RESEARCH ARTICLE

Identification of the Yeast-like Fungus *Acaromyces ingoldii* Newly Recorded in Korea

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ABSTRACT

The fungal strain, designated KNUF-25-GA222, was collected from Miryang-si, Gyeongnam province, Korea and was identified as a previously unreported species within the genus *Acaromyces*. When cultivated on potato dextrose agar and yeast extract peptone glucose agar, blastoconidial production was observed, and the strain KNUF-25-GA222 was determined to be a yeast-like fungus based on its morphological characteristics. Molecular phylogenetic analyses were performed using concatenated nucleotide sequences from the internal transcribed spacer (ITS) region and 28S rRNA (LSU) gene to determine its taxonomic position and evolutionary relationships. The results revealed that the strain belonged to the genus *Acaromyces*, with high sequence similarities of 99.6% and 100% to *A. ingoldii* CBS 10536 in the ITS region and the LSU gene, respectively. Phylogenetic and morphological evidence collectively supports the identification of KNUF-25-GA222 as the asexual morph of *A. ingoldii*. To the best of our knowledge, *A. ingoldii* was confirmed as a previously unreported species in Korea.

Keywords: *Acaromyces ingoldii*, Morphology, Phylogenetic analyses, Yeast-like fungi

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INTRODUCTION

Yeast-like fungi are so named because they produce yeast-like daughter cells, called blastoconidia, by budding from the mycelium [1]. These fungi are phylogenetically related to filamentous ascomycete taxa rather than to true yeasts [2]. Yeast-like fungi belong to various taxonomic groups, including genera belonging to Ascomycota, such as *Ascoidea* and *Cephaloascus* or genera belonging to Basidiomycota, such as *Inopinatum*, *Meira*, and *Acaromyces* [3–5]. Exobasidiales, which include yeast-like fungi belonging to the Basidiomycota, are mostly plant-associated fungi, including phytopathogenic, endophytic, and epiphytic species, some of which interact with insects [6]. Despite their wide distribution and morphological and physiological diversity, our understanding of yeast-like fungi remains limited [1]. The order Exobasidiales includes several families, such as Cryptobasidiaceae and Exobasidiaceae; several species within these families have been reported to form galls on the stems or trunks of host plants [7–10]. Cryptobasidiaceae, which occurs mainly in the Lauraceae, is characterized

by internal sporulation, forming elongated gastroid basidia inside the host tissues [7,10]. The genus *Acaromyces*, classified within the order Exobasidiales and family Cryptobasidiaceae, is regarded as a yeast-like fungus that produces yeast-like daughter cells known as blastoconidia [1,5]. Following recent taxonomic revisions, *Acaromyces* currently comprises only one valid species: *A. ingoldii*. Based on previous studies, *A. ingoldii* has been reported from a variety of habitats worldwide, ranging from mite cadavers in the coastal plain of Israel to marine sediments in the South China Sea [11–13]. Historically, species of the genus *Acaromyces* have been recognized for their pathogenicity toward a wide range of mite species [14]. Until recently, this species had not been reported in Korea. The present study aimed to clarify the taxonomic status of previously unrecorded species in Korea through detailed morphological examinations and phylogenetic analyses.

MATERIALS AND METHODS

Sample collection and fungal isolation

Healthy apple leaf samples were collected from Miryang-si, Gyeongnam province, Korea (35°35'39.6"N, 128°57'1.8"E). To isolate fungi from the leaf surface, apple leaves were sterilized in 70% ethanol for 1 min and in a 2% sodium hypochlorite solution for 1 min. The samples were placed in double-distilled water (DDW) and washed three times for 30 s each. The sterilized samples were dried on a filter paper for 30 min. Then 100 μL of DDW was added to the surface and mixed thoroughly. The suspension was then spread onto potato dextrose agar (PDA; Difco, Detroit, MI, USA) plates and incubated for 2–3 days at 25℃. Single colonies were then transferred to fresh PDA plates and incubated at 25℃. The isolated fungi were designated KNUF-25-GA222 and selected for molecular, cultural, and morphological analyses.

Cultural and morphological characterization

The strain was cultured at 25°C on PDA and yeast extract peptone glucose agar (YPGA; yeast extract, 10 g; peptone, 5 g; glucose, 40 g; agar, 15 g; and distilled H_2O, 1,000 mL) for morphological and cultural characterization. Cultures were incubated for 7 and 21 days in the dark, and characteristics such as the size, color, and shape of the mycelium, and details of the colonies were observed. The cultures were identified by visual observation and examination under a light microscope (BX-50; Olympus, Tokyo, Japan).

Genomic DNA extraction, PCR amplification, and sequencing

For phylogenetic analysis, genomic DNA was extracted from cultures grown on PDA plates using the HiGene™ Genomic DNA Prep Kit (Biofact, Daejeon, Korea), according to the manufacturer's instructions. Phylogenetic analysis was conducted based on internal transcribed spacer (ITS) regions and 28S rRNA gene (LSU), which were amplified using the primer pairs ITS1F/ITS4 and LR0R/LR5, respectively [15–17]. The amplified products were purified using ExoSAP-IT (Thermo Fisher Scientific, Waltham, MA, USA)

and submitted to Macrogen (Seoul, Korea) for sequencing. The sequences of the obtained strains were deposited in the National Center for Biotechnology Information (NCBI) GenBank database (Table 1).

Table 1. GenBank accession numbers of strains used in this study

Species	Strain numbers	GenBank accession No.	
		ITS	LSU
Acaromyces ingoldii	**KNUF-25-GA222**	**LC901949**	**LC901950**
Acaromyces ingoldii	CBS 10536	AM991023	AM991022
Acaromyces ingoldii	CBS 110050^{T}	AY158671	AY158665
Clinoconidium onumae	HM16-730C	LC522970	LC523831
Erythrobasidium hasegawianum	JCM 1545^{T}	NR_111008	AF131058
Exobasidium gracile	DSM 4460	DQ663700	DQ663699
Exobasidium rhododendri	CBS 101457	DQ667153	DQ667151
Exobasidium vaccinii	TUB 019109	AB180362	FJ644526
Laurobasidium hachijoense	MAFF238665	AB180359	AB177562
Laurobasidium lauri	M.P. 2371	MZ159755	AF487403
Meira argovae	AS006	AY158676	AY158670
Meira argovae	CBS 110053^{T}	AY158675	AY158669
Meira geulakonigii	PM1	GQ917049	GQ917048
Meira geulakonigii	CBS 110052^{T}	AY158674	AY158668
Meira miltonrushii	MCA 3882^{T}	NR_120190	JX432962
Meira nashicola	CBS 117161^{T}	AB185159	AB185157

^{T}type strain. ITS: internal transcribed spacer regions; LSU: 28S rRNA gene.
The strains identified in this study are indicated in bold.

Phylogenetic analyses

The sequence of strain KNUF-25-GA222 was analyzed for similarity against datasets in the NCBI database using the Basic Local Alignment Search Tool (BLAST). Several related sequences were retrieved from the database for phylogenetic analysis. Phylogenetic trees were constructed using the concatenated sequences of the ITS regions and the LSU gene, employing the maximum-likelihood (ML) method with Molecular Evolutionary Genetics Analysis (MEGA) 11.0 software [18]. An evolutionary distance matrix analysis was performed using the Tamura–Nei model, and bootstrap values were based on 1,000 replications [19].

RESULTS

Acaromyces ingoldii Boekhout, Scorzetti, Gerson & Sztejnb. ex Denchev & T. Denchev, Mycobiota 11:5 (2021) [MB#558279]

Cultural and morphological characteristics of strain KNUF-25-GA222

Cultures on PDA after 7 days at 25°C, measured 12 mm in diameter. The colonies were tough, pale yellow, velvety, and pruinose with a ridged lamelliform surface, flat marginal zone, and an eroded outermost

margin. After 21 days, the colonies grew 24 mm in diameter, appeared greyish brown, were covered with white velvety patches, and the reverse side of the colony was brown (Fig. 1A). Under the same conditions, the colonies were cultured on YPGA for 7 days and were 14 mm in diameter, firm and ridged, whitish, velvety surface, pulvinoid, and furrowed, with the margin eroded. After 21 days, the colonies were 23 mm in diameter, the surface was pruinose and cerebriform, and the reverse side of the colony was yellowish-brown (Fig. 1B). The production of blastoconidia within the mycelium was clearly observed (Fig. 1C). These blastoconidia originated from sterigma-like structures measuring 13–61 × 2–5 μm in size (Fig. 1D and E). Blastoconidia were formed acropetally in chains, became smaller toward the apex of the conidial chain, and were usually formed near the septa of narrow hyaline hyphae (Fig. 1F) [5,20,21]. The strains *Acaromyces ingoldii* KNUF-25-GA222 and *A. ingoldii* CBS 110050^{T} exhibited similar cultural characteristics on PDA and YPGA media and shared morphological traits such as velvety pruinose and furrowed colonies, and blastoconidia formed from lateral sterigma-like structures (Table 2).

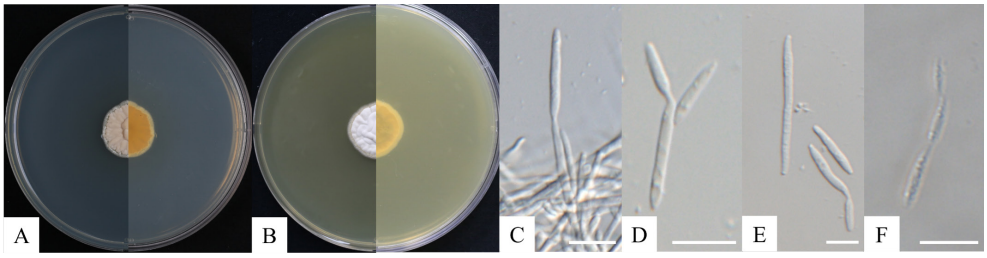

Fig. 1. Cultural and morphological characteristics of *Acaromyces ingoldii* KNUF-25-GA222. A, B: obverse and reverse view of the colony at 25°C after 21 days on potato dextrose agar (PDA) and yeast extract peptone glucose agar (YPGA), respectively; C: blastoconidia arising from the hyphae; D, E: blastoconidia, F: blastoconidia formed in chains. Scale bars = 10 μm.

Table 2. Cultural and morphological characteristics of the isolated strain KNUF-25-GA222 with reference to *Acaromyces* species

Characteristics	*Acaromyces ingoldii* KNUF-25-GA222[a]	*Acaromyces ingoldii* CBS 110050[Tb]
Colony on PDA	24 mm diam, after 21 days at 25°C in the dark velvety pruinose, ridged, furrowed, pale yellow at first, becoming greyish brown	25 mm diam, after 21 days at 25°C in the dark velvety pruinose, pulvinate, furrowed, whitish at first, becoming greyish brown
Colony on YPGA	23 mm diam, after 21 days at 25°C in the dark; velvety, thin pruinose, pulvinoid, furrowed, margin eroded, whitish	16 mm diam, after 21 days at 25°C in the dark; velvety, thin pruinose, pulvinoid, furrowed, margin eroded, whitish
Blastoconidia	13–61 × 2–5 μm; sterigma-like, fusiform, aseptate, hyaline	20–35 × 2–3 μm; sterigma-like, fusiform, shorter near the apex of the chain, hyaline

[a]fungal strain used in this study; [b]source of descriptions [4]; [T]type strain.
PDA: potato dextrose agar; YPGA: yeast extract peptone glucose agar.

Molecular phylogeny of strain KNUF-25-GA222

Amplification of the ITS region and the LSU gene for the identification of the isolated fungus KNUF-25-GA222 yielded sequences of 556 and 806 bp, respectively. The ITS region exhibited 99.6% similarity with *Acaromyces ingoldii* CBS 10536 and 98.4% similarity with *Laurobasidium hachijoense* MAFF238330. In

the case of the LSU gene sequence, the strain exhibited 100% similarity to strain *A. ingoldii* CBS 10536 and 99.9% similarity to *L. hachijoense* MAFF238665. The ML phylogenetic tree was generated using concatenated sequences of the ITS regions and the LSU gene; strain KNUF-25-GA222 clustered together with *A. ingoldii* CBS 10536. Thus, based on the morphological and phylogenetic analyses, strain KNUF-25-GA222 was identified as *A. ingoldii* (Fig. 2).

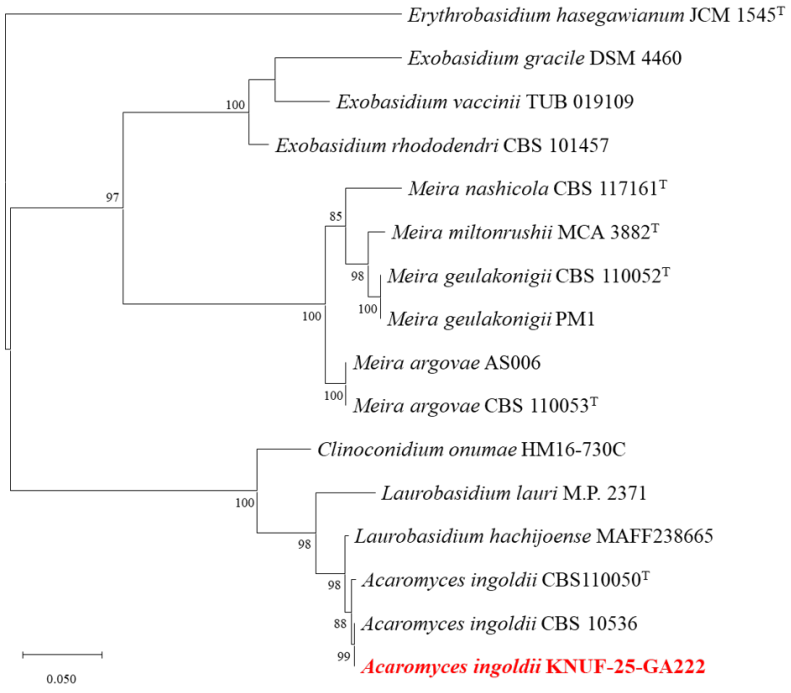

Fig. 2. Maximum-likelihood phylogenetic tree based on a combined dataset of partial sequences of internal transcribed spacer (ITS) regions and 28S rRNA gene (LSU) showing the phylogenetic position of the strain KNUF-25-GA222 among Exobasidiales. Bootstrap values greater than 80% (percentage of 1,000 replications) are shown at branching points. The strain isolated in this study is in bold and red. The tree was rooted using *Erythrobasidium hasegawianum* JCM 1545^{T} as an outgroup. Bar = 0.050 substitutions per nucleotide position.

DISCUSSION

The class Exobasidiomycetes comprises seven genera of yeasts and yeast-like fungi, including *Acaromyces* and *Meira*. Since protein-coding genes, as well as small subunit rRNA gene and ITS region, are not available at present, only LSU gene sequences have been used to analyze the phylogenetic relationships of yeast species with teleomorphic species in Exobasidiomycetes [22]. Members of the genus *Acaromyces* are yeast-like fungi belonging to the Exobasidiales order of Exobasidiomycetes and are rarely recorded worldwide [23]. Previous studies have described *A. ingoldii* as a yeast-like basidiomycete that produces blastoconidia, and the morphological and molecular characteristics observed in the Korean isolate were consistent with those of previously reported *A. ingoldii* strains [5]. To date, two species belonging to the genus *Acaromyces* have been documented worldwide. *A. laviae* was originally described as a yeast-like

organism associated with the death of *Acarapis woodi*, a mite responsible for acarine disease in honeybees [14]. This association suggests the potential pathogenicity of *A. laviae* in mites. However, since *A. laviae* has not been formally described, it is considered invalid, whereas *A. ingoldii* is the only validly reported species. *A. ingoldii* is a well-known entomopathogenic fungus that exhibits pathogenicity against phytophagous mites [11]. Other studies have revealed that this species was first described based on a culture isolated from the citrus rust mite infesting grapefruit leaves (*Citrus paradisi*) and was subsequently assayed against several citrus mite species, *Eutetranychus orientalis*, *Tetranychus urticae*, *Phyllocoptruta oleivora*, and *Panonychus citri* [5,11,12]. Because the pathogenicity of this species against mites has been demonstrated in previous studies, further studies will be conducted to apply this strain to citrus rust mites and other pest mite species to confirm mortality and evaluate its mite-inhibitory activity. In addition, *A. ingoldii* produces secondary metabolites that inhibit *Raffaelea lauricola*, the causal agent of laurel wilt, which is an important disease that affects members of the Lauraceae family, including sassafras (*Sassafras albidum*). In addition, *A. ingoldii* suppresses the growth of wood-decaying fungi, including brown and white rot species [24,25]. However, related species of *A. ingoldii,* including *Laurobasidium hachijoense*, *Clinoconidium onumae*, and *Laurobasidium lauri* have been reported to produce gall structures [8–10]. This suggests that *A. ingoldii* may also be capable of forming galls on hosts of the Lauraceae family, warranting further investigation into the pathogenicity, host specificity, and ecological roles of *A. ingoldii*. These findings indicate that *A. ingoldii* deserves further investigation as a potentially novel plant pathogenic fungus, as a biocontrol agent against phytophagous mites, and as a source of useful secondary metabolites. This study is the first report of *A. ingoldii* in Korea, expands the known geographical distribution of this species, and provides additional taxonomic insights into *Acaromyces*.

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CONFLICT OF INTEREST

No conflict of interest was reported or declared by the authors.

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