

RESEARCH ARTICLE

First Report of *Chrysosporium articulatum* Isolated from Soil in Korea

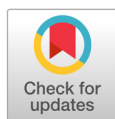
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ABSTRACT

As part of a fungal taxonomic study in Korea, strain KNU-YC-1803F was isolated from soil collected in Gogyeong-myeon, Yeongcheon-si, Gyeongsangbuk-do. When cultivated on potato dextrose agar and Sabouraud dextrose agar, the colonies appeared fluffy white with a pale yellow reverse. Microscopic examination revealed the presence of aleurioconidia that were hyaline, clavate to elongated-pyriform, measuring approximately $9.2 \times 6.1 \mu\text{m}$. Additionally, arthroconidia were abundantly formed in chains, cylindrical to navicular in shape, and measured around $7.6 \times 5.7 \mu\text{m}$. Sequence analysis of the internal transcribed spacer (ITS) region showed 100% similarity with *Trichophyton verrucosum* and 99.8% similarity with *Chrysosporium articulatum* and *Aphanoascus reticulisporus*. Complementary analysis of the large subunit (LSU) of the nuclear ribosomal DNA revealed high similarity to several related taxa. When trimmed for phylogenetic analysis, the LSU sequence exhibited 100% similarity to multiple species, including *C. articulatum*. The phylogenetic tree, constructed from concatenated ITS and LSU sequences, revealed that the isolate clustered within a clade containing *C. articulatum*, distinctly separated from other closely related taxa. Taken together, the morphological characteristics and molecular phylogenetic evidence confirm that strain KNU-YC-1803F is *C. articulatum*, marking the first documented occurrence of this species in Korea.

Keywords: *Chrysosporium articulatum*, Molecular phylogeny, Morphological characterization, Phylogenetic analysis, Soil-derived fungi



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INTRODUCTION

The family Onygenaceae (Ascomycota) is characterized by hyaline mycelia and the formation of arthrospores and aleuriospores, typically producing hyaline conidia. Members of this family are frequently isolated from soil, plant debris, and keratin-rich substrates and include genera such as *Chrysosporium*, *Aphanoascus*, and *Onygena* [1].

The genus *Chrysosporium*, first described by Corda in 1833 [2], currently encompasses approximately 100 recognized species worldwide [3]. Species of this genus typically form white to pale colonies and produce simple conidia that are subglobose, pyriform, or clavate in shape [4]. However, morphological

similarities among Onygenaceae species often complicate accurate identification. To address this issue, molecular phylogenetic analyses—particularly those based on internal transcribed spacer (ITS) sequences—are now widely employed alongside traditional morphological observations [5]. Consequently, several species originally classified under *Chrysosporium* have been reallocated to related genera such as *Aphanoascus* or *Keratinophyton* [6].

Chrysosporium species are widely distributed across diverse environments, including terrestrial soils, animal-associated habitats, and marine ecosystems [7]. They are often recovered from keratin-rich materials such as feathers, hair, and skin, reflecting their ability to secrete keratin-degrading enzymes and other secondary metabolites [3,8,9]. These properties facilitate the degradation of keratinous waste, promote nutrient recycling in ecosystems, and offer considerable potential for industrial applications. In Korea, several *Chrysosporium* species have recently been reported, most of which were isolated from soil [4,10], while others were recovered from freshwater sediment [11].

During a survey of fungal diversity in Korean soils, strain KNU-YC-1803F was isolated. This study presents the first record of this species in Korea and aims to determine its precise taxonomic placement. To achieve reliable identification, detailed morphological characterization and molecular phylogenetic analyses were conducted.

MATERIALS AND METHODS

Sample collection and fungal isolation

The fungal strain described in this study was isolated from a soil sample collected in Gogyeong-myeon, Yeongcheon-si, Gyeongsangbuk-do, Republic of Korea (35°57'56.39"N, 128°59'27.53"E). Isolation was performed using the serial dilution method. A 100 µL aliquot of the soil suspension was spread onto potato dextrose agar (PDA; Difco, Detroit, MI, USA) and incubated at 25°C under dark conditions for two weeks [12]. Subsequently, distinct single colonies were selected and transferred to fresh PDA plates for purification. Preliminary identification was conducted based on morphological characteristics, genomic DNA extraction, PCR amplification, and sequencing of the ITS regions. The isolate, designated KNU-YC-1803F, was tentatively identified as an unrecorded species in Korea. Strain KNU-YC-1803F (NIBRFGC000502236) has been deposited in a metabolically inactive state at the National Institute of Biological Resources (NIBR), Republic of Korea.

Cultural and morphological characterization

Strain KNU-YC-1803F was cultured on PDA and Sabouraud dextrose agar (SDA; MCell, Seoul, Korea) at 25°C under dark conditions. To observe colony morphology, the strain was incubated for four weeks. Colony features, including color, texture, and growth pattern, were documented using a Canon EOS 5D Mark III digital camera (Canon, Tokyo, Japan). To observe microscopic morphological features such

as spore shape and size, the strain was cultured on a glass slide for 1–2 weeks and examined using a light microscope (BX-50, Olympus, Tokyo, Japan).

DNA extraction, PCR, and sequencing

Genomic DNA of strain KNU-YC-1803F was extracted using the HiGene Genomic DNA Prep Kit (BIOFACT, Daejeon, South Korea), following the manufacturer's instructions. The ITS regions were amplified using primers ITS1F and ITS4 [13], while the large subunit (*LSU*) of the nuclear ribosomal RNA gene was amplified using the primers LR0R and LR7 [14]. PCR products were purified using the ExoSAP-IT reagent (Thermo Fisher Scientific, Waltham, MA, USA) and sequenced by Macrogen (Seoul, South Korea). Sequences of the ITS region and *LSU* gene were deposited in GenBank under accession numbers LC880189 and LC880190, respectively.

Phylogenetic analysis

For molecular phylogenetic analysis, the sequences of strain KNU-YC-1803F were compared with reference sequences available in the GenBank database of the National Center for Biotechnology Information (NCBI) using the Basic Local Alignment Search Tool (BLAST). Closely related sequences were retrieved from GenBank for further analysis (Table 1). Multiple sequence alignment was performed using ClustalX version 2.0 (European Bioinformatics Institute, Hinxton, United Kingdom). Phylogenetic analyses were conducted using MEGA version 7.0 (Institute of Molecular Evolutionary Genetics, Pennsylvania State University, University Park, PA, USA) [15]. A concatenated dataset of ITS and *LSU* sequences was used to construct the phylogenetic tree. The tree was inferred using the maximum likelihood (ML) method with 1,000 bootstrap replicates. Genetic distances between taxa were calculated using Kimura's two-parameter model [16].

Table 1. GenBank accession numbers of sequences used for phylogenetic analyses in this study

Species name	Strain	GenBank accession numbers	
		ITS	<i>LSU</i>
<i>Chrysosporium articulatum</i>	FMR 19012	ON720201	ON720740
<i>Chrysosporium articulatum</i>	KNU-YC-1803F	LC880189	LC880190
<i>Chrysosporium fluviale</i>	FMR 6005 ^T	AJ005367	MT875000
<i>Chrysosporium indicum</i>	CBS 117.63 ^T	MH858234	MH869835
<i>Chrysosporium irregularum</i>	GZAC.J1.1	MW488042	MW485693
<i>Chrysosporium keratinophilum</i>	CBS 104.62 ^T	MH858113	MH869689
<i>Chrysosporium longisporum</i>	UTHSC R-4380 ^T	HF547873	HF547858
<i>Chrysosporium lucknowense</i>	CBS 143.77 ^T	MH861041	NG_063936
<i>Chrysosporium mephiticum</i>	CBS 320.86 ^T	MH861959	MH873650
<i>Chrysosporium minutisporosum</i>	IMI 379912 ^T	AJ131689	MT875001
<i>Chrysosporium qinghaiense</i>	CGMCC 3.20872	OM952134	OM952122
<i>Chrysosporium queenslandicum</i>	FMR 18702	ON721311	ON720784
<i>Chrysosporium siglerae</i>	UAMH 6541 ^T	AJ131684	MT875002
<i>Chrysosporium submersum</i>	CBS 101575	MH862740	MH874348
<i>Chrysosporium tropicum</i>	CBS 171.62	MH858134	MH869718
<i>Chrysosporium zonatum</i>	CBS 437.88 ^T	KT155898	KC989735

RESULTS

Strain KNU-YC-1803F formed colonies measuring 21.6–23.2 mm (mean = 22.4 ± 0.6 mm, $n = 6$) in diameter after 7 days and 41.7–45.4 mm (mean = 44.2 ± 1.3 mm, $n = 6$) after 14 days of incubation on PDA. On SDA medium, the colony diameter reached 26.1–26.8 mm (mean = 26.6 ± 0.4 mm, $n = 4$) after 7 days and 40.8–43.6 mm (mean = 42.0 ± 1.4 mm, $n = 4$) after 10 days of incubation. Colonies on both PDA and SDA were white and fluffy in appearance, with a pale yellow coloration on the reverse side (Fig. 1 A–D).

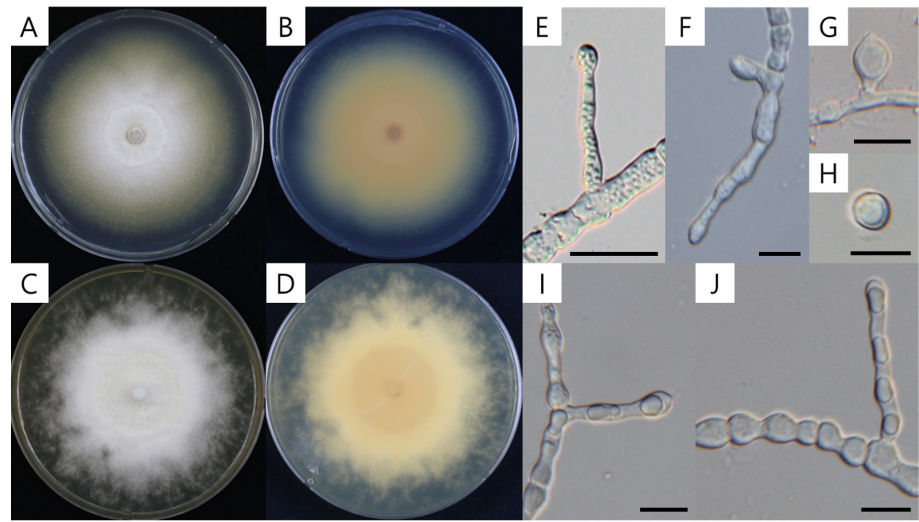


Fig. 1. Culture and morphological characteristics of *Chrysosporium articulatum*. A: Surface of colony on potato dextrose agar (PDA); B: Reverse of colony on PDA; C: Surface of colony on Sabouraud dextrose agar (SDA); D: Reverse of colony on SDA; E–H: Aleurioconidia; I and J: Arthroconidia. Scale bars: E, 20 μ m; F–J, 10 μ m.

Microscopic examination revealed aleurioconidia that were hyaline, clavate, and elongated-pyriform in shape, measuring $4.1\text{--}14.0 \times 4.4\text{--}9.2$ μ m (mean = $9.2 \pm 2.4 \times 6.1 \pm 1.2$ μ m, $n = 30$) (Fig. 1 E–H). Arthroconidia were abundantly produced, cylindrical to navicular in shape, and formed in chains, with dimensions of $4.7\text{--}11.9 \times 3.6\text{--}7.7$ μ m (mean = $7.6 \pm 1.7 \times 5.7 \pm 0.9$ μ m, $n = 60$) (Fig. 1 I & J). The colony morphology and microscopic features of KNU-YC-1803F closely resembled those of *C. articulatum* [8,17] (Table 2). In contrast, *C. keratinophilum* forms fluffy yellow colonies with a cream to brown reverse, and its aleurioconidia are larger ($9\text{--}13 \times 6\text{--}9$ μ m) than those of KNU-YC-1803F [18]. Additionally, arthroconidia are rarely observed in *C. keratinophilum*. Based on these morphological distinctions, KNU-YC-1803F is most similar to *C. articulatum* and can be clearly differentiated from *C. keratinophilum*.

Table 2. Comparison of morphological and cultural characteristics between KNU-YC-1803F and the phylogenetically closest species of the genus *Chrysosporium*

Characteristics		<i>Chrysosporium articulatum</i> KNU-YC-1803F ^a	<i>Chrysosporium articulatum</i> ^b	<i>Chrysosporium keratinophilum</i> ^c
Colony	Color	SDA: Fluffy white, reverse pale yellow	SDA: Fluffy white, reverse pale creamy yellow	SDA: Fluffy yellow, reverse cream to brown
	Growth (mm)	SDA: 26.6 (7 d), 42.0 (10 d)	SDA (28°C): 32–37 (7 d)	SDA: 44–54 (10 d)
Aleurioconidia	Shape	Hyaline, clavate, pyriform	Hyaline, clavate, elongated-pyriform	Hyaline, pyriform
	Size (μm)	9.2 × 6.1	9.7–11.2 × 5–5.2	9–13 × 6–9
Arthroconidia	Shape	Abundant, cylindrical, navicular, produced in chains	Abundant, cylindrical, navicular, produced in chains	Rare
	Size (μm)	7.6 × 5.7	9–19.5 × 4.5–5.2	Size not reported

^aFungal strain characterized in this study; ^bSource of description [8,17]; ^cSource of description [18].

The ITS sequence of KNU-YC-1803F was determined to be 618 bp in length. BLAST analysis using the NCBI database revealed 100% similarity with *Trichophyton verrucosum* (NIAS CH101; ON843702) and 99.8% similarity with *Chrysosporium articulatum* (UAMH 4320; AJ007841) and *Aphanoascus reticulisporus* (IHEM:23961; OW987155). The ITS match with *T. verrucosum* likely reflects limitations of the sequences available in the NCBI database, rather than indicating a close phylogenetic relationship. Although recent studies have relied solely on ITS sequences for species delimitation within the genus *Chrysosporium* [5], the present study indicates that ITS alone may be insufficient for reliable genus- and species-level identification. Therefore, *LSU* sequencing was performed to enable multi-locus phylogenetic analysis.

The *LSU* sequence of KNU-YC-1803F was determined to be 1,317 bp in length. BLAST analysis of the *LSU* sequence showed 99.9% similarity to *Aphanoascus guizhouensis* (ZY 22.044; OR680590) and 96.8% similarity to *Keratinophyton sichuanense* (CGMCC 3.20871; NG_149049) and *Keratinophyton hubeiense* (CGMCC 3.20870; OM952116). After trimming for phylogenetic analysis, the *LSU* sequence showed 100% similarity to *A. reticulisporus* (NBRC 32372; JN941549), *Chrysosporium keratinophilum* (CBS 392.67; AY176730.1), and *C. articulatum* (FMR 19012; ON720740).

To determine the phylogenetic placement of strain KNU-YC-1803F, a phylogenetic tree was constructed from concatenated ITS and *LSU* sequences using the ML method. The results showed that KNU-YC-1803F clustered with *C. articulatum* and was clearly separated from *C. keratinophilum*, supporting its identification as *C. articulatum* (Fig. 2).

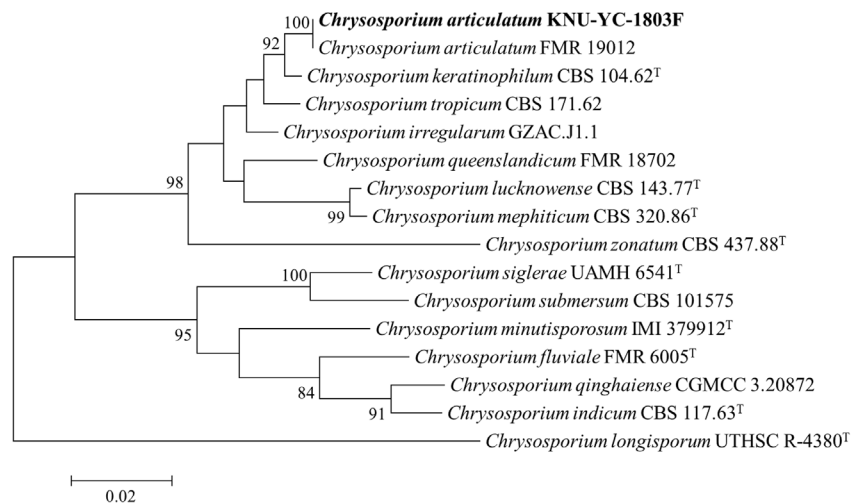


Fig. 2. Maximum likelihood phylogenetic tree based on internal transcribed spacer (ITS) regions and large subunit (*LSU*) gene sequences showing the phylogenetic position of KNU-YC-1803F within the genus *Chrysosporium*. *Chrysosporium longisporum* UTHSC R-4380^T was used as the outgroup. The isolate from this study is highlighted in bold. The numbers above the branches indicate bootstrap values (>80%) from 1,000 replicates. Bar = 0.02 substitutions per nucleotide position.

DISCUSSION

Previously, *C. articulatum* and *Chrysosporium queenslandicum* were considered conspecific, based on their presumed connection to the teleomorph *Apinisia queenslandica* [2]. However, this classification relied primarily on morphological similarity and lacked molecular phylogenetic evidence, rendering it a limited and provisional interpretation. Later, Sigler reclassified the two species as distinct based on clear morphological differences, including the arrangement of arthroconidia and the size and structural characteristics of conidia [19]. More recently, molecular phylogenetic analysis based on ITS sequences further supported this distinction, demonstrating that the two species occupy separate clades consistent with their morphological divergence [5]. Furthermore, phylogenetic analyses based on concatenated ITS and *LSU* sequences placed KNU-YC-1803F within the same clade as *C. articulatum*, clearly separating it from *C. queenslandicum* and supporting its identification as *C. articulatum* (Fig. 2).

Chrysosporium articulatum was previously reported from an unidentified marine sponge in Korea [20]; however, that study lacked definitive morphological, molecular, and cultural evidence, rendering the identification uncertain. In the present study, the species was confirmed as *C. articulatum* through detailed morphological observation and multi-locus phylogenetic analysis based on ITS and *LSU* sequences. Accordingly, this strain is herein reported as the first confirmed record of *C. articulatum* in Korea, carrying significant taxonomic value.

The genus *Chrysosporium* is known to produce a wide array of secondary metabolites, with approximately 66% identified as novel natural compounds. These include alkaloids, polyketides, lactones, and furan derivatives, many of which exhibit notable bioactivities such as cytotoxicity, antibacterial, antifungal, and enzyme inhibitory effects [7]. In particular, *C. articulatum* and several related species have been reported to produce keratinase, an enzyme capable of degrading animal keratin such as feathers and

hair [8], suggesting its potential application in keratin waste management and industrial enzyme production.

The domestic report of this strain extends beyond mere identification, representing a meaningful contribution to the expansion of national fungal biodiversity from soil environments and serving as a foundational resource for strain banking and future biotechnological utilization.

CONFLICT OF INTERESTS

The authors declare that they have no potential conflicts of interest.

ACKNOWLEDGEMENTS

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