RESEARCH ARTICLE

Isolation and Characterization of *Humicola phialophoroides* from Red Pepper in Korea

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ABSTRACT

The fungal strain KNUF-25-YP1 was isolated from the root collar of wilted red pepper in Gyeongbuk Province, Korea. The strain was characterized by the production of *Phialophora*-like conidiophores and *Humicola*-like aleuriospores. Its initial classification was based on its observable characteristics, including morphological and cultural characteristics. To confirm its identification and elucidate its evolutionary relationships, molecular phylogenetic analyses were conducted using the internal transcribed spacer (ITS) regions and partial sequences of the large subunit 28S rRNA (LSU) gene. The results revealed that the strain belongs to the genus *Humicola*. Its ITS regions and LSU gene exhibited 99.81% and 99.75% similarities, respectively, to *Humicola phialophoroides* CBS 125784[T]. Integrated analysis of morphological and phylogenetic data led to the identification of strain KNUF-25-YP1 as *Humicola phialophoroides.* To the best of our knowledge, this study represents the first report of *H. phialophoroides*, a previously unrecorded species in Korea, thereby enhancing our understanding of its taxonomy.

Keywords: *Humicola phialophoroides*, *Humicola*-like aleuriospores, Morphological characterization, *Phialophora*-like conidiophores, Phylogeny

OPEN ACCESS

pISSN : 0253-651X
eISSN : 2383-5249

Kor. J. Mycol. 2026 June, 54(2):155–163
https://doi.org/10.4489/kjm.2026.54.2.5

Received: April 16, 2026
Revised: June 02, 2026
Accepted: June 04, 2026

INTRODUCTION

Fungi, which are eukaryotic organisms, play a significant role in maintaining the balance of ecosystems and provide various ecological services, from nutrient cycling to symbiotic relationships with plants and animals [1]. The family Chaetomiaceae, which belongs to the phylum Ascomycota, substantially influences agriculture, ecosystems, biotechnology, and the health of living beings by producing a wide range of structurally diverse metabolites [2]. The genus *Humicola*, found in the family Chaetomiaceae, was first introduced by Traaen in 1914 for two species of *H. fuscoatra* and *H. grisea* [3]. Members of the genus *Humicola* possess diverse bioactive metabolites and produce thermostable enzymes, thereby exhibiting significant biotechnological and industrial potential [4–8]. More than 50 species of this genus have been described and most of these species are commonly found in soil, decomposing plant matter, caves, and indoor spaces [7,9]. Moreover, some species of this genus are mycoparasites that colonize and invade the

hyphae or survival structures, i.e., sclerotia, of other fungal species [4,6,7]. Although some species can control plant diseases caused by other fungal species, such as *Phytophthora capsici*, a few species can cause infections in humans [10–12]. Certain species belonging to this genus also act as biosorbents for heavy metals [13–15]. In Korea, studies on the genus *Humicola* remain limited, and only a few species, including *H. olivacea* and *H. alopallonella*, have previously been reported [16–18]. Members of this genus are known to inhabit diverse ecological niches, including soil, decaying plant materials, and plant-associated environments.

The discovery of previously unreported *Humicola* species not only expands our understanding of fungal diversity but also highlights the importance of continued exploration in varied ecosystems in South Korea. By defining an unreported species, our study contributes to the broader understanding of the taxonomy, ecology, and potential biotechnological applications of fungi. This study reports the cultural and morphological characteristics of a previously unreported species, providing detailed information regarding its importance within the genus.

MATERIALS AND METHODS

Sample collection and fungal isolation

The fungal strain KNUF-25-YP1 was isolated from internal tissues of the root collar of wilted red pepper plants collected from a red pepper field located in Yeongyang-gun, Gyeongbuk Province. Collected tissues were directly cultured on potato dextrose agar (PDA; Difco, Detroit, MI, USA) and incubated at 25°C for 7 days. The fungal strain KNUF-25-YP1 was selected for further molecular analyses, as well as cultural and morphological evaluations. The isolate was preserved at the Korean Agricultural Culture Collection under the accession number KACC 411310.

Cultural and morphological characterization

The isolate was cultured on PDA (Difco, Detroit, MI, USA), oatmeal agar (OA; Difco, Detroit, MI, USA), malt extract agar (MEA; Difco, Detroit, MI, USA), corn meal agar (CMA; Difco, Detroit, MI, USA), and potato carrot agar (PCA; HiMedia, Mumbai, India) for morphological and cultural characterization [9,10]. The cultures were maintained for 7 days at 25°C in the dark, and various morphological characteristics, such as size, color, mycelial shape, and details of the colony, including morphological features such as conidiophores, aleuriospores, chlamydospores, phialides, and conidia were observed. Morphological characteristics were examined under a light microscope (BX-50; Olympus, Tokyo, Japan).

Genomic DNA extraction, PCR amplification and sequencing

For molecular identification, total genomic DNA was extracted from the KNUF-25-YP1 strain using the HiGene™ Genomic DNA Prep Kit (Biofact, Daejeon, Korea), according to the manufacturer's

instructions. Phylogenetic analysis was conducted based on two non-coding regions, namely the internal transcribed spacer (ITS) regions and partial sequences of the large subunit 28S rRNA (LSU) gene, which were amplified using the primer pairs ITS1F/ITS4 and LR0R/LR5, respectively [19–21]. Successful amplification was confirmed through electrophoresis using 1.0% HP agarose gels (Biopure, Cambridge, MA, USA). The amplified products were purified utilizing ExoSAP-IT (Thermo Fisher Scientific, Waltham, MA, USA) and then submitted to Macrogen (Seoul, Korea) for sequencing.

Phylogenetic analyses

The acquired sequences were analyzed for similarity using the Basic Local Alignment Search Tool (BLAST) available in the National Center for Biotechnology Information (NCBI) database (Table 1). Phylogenetic trees were constructed based on the combined sequences of the ITS regions and LSU gene, using the maximum likelihood (ML) method in MEGA X [22]. Evolutionary distances for the ML analysis were calculated using the Tamura-Nei model, with bootstrap values derived from 1,000 replications [22].

Table 1. List of species and GenBank accession numbers of their sequences used for the phylogenetic analyses in this study

Species name	Strain	GenBank Accession Number	
		ITS	LSU
Humicola phialophoroides	**KNUF-25-YP1**	**PZ256383**	**PZ256400**
Humicola phialophoroides	CBS 125784^T	NR171742	MH875195
Humicola fuscoatra	MTCC 6329	EF550969	EU257376
Humicola floriformis	CBS 815.97^T	NR172533	MH862678
Humicola floriformis	PSSCE 126	PP851769	PP851769
Humicola ampulliella	IHEM 24695	OW987292	OW987292
Humicola ampulliella	CBS 116735^T	NR172956	LT993568
Humicola leptodermospora	CBS 120095^T	NR172960	LT993584
Humicola cuyabenoensis	CBS 398.97^T	NR160060	LT993573
Humicola degenerans	CBS 232.65^T	NR172958	LT993574
Humicola homopilata	CBS 157.55^T	NR168166	KM655364
Phialocephala lagerbergii	CBS 266.33	NR119426	MH866885

ITS: internal transcribed spacer regions; LSU: partial sequences of the large subunit 28S rRNA.
^{T}Type strain.
The strain used in this study is indicated in bold.

RESULTS

Humicola phialophoroides W.H. Ko, Ching H. Yang, Mei J. Lin, Chi Y. Chen, & Y.J. Tsou: 200 (2011) [MB#581623]

Cultural and morphological characteristics of strain KNUF-25-YP1

Five different media were used to culture fungal colonies at 25°C for 7 days. Colonies on PDA measured 45–47 mm in diameter, and the observed side was an effused circular colony with an entire margin, a

cottony texture, and abundant white-colored aerial hyphae. The reverse side of the colony was initially light yellow and gradually became light brown with irradiation (Fig. 1A). Colonies on OA measured 58–60 mm in diameter and exhibited a circular form with a slightly filamentous, even margin. White-colored aerial hyphae with a cottony texture were observed on the upper side, and an opaque appearance was observed on the reverse side (Fig. 1B). The colonies on CMA measured 49–51 mm in diameter and appeared circular and flat with an entire margin. The colonies had translucent opacity with surface hyphae and exhibited white-colored mycelia throughout the media and yellowish white-colored mycelia at the radius. Brownish yellow-colored mycelia appeared around the central inoculation point on the reverse side (Fig. 1C). On MEA, the colonies measured 34–36 mm in diameter and had a slightly filamentous, flat, irregular margin. White-colored surface hyphae were observed on the upper side, while the reverse side of the colony was pale yellow (Fig. 1D). On PCA, the colonies measured approximately 55–57 mm in diameter. The colonies were circular, had a smooth margin, and were slightly filamentous at the edge. A cottony texture with abundant white aerial hyphae was noted on the front side. The color on the reverse side was initially pale yellow and later became deep yellow to light orange, with even coloration extending to the radius of the colony (Fig. 1E). Dichotomously branched conidiophores with a pigmented basal portion measured 22–87 μm in length (n = 30) from the basal cell to the top of the phialides (Fig. 1F). Phialides were terminal or lateral and measured 8.4–13.9 μm in length; they were 2.4–3.5 μm broad at the base and 0.8–1.4 μm broad at the apical collarette (n = 30; Fig. 1K and L). Conidia were hyaline, unicellular, and spherical; they measured 1.6–2.6 μm in diameter (n = 50; Fig. 1H). Some conidia were arranged along with hyphae and accumulated as a ball-like mass at the tip of the phialides (Fig. 1M). Aleuriospores were spherical, unicellular, 6.7–10.2 μm in diameter (n = 40), and pigmented with light brown to dark brown color (Fig. 1G). Intercalary chlamydospores were oblong or obovate, measuring 9.2–11.8 × 4.8–5.5 μm (length × width, L × W) (Fig. 1I and J).

Note: Comparing the strain KNUF-25-YP1 and *Humicola phialophoroides* CBS 125784^{T}, both strains exhibit similar cultural characteristics on PDA media. Both strains produced colonies with entire margins and a floccose to cottony texture. In addition, the mycelial growth of KNUF-25-YP1 was larger than that of *H. fuscoatra* on OA, CMA, MEA, and PCA media. For the morphological characteristics, KNUF-25-YP1 exhibited hyaline, spherical conidia, dichotomously branched conidiophores with pigmented basal portions, phialides with apical collarettes, and intercalary chlamydospores, which were comparable to those of the type strain (Table 2).

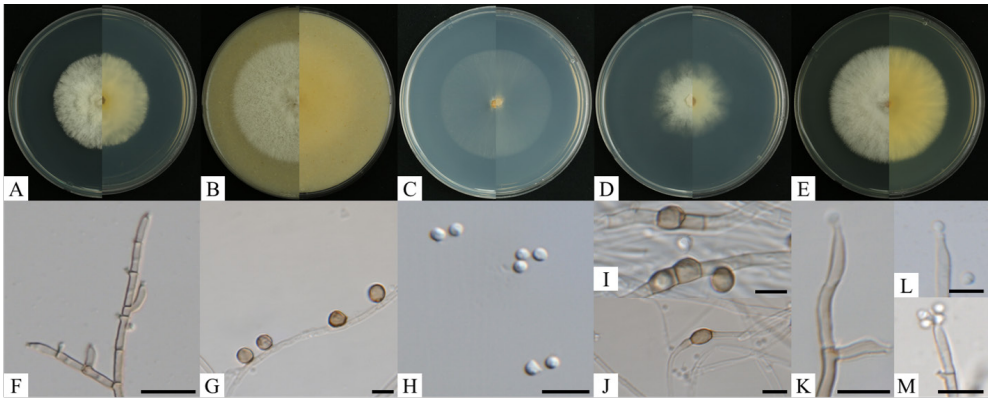

Fig. 1. Cultural and morphological characteristics of *Humicola phialophoroides* KNUF-25-YP1. A–E: obverse and reverse sides of the colony at 25°C after 7 days on PDA, OA, CMA, MEA, and PCA, respectively; F: Dichotomously branched conidiophore; G: Aleuriospores; H: Conidia; I,J: Chlamydospores; K,L: Phialides; M: Mass of conidia arranged on the tip of the phialide. Scale bars = 10 μm.

Table 2. Comparison of cultural and morphological characteristics of *Humicola phialophoroides* KNUF-25-YP1 with those of reference strains

Characteristic	*Humicola phialophoroides* KNUF-25-YP1[a]	*Humicola phialophoroides* CBS 125784[Tb]	*Humicola fuscoatra* CGMCC 3.13428[c]
Colony on PDA	45–47 mm at 25°C for 7 days; Entire margin, floccose to cottony; reverse light yellow to light brown	7 mm/day at 32°C; White with abundant aerial hyphae	N/A
Colony on OA	58–60 mm at 25°C for 7 days; Even margin, cottony, floccose; reverse opaque	N/A	29–35 mm; Entire, olivaceous; reverse pale olivaceous gray
Colony on CMA	49–51 mm at 25°C for 7 days; Flat, entire margin, white to yellowish; reverse brownish yellow	N/A	24–30 mm; Slightly crenate; reverse pale olivaceous gray with soluble pigment
Colony on MEA	34–36 mm at 25°C for 7 days; Flat, irregular to slightly filamentous margin; reverse pale yellow	N/A	18–24 mm; Entire to slightly lobate, grayish sepia to fuscous black; reverse grayish sepia
Colony on PCA	55–57 mm at 25°C for 7 days; Margin smooth to slightly filamentous, floccose; reverse pale yellow	N/A	17–23 mm; Entire, grayish sepia to olivaceous at the center; reverse similar
Conidia	1.6–2.6 μm; Hyaline, spherical	1.8–2.6 μm; Hyaline, spherical, clustered mass at the phialide apex	N/A
Conidiophore	22–87 μm; Dichotomously branched, pigmented at the base	31–72 μm; Dichotomously branched, pigmented at the base	N/A
Aleuriospore	6.7–10.2 μm; Light brown to dark brown, spherical, unicellular	8.8–12.5 μm; Dark colored, spherical, unicellular	6.5–9 × 7–9 μm; Olivaceous brown, globose, subglobose to oblate
Phialide	8.4–13.9 μm; Terminal or lateral, apical collarette present	9.5–15.5 μm; Lageniform, attenuate sursum	N/A
Chlamydospore	9.2–11.8 × 4.8–5.5 μm (L × W); Intercalary, oblong to obovate	9.0–12.1 × 5.6 μm (L × W); Intercalary, oblong to obovate, dark colored	N/A

PDA: potato dextrose agar; OA: oatmeal agar; CMA: corn meal agar; MEA: malt extract agar; PCA: potato carrot agar; L: length; W: width.
[a]Fungal strain used in this study; [b]Source of description [10]; [c]Source of description [9]; [T]Type strain; N/A: Not available.

Molecular phylogeny of strain KNUF-25-YP1

For the molecular identification of the isolated fungal strain, total genomic DNA was amplified to obtain sequences for the ITS regions and LSU gene, yielding lengths of 558 and 795 bp, respectively. The ITS regions exhibited 99.81% similarity to *H. phialophoroides* CBS 125784^{T}, whereas the LSU exhibited 99.75% similarity to *H. phialophoroides*. *H. fuscoatra* and *H. homopilata* exhibited 94.66% and 93.91% similarities, respectively, to the combined sequence of the ITS regions and LSU gene, indicating their close relationship to *H. phialophoroides*. Thus, they were closely related to strain KNUF-25-YP1. A phylogenetic tree was constructed using the ML method based on the concatenated sequence of the ITS regions and LSU gene (Fig. 2).

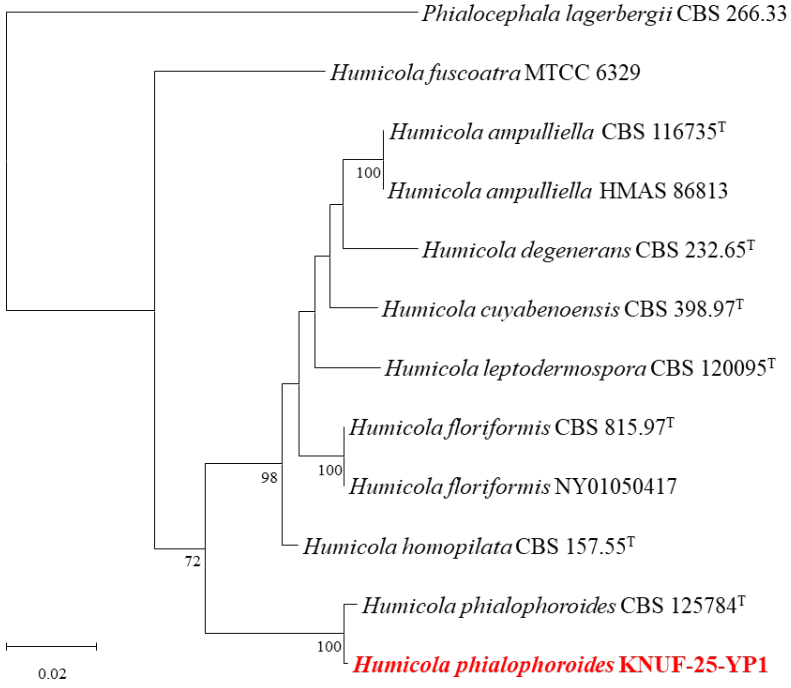

Fig. 2. Maximum likelihood tree based on combined dataset of partial sequences, internal transcribed spacer (ITS) regions, and partial sequences of the large subunit 28S rRNA (LSU) gene, illustrating the phylogenetic placement of the strain KNUF-25-YP1 within the genus *Humicola*. Bootstrap values greater than 70% (based on 1,000 replications) are shown at branching points. The strain isolated in this study is indicated in bold and red. The tree was rooted using *Phialocephala lagerbergii* CBS 266.33 as an outgroup. Scale bar = 0.02 substitutions per nucleotide position.

DISCUSSION

This study provides the first morphological and phylogenetic evidence of *H. phialophoroides* in Korea. It was first described as a hyphomycete fungus that could produce pigmented, thick-walled, single-celled spores that appeared terminally or laterally on hyphae or on minimally differentiated conidiophores [9]. A comprehensive study between some *Chaetomium* species and *Humicola* species was conducted based on phylogenetic analyses by Wang et al. [9]. *Humicola*, previously known as an asexual genus in the family

Chaetomiaceae, has been recently redefined to be capable of producing not only asexual spores, conidia, and aleuriospores but also sexual spores [9]. *Humicola* species exhibit biotechnological and industrial potential and are a thriving source of distinct and structurally diversified metabolites with a wide range of bioactivities [4]. Cerebrosides, terpenoids, xanthones, xanthoquinones, depsides, and benzenediol lactones are the compounds that have been detected in *Humicola* species. These compounds exhibit significant bioactivities, including antioxidant, antimalarial, anticancer, antimicrobial, antidiabetic, antiallergic, anticoagulant, insecticidal, anticoccidial, antihuman immunodeficiency virus (anti-HIV), and diacylglycerol acyltransferase inhibitory activities [4,5]. Moreover, *Humicola* species can produce thermostable enzymes, such as cellulases, lipases, xylanases, feruloyl esterases, pectate lyases, and beta-xylosidases, which have biotechnological and industrial importance [4,8]. Some *Humicola* species can also cause allergic reactions. For instance, *H. pulvericola* and *H. sardiniae* have been reported to cause fungal keratitis in the human eye [11,12]. In one study, *H. fuscoatra*, isolated from a sediment sample, was found to produce secondary metabolites that reactivated latent HIV type-1 (HIV-1) expression in an in vitro model [23]. Some species within the genus *Humicola* produce silver nanoparticles on reacting with aqueous silver nitrate solutions, resulting in the generation of extracellular nanoparticles with good dispersity and stability [24].

In the early stage of growth, *H. phialophoroides* forms branched *Phialophora*-like conidiophores bearing phialides with saucer-shaped tops. In the later stage, it produces *Humicola*-like aleuriospores [10]. Although several features of this species are similar to those of species belonging to the genera *Phialophora* and *Humicola*, their key fungal characteristics differ. *H. phialophoroides* is phylogenetically closer to the genus *Humicola* than to the genus *Phialophora* [10,25–28]. On preheating with sodium hydroxide, *H. phialophoroides* has been found to exhibit the greatest biosorption capacity for zinc and chromium [14,15]. So far, its pathogenicity in humans or plants has not been reported. Rather, it has been identified to have beneficial effects on the environment, including the potential suppression of fungal plant diseases, such as Phytophthora blight of pepper and leaf spot of spoon cabbage [10,13]. This fungus has a distinct ability to produce substances that can induce resistance against *P. capsici* by inhibiting zoospore germination or germ tube growth, with the active substances retained in the mycelium [13].

To the best of our knowledge, this is the first report of *H. phialophoroides* in Korea. In addition, although strain KNUF-25-YP1 was isolated from wilted red pepper plants, further studies are required to clarify its ecological role, including whether the species is saprophytic, endophytic, or associated with plant disease symptoms. The identification of this previously unreported species will contribute to the understanding and conservation of domestic biodiversity.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

This work was carried out with the support of "Cooperative Research Program for Agriculture Science & Technology Development (Project No. RS-2025-02183115)" Rural Development Administration, Republic of Korea.

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