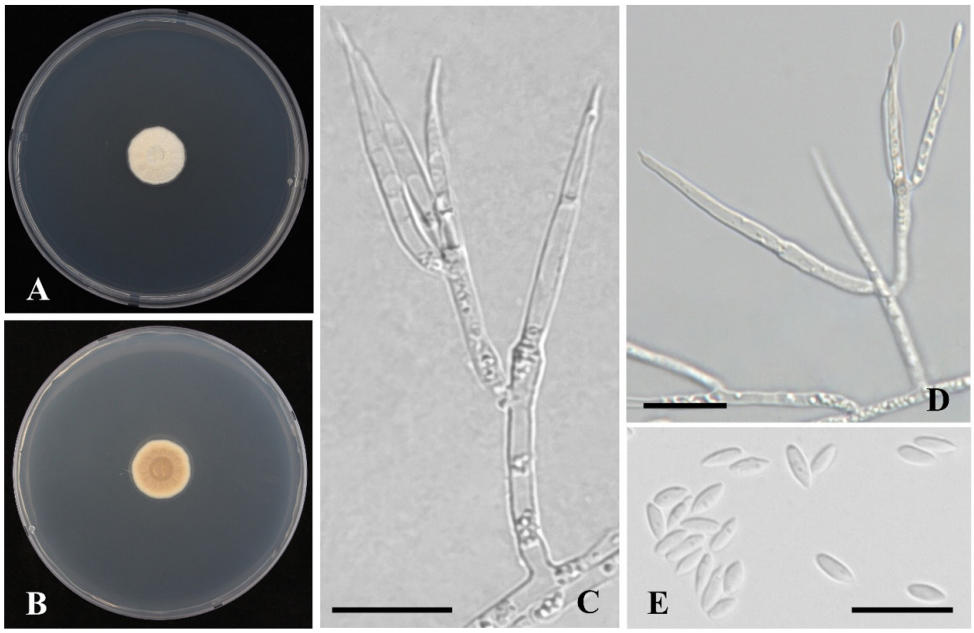

Fig. 3. *Chlorocillium lepidopterorum* MF340. A-B: Obverse (A) and reverse (B) of colony on potato dextrose agar (PDA) after incubation for 14 d at 25℃. C, D: Conidiophores; E: Conidia (scale bar: C–E = 10 μm).

(5B3). Hyphae hyaline, smooth, and septate (Fig. 3A, B). Conidiophores typically emerge from aerial hyphae. Phialides occur singly or in two to three groups, with a cylindrical basal that gradually narrows into a short, well-defined neck, 17.5–30.3 × 1.2–1.5 μm. Conidia, hyaline, fusiform, smooth-walled, 3.1–4.5 × 1.4–1.6 μm (Fig. 3C–E).

Strains examined: Jinwi-myeon, Pyeongtaek-si, Gyeonggi-do, Republic of Korea, isolated from the fruiting bodies of *C. militaris*; strains MF340 (NIBRFGC000510859) and MF354.

Note: *Chlorocillium lepidopterorum* is phylogenetically close to *C. araneogenum*, *C. griseum* and *C. lepidopterorum* within Clavicipitaceae. *Chlorocillium lepidopterorum* is easily distinguished from other species within Clavicipitaceae by size of phialide and conidia [16]. *Chlorocillium lepidopterorum* can be distinguished from *C. araneogenum* (8.3–23.3 × 1.3–2.2 μm) by slight larger phialides. *Chlorocillium lepidopterorum* produces smaller conidia than *C. griseum* (4.5–6 × 1.0–1.5 μm).

CONFLICT OF INTERESTS

No conflicts of interest were reported by the authors.

ACKNOWLEDGEMENT

This research was supported by grants from the National Institute of Biological Resources (NIBR) funded by the Ministry of Environment (MOE) of the Republic of Korea (NIBR202002104 and NIBR202511101).

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