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Research Article

Characterization of Two Species of *Acremonium* (Unrecorded in Korea) from Soil Samples: *A. variecolor* and *A. persicinum*

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Abstract During a survey of fungal diversity of the order Hypocreales in Korea, two *Acremonium* isolates, CNUFC-1YSRS2-4 and CNUFC-GSNPF3-1, were isolated from soils collected on a bank of the Yeongsan River, Naju, and in a forest on the Mt. Daegak located on Sinsi Island, Gunsan, South Korea, respectively. Based on the morphological characteristics and sequence analysis of the internal transcribed spacer and D1/D2 domains of 28S ribosomal DNA, the isolates CNUFC-1YSRS2-4 and CNUFC-GSNPF3-1 were identified as *A. variecolor* and *A. persicinum*, respectively. These 2 species represent novel Hypocreales isolates in Korea.

Keywords *Acremonium persicinum*, *Acremonium variecolor*, Diversity, Hypocreales

The anamorph *Acremonium* Link is the largest genus within the order Hypocreales. The genus *Acremonium*, formerly called *Cephalosporium*, includes approximately 100 species; these organisms are known to be saprobic on dead plants or soil dwellers [1]. In 1971, the taxon *Acremonium* fulfilled the criteria of the standard taxonomical nomenclature and was designated as a separate genus [2]. Some of *Acremonium* species are known to be opportunistic pathogens of humans and animals [3], causing eumycetoma, onychomycosis, and hyalohyphomycosis. Infections by *Acremonium* in humans are rare but could result in clinical manifestations of hyalohyphomycosis, such as arthritis, osteomyelitis, peritonitis, endocarditis, pneumonia, cerebritis, and subcutaneous infection [4]. Of note, many species of this genus have been identified as producers of useful metabolites. Cephalosporins, which belong to the β-lactam class of antibiotics, were derived from *Acremonium strictum* W. Gams (syn. *Cephalosporium acremonium* Corda). In Korea, anti-inflammatory sesquiterpenoids from a sponge-derived *Acremonium* sp. were reported [5]. In addition, phthalide and isocoumarin derivatives have been reported to be produced by *Acremonium* sp. isolated from a mangrove [6]. *Acremonium cellulolyticus* is known to be a possible cellulase producer [7, 8].

In terms of taxonomy, this genus is morphologically simple; thus, classification at the species level is difficult. Their morphological features include septate hyphae with simple, tapered, lateral phialides produced singly or in groups and unicellular, globose-to-cylindrical conidia, which are mostly aggregated in slimy heads at the apex of the phialide [9]. Nonetheless, their taxonomy has not been firmly resolved due to the absence of clear-cut morphological differences at the species level and the absence of reliable sequences in public databases [10, 11].

In 2011, Summerbell *et al.* [12] presented the results on the phylogenetic analyses of the D1/D2 domain of large subunit and small subunit ribosomal DNA (rDNA) to classify the majority of *Acremonium* species. In that study, some species of *Acremonium* were reclassified into the genus *Gliomastix* and included into *Sarocladium*.

Only 5 species of *Acremonium*, namely *A. strictum*, *A. acutatum*, *A. cellulolyticus*, *A. zonatum*, and *A. sclerotigenum*, have been recorded in Korea since 2006 [9, 13-16], but *A. cellulolyticus* (GenBank accession No. AB474749) was transferred to the genus *Talaromyces* by Fujii *et al.* [17]. In the present study, we sought to investigate the morphological characteristics of *Acremonium* isolates obtained from soil

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samples in Korea and to determine phylogenetic positions of *A. variecolor* and *A. persicinum*.

MATERIALS AND METHODS

Extraction of fungal strains from soil samples. The strains used in this study were *Acremonium* species isolated from soil samples collected on the Yeongsan riverside in Naju (GPS 35.0022967, 126.6832094) and on Sinsi Island (*Sinsido*) in Gunsan (GPS 35.818669, 126.473644), Korea. The soil samples were placed in conical tubes and kept at ambient temperature until cultured. Fungi were isolated by the serial dilution plating method. Briefly, 1 g of soil was mixed with 9 mL of sterile distilled water and shaken for 15 min at room temperature; serial dilutions ranging from 10^{-3} to 10^{-5} were then prepared. An aliquot of 0.1 mL from each dilution was transferred onto potato dextrose agar (PDA; 39 g PDA in 1 L of deionized water; Becton, Dickinson and Co., Sparks, MD, USA) and incubated at 25°C for 3–7 days.

Hyphal tips were transferred to new PDA plates under a

Table 1. Information on the sequences of *Acremonium* and related genera used in the present study

Taxon name	Collection No. (isolate No.)	GenBank accession No.	
		ITS	D1/D2 domains of 28S rRNA gene
Acremonium alternatum	CBS 407.66 (T)	HE798150	-
A. antarcticum	CBS 987.87	DQ825970	-
A. asperulatum	CBS 130362 (T)	HE608641	HE608649
A. blochii	CBS 993.69	HE608636	-
A. borodinense	CBS 101148 (T)	HE608635	-
A. butyri	CBS 301.38 (T)	DQ286652	-
A. curvulum	CBS 430.66 (T)	HE608638	HE608656
A. egyptiacum	CBS 114785 (T)	FN706550	-
A. exuviarum	CBS 113360 (T)	AY882946	-
A. fuci	CBS 112868 (T)	AY632653	-
A. fusidioides	CBS 840.68 (T)	FN706542	-
A. glaucum	CBS 796.69 (T)	FN691454	HE608657
A. hansfordii	CBS 390.73	AB540578	-
A. implicatum	MUCL 1412	FN706553	HE608659
A. kiliense	MUCL 9724 (T)	FN691446	-
A. persicinum	CBS 310.59 (T)	FN706554	-
A. persicinum	MEF020	KT315410	-
A. persicinum	SC0105	KM086711	-
A. persicinum	**CNUFC-GSNPF3-1-1**	**MF977727**	**MF977731**
A. persicinum	**CNUFC-GSNPF3-1-2**	**MF977728**	**MF977732**
A. polychrorum	MUCL 9834 (T)	FN706547	-
A. sclerotigenum	CBS 124.42 (T)	FN706552	-
A. spinosum	CBS 136.33 (T)	HE608637	HE608655
A. variecolor	CBS 130360 (T)	HE608647	HE608651
A. variecolor	CBS 130361	HE608648	HE608652
A. variecolor	**CNUFC-1YSRS2-4-1**	**MF977725**	**MF977729**
A. variecolor	**CNUFC-1YSRS2-4-2**	**MF977726**	**MF977730**
A. zeae	CBS 800.69 (T)	FN691451	-
Bulbithecium hyalosporum	CBS 318.91 (T)	HE608634	HE608661
Cosmospora coccinea	CBS 114050	FJ474072	-
Geosmithia morbida	CBS 124663	FN434082	-
Gibellulopsis nigrescens	CBS 120949	EF543857	-
G. piscis	CBS 892.70 (T)	DQ825985	-
Leucosphaerina arxii	CBS 737.84 (T)	HE608640	HE608662
Nalanthamala diospyri	CBS 430.89	AY554209	-
Nectria fuckeliana	-	AJ557573	-
N. haematococca	-	HQ651171	-
Sarocladium bacillisporum	CBS 425.67 (T)	HE608639	HE608658
Verticillium suchlasporium	CBS 248.83 (T)	AJ292406	-

Bold letters represent strains used in this study.

ITS, internal transcribed spacer; CBS, Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; T, ex-type strain; MUCL, Mycothéque de l'Université Catholique de Louvain, Louvain-la Neuve, Belgium; MEF and SC, strain or voucher number absent; CNUFC, Environmental Microbiology Laboratory Fungarium, Chonnam National University, Gwangju, South Korea.

stereomicroscope. To obtain each pure culture, individual colonies of varied morphologies were transferred to PDA plates. Pure isolates were maintained in PDA slant tubes and stored in 20% glycerol at −80°C in the Environmental Microbiology Laboratory Fungarium, Chonnam National University, Gwangju, Korea.

Morphological analyses. To obtain samples for microscopic examination and growth rate determination, isolates CNUFC-1YSRS2-4 and CNUFC-GSNPF3-1 were cultured on each of the 3 media: PDA, malt extract agar (MEA; 33.6 g MEA in 1 L of deionized water; Becton, Dickinson and Co.), and oatmeal agar (OA; 1.5% oatmeal and 1.5% agar in 1 L of deionized water; Junsei, Tokyo, Japan). The plates were incubated at 25°C in the dark for 7 days. Samples were mounted in a drop of distilled water on a glass slide and were examined under an Olympus BX51 microscope with DIC optics (Olympus, Tokyo, Japan). Fine structures of the fungi were analyzed by scanning electron microscopy (Hitachi S4700 field emission scanning electron microscope; Hitachi, Tokyo, Japan). Samples were fixed in 2.5% paraformaldehyde-glutaraldehyde buffer with 0.05 M phosphate (pH 7.2) (Junsei) for 2 hr and washed in cacodylate buffer (Junsei). Cellular membranes were preserved by fixing the samples in 1% osmium tetroxide (diluted in cacodylate buffer; Electron Microscopy Sciences, Hatfield, PA, USA) for 1 hr, by washing again in cacodylate buffer, dehydrating in a graded series of ethanol solutions (Emsure, Darmstadt, Germany) and isoamyl acetate (Junsei), and by drying in a fume hood. Finally, samples were covered with gold in a sputter coater and examined at Korea Basic Science Institute, Gwangju, Korea.

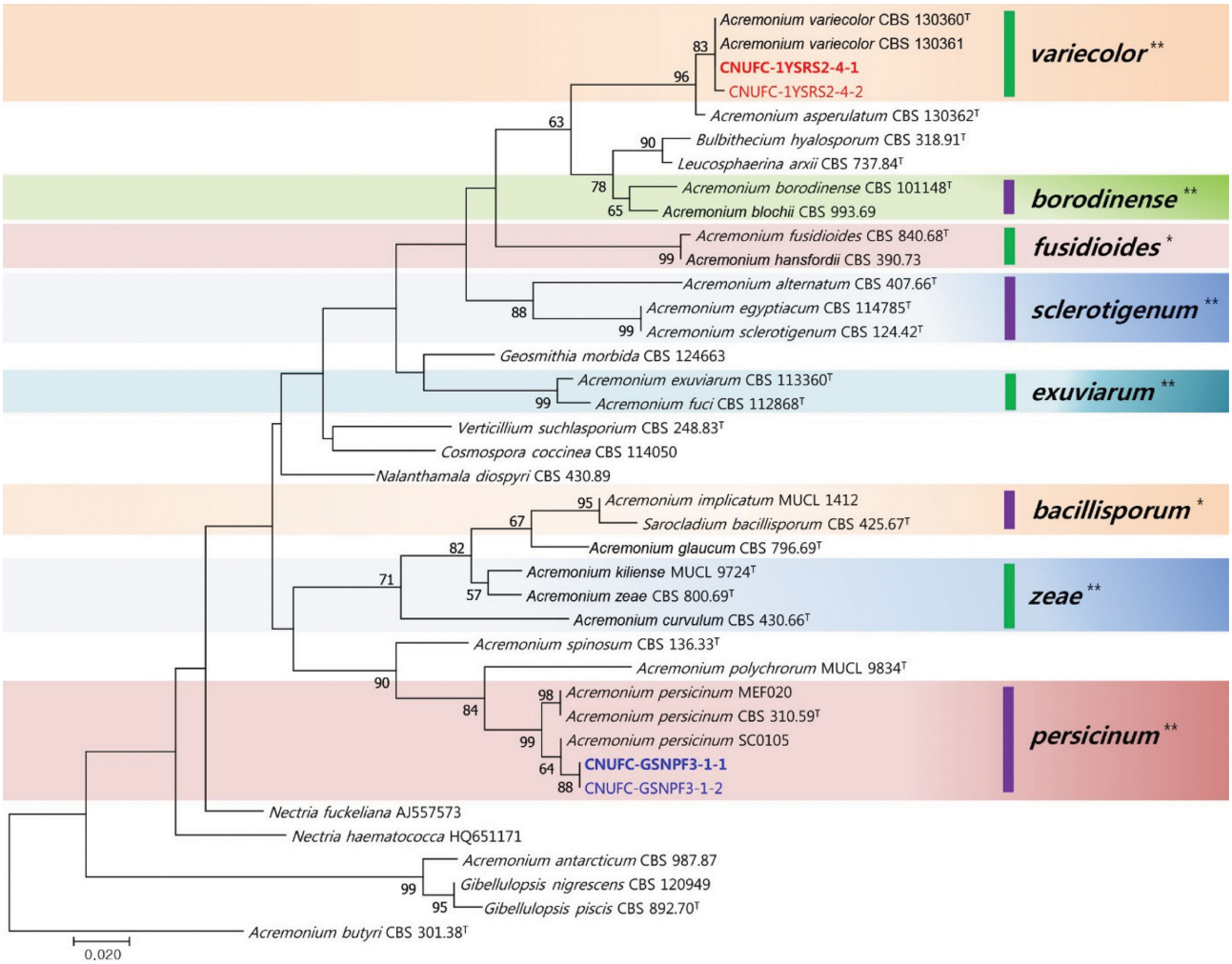

Fig. 1. A phylogenetic tree based on the maximum likelihood method analysis of the internal transcribed spacer rDNA region for *Acremonium variecolor* CNUFC-1YSRS2-4-1, *A. variecolor* CNUFC-1YSRS2-4-2, *A. persicinum* CNUFC-GSNPF3-1-1, and *A. persicinum* CNUFC-GSNPF3-1-2. *Acremonium butyri* served as an outgroup. Numbers at the nodes indicate the bootstrap values (> 50%) from 1,000 replications. The scale bar indicates the number of substitutions per position. The thick colored clade lines with the asterisk (*) were constructed by means of a phylogenetic system described by Summerbell *et al.* [12], whereas the clade lines marked with the asterisk (**) are suggested by the present authors.

DNA extraction, PCR, and sequencing. Total genomic DNA was directly extracted from mycelia using the Solg Genomic DNA Prep Kit for fungi (SolGent, Daejeon, Korea). The internal transcribed spacers (ITS1 and ITS2) and the 5.8S gene were amplified with primers ITS5 (5′-GGAAGTAAAAGTCGTAACAAGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) by the method of Cano *et al.* [18]. D1/D2 domains of the 28S rRNA gene were amplified with primers NL1 (5′-GCATATCAATAAGCGGAGGAAAAG-3′) and NL4 (5′-GGTCCGTGTTTCAAGACGG-3′) according to the method of Gilgado *et al.* [20]. The PCR amplification mixture (total volume 20 μL)

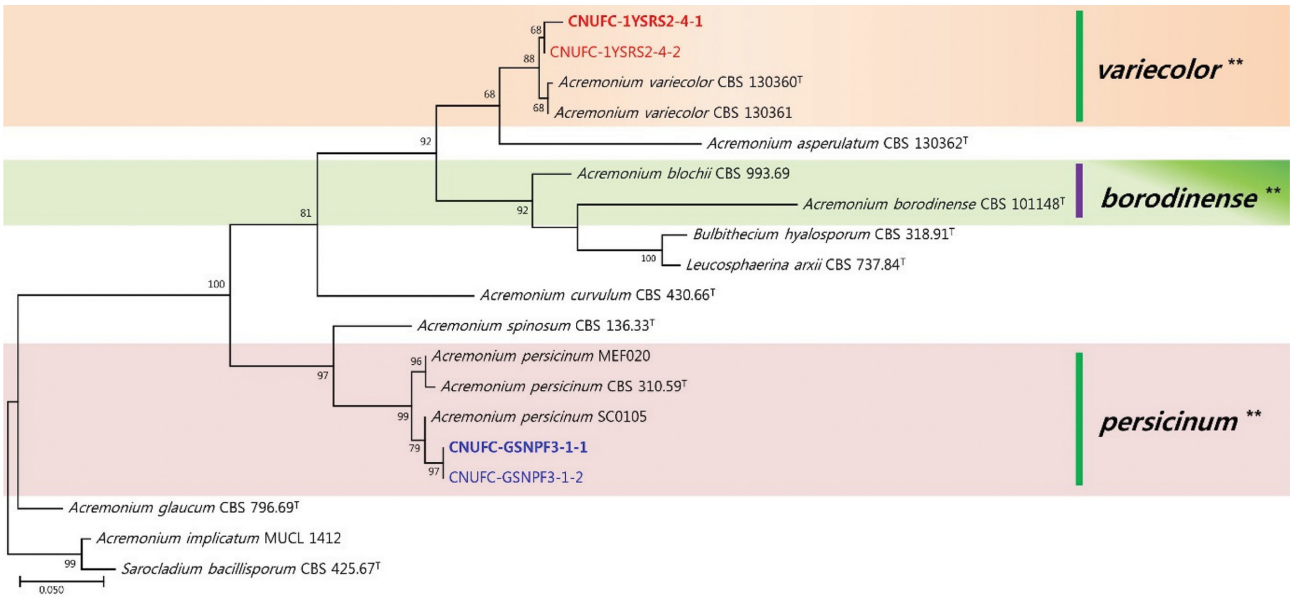

Fig. 2. A phylogenetic tree based on application of the maximum likelihood method to multiple loci including internal transcribed spacer region and D1/D2 rDNA sequence for *Acremonium variecolor* CNUFC-1YSRS2-4-1, *A. variecolor* CNUFC-1YSRS2-4-2, *A. persicinum* CNUFC-GSNPF3-1-1, and *A. persicinum* CNUFC-GSNPF3-1-2. *Acremonium implicatum* and *Sarocladium bacillisporum* served as outgroups. Numbers at the nodes indicate the bootstrap values (> 50%) from 1,000 replications. The bar indicates the number of substitutions per position. The thick colored clade lines with the asterisk (**) are suggested by the authors.

Table 2. Morphological characteristics of CNUFC-1YSRS2-4 and of the reference species *Acremonium variecolor* when grown on the potato dextrose agar medium at 25°C

	CNUFC-1YSRS2-4	*Acremonium variecolor*[a]
Colony	Yellowish white to grayish yellow, zonate, velvety at the center, slightly cottony toward the margin, pigment pale and beige in the periphery, reaching 62 mm diameter after 15 days in the dark at 25°C	Yellowish white to grayish yellow, often zonate, radially folded, velvety at the center, slightly cottony toward the periphery, reaching 41–44 mm diameter after 14 days in the dark at 25°C
Phialide	Lateral, acicular, thin, very variable in length, slightly straight, often curved near the conidia and apex, collarette inconspicuous, smooth or not smooth walled, sometimes deep surface, hyaline, 1–2 μm wide × 18–95 μm long at the base	Terminal or lateral, straight, acicular, with periclinal thickening at the apex, collarette inconspicuous, thin and smooth walled, hyaline, 1–2 μm wide × 18–95 μm long at the base
Conidia	Unicellular, subglobose or ovoid, sometimes long and thin, hyaline to subhyaline, slightly rough and wrinkled surface, arranged in slimy heads, 2.25–4.35 μm wide × 2.54–5.08 μm long	Unicellular, subglobose or ovoid, slightly apiculate base, hyaline to subhyaline, thick- and smooth-walled, arranged in slimy heads, 3–4(–5) μm wide × 2–4 μm long
Conidiophore	Erect, branched, bearing whorls of up to 5 phialides, septate, hyaline, not smooth, slightly wrinkled, thick walls, sometimes caved on the surface, up to 225 μm long	Erect, mostly branched, bearing whorls of 2–5 phialides, septate, hyaline, smooth, walls usually thicker than those of the vegetative hyphae, up to 290 μm long

[a] From the description by Giraldo *et al.* [19].

contained 10 ng of a fungal DNA template, 5 pmol/μL each primer, and the Accupower PCR Premix (*Taq* DNA polymerase, dNTPs, buffer, and a tracking dye; Bioneer Corp., Daejeon, Korea). The PCR products were purified by means of the Accuprep PCR Purification Kit (Bioneer Corp.). DNA sequencing was performed on an ABI 3700 Automated DNA sequencer (Applied Biosystems Inc., Foster City, CA, USA).

Phylogenetic analyses. Fungal sequences (Table 1) were initially aligned in Clustal_X v.2.1 [21] and BioEdit v.7.2.6 software [22]. Phylogenetic analyses were conducted in MEGA 7.0.26 with the default settings [23]. Phylogenetic trees were constructed by the maximum likelihood method with the ITS rDNA sequences and a combined dataset of the ITS and D1/D2 sequences. *Acremonium butyri* (J. F. H. Beyma) W. Gams served as an outgroup in the phylogenetic tree of the ITS sequences, whereas *Acremonium implicatum* (J. C. Gilman & E. V. Abbott) W. Gams and *Sarocladium bacillisporum* (Onions & G. L. Barron) Summerb., served as an outgroup in the combined tree of ITS regions and D1/D2 datasets. The sequence identity was determined by

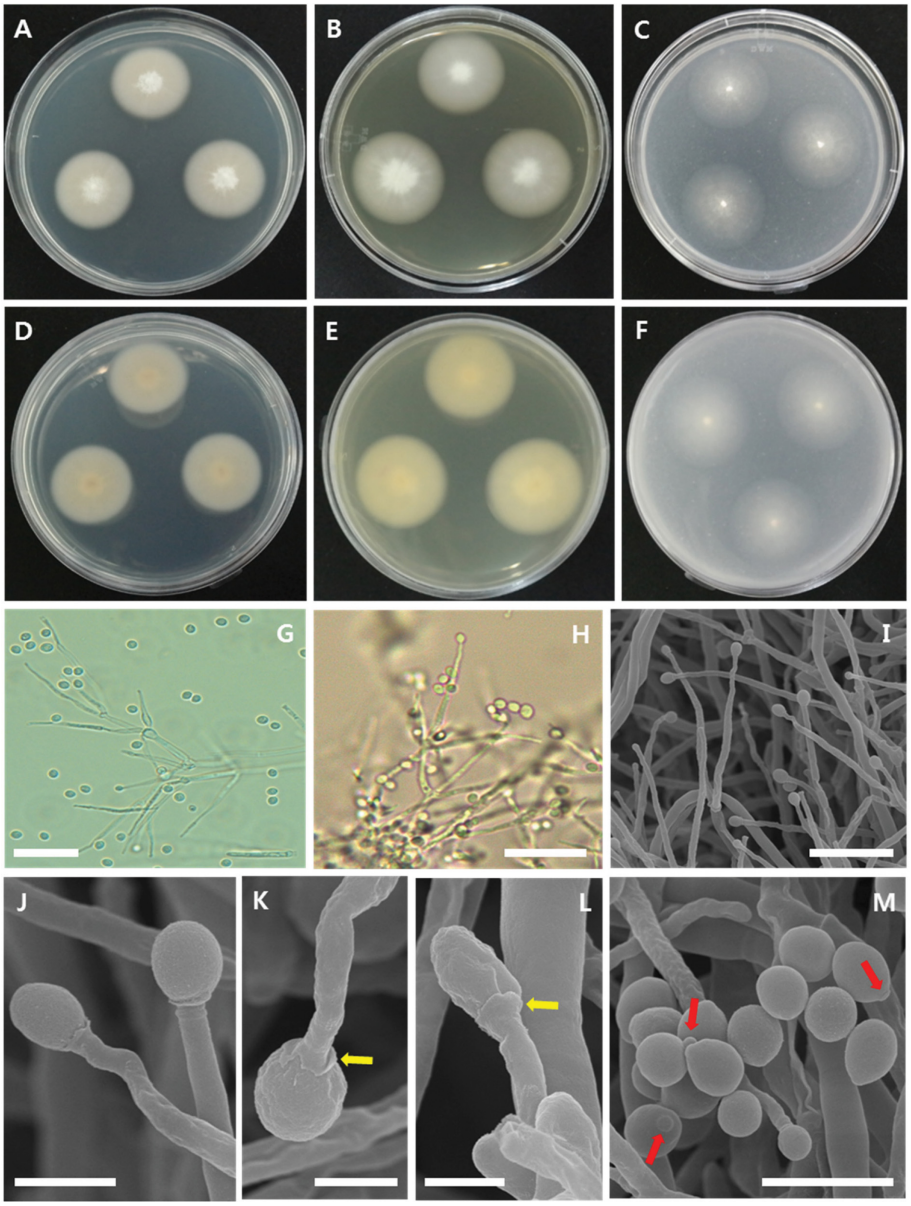

Fig. 3. Morphology of *Acremonium variecolor* CNUFC-1YSRS2-4-1. A–F, Colonies on potato dextrose agar (A, D), malt extract agar (B, E), or oatmeal agar (C, F) (A–C, obverse view; D–F, reverse view); G–I, Branched conidiophores, an acicular phialide, and oval conidia; J–L, A curved or straight phialide and globose to subglobose or ovoid conidia supported by a calyxlike structure (arrows); M, Conidia detached from slimy heads and conidia with an apiculate base (arrows) (scale bars: G, H = 20 μm, I = 10 μm, J = 2.5 μm, K, L = 1.5 μm, M = 5 μm).

means of the National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool for nucleotides (BLASTn).

RESULTS

Phylogenetic analysis. The ITS and 28S sequences of strains CNUFC-1YSRS2-4-1, CNUFC-1YSRS2-4-2, CNUFC-GSNPF3-1-1, and CNUFC-GSNPF3-1-2 were deposited in the NCBI database under accession numbers shown in Table 1.

A BLASTn search revealed that the ITS rDNA sequences of CNUFC-1YSRS2-4-1 and CNUFC-GSNPF3-1-1 have high sequence identities of 99.1% (472/476 bp) and 98.3% (520/529 bp) with *A. variecolor* (GenBank accession No. LN714516) and *A. persicinum* (KF993390), respectively. The phylogenetic tree of the ITS region with high bootstrap values (96% and 99%) confirmed that the CNUFC-1YSRS2-4-1 and CNUFC-GSNPF3-1-1 isolates are *A. variecolor* and *A. persicinum*, respectively (Fig. 1).

In the analysis of the D1/D2 domains of the 28S rDNA sequences, CNUFC-1YSRS2-4-1 and CNUFC-GSNPF3-1-1 strains showed 100% (488/488 bp) and 99.5% (544/547 bp) sequence identities with *A. variecolor* (LN714516) and *A. persicinum* (AB920179), respectively. Moreover, in a combined analysis of the ITS and D1/D2 sequence regions, the 2 strains within the order Hypocreales, like other *Acremonium* species, showed well-supported branch values (Fig. 2).

Taxonomy of CNUFC-1YSRS2-4-1.

Acremonium variecolor Giraldo, Guarro, Gené & Cano, Mycologia 104: 1463 (2012) (Table 2, Fig. 3).

Description: Colonies were initially white and then changed to brown, reaching 62 mm in diameter after 15 days in culture at 25°C on PDA. The colony reverse was pale yellow and regularly zonate. Conidiophores were erect, mostly branched, bearing whorls of up to 5 phialides. Phialides were straight or acicular, measuring 1.0–2.0 μm wide by 18.0–95.0 μm long at the base. Conidia were subglobose or ovoid, measuring 2.25–4.35 μm wide × 2.54–5.08 μm long.

Taxonomy of CNUFC-GSNPF3-1-1.

Acremonium persicinum (Nicot) W. Gams, *Cephalosporium*-artige Schimmelpilze: 75 (1971) (Table 3, Fig. 4).

= *Paecilomyces persicinus* Nicot, Bulletin de la Société Mycologique de France 74: 222 (1958).

= *Cephalosporium purpurascens* Sukapure & Thirum, Mycologia 55: 563 (1963).

Description: Colonies were initially white and then changed to light pinkish with age, reaching 53 mm in diameter after 15 days in culture at 25°C on PDA. The middle of the colony obverse was initially dark white and became pale and formed a crumpled shape as the colony grew toward the edge. On MEA and OA, the colony reverse was light yellow. Conidiophores were erect, simple, or branched. Phialides were lateral and acicular, measuring 1.5–2.5 μm wide × 18–43 μm long at the base. Conidia were oval, measuring 1.0–2.0 μm wide × 2.5–3.0 μm long.

Mycelial growth. The growth varied with strains and media. The average growth rates of CNUFC-1YSRS2-4-1 on the PDA, MEA, and OA media at 25°C were 53, 73, and 75 mm per 12 days, respectively. The growth rates of CNUFC-GSNPF3-1-1 were 36, 51, and 53 mm after 12 days, respectively. The *A. variecolor* CNUFC-1YSRS2-4-1 strain grew faster than did the *A. persicinum* CNUFC-GSNPF3-1-1 strain in the 3 media, and both strains grew

Table 3. Morphological characteristics of CNUFC-GSNPF3-1 and of the reference species *Acremonium persicinum* on potato dextrose agar medium at 24–25°C

Character	CNUFC-GSNPF3-1	*Acremonium persicinum*[a]
Colony	Light color at slightly crumpled margin and pale yellowish in the center, zonate, grows slowly and reaches 53 mm diameter after 15 days in the dark at 25°C	Fluffy mycelium, initially white, changes to light brown with age, grows slowly and reaches 20 mm diameter after 8 days in the dark at 24°C
Phialide	Lateral, straight, or slightly curved, acicular, thickening at the conidiophore, hyaline or subhyaline, thick and crumpled surface, not smoothed, 1.5–2.5 μm wide × 18–43 μm long at the base	Lateral, slightly straight, not acicular, thickening at the conidiophore, hyaline or subhyaline, thick and smooth walled, 4.7–26.7 μm long, gradually tapering from 0.7–1.0 μm at the base to 0.2–0.5 μm
Conidia	Unicellular, slightly ovoid, not completely spherical, hyaline, protruding and slightly short base, bumpy surface, not smooth, arranged in slimy heads, not arranged in chains, chlamydospores absent, 1.0–2.5 μm wide × 2.5–3.0 μm long	Unicellular, spherical to globose, obovoid, thin-walled, hyaline, usually smooth or occasionally rugose, protruding and slightly truncate base, arranged in chains or in masses, chlamydospores absent, 1.5–3.5 μm wide × 2.0–3.0 μm long
Conidiophore	Erect, simple, long, branches present, non-septate, hyaline, neither tapering at the tip nor swollen at the base, 15.0–100.0 μm long	Erect, simple, short, unbranched, non-septate, neither tapering at the tip nor swollen at the base, 1.5–2.5 μm wide × 15.0–25.0 μm long

[a] From the descriptions by Perdomo *et al.* [11], Sukapure *et al.* [24], and Lo Piccolo *et al.* [25].

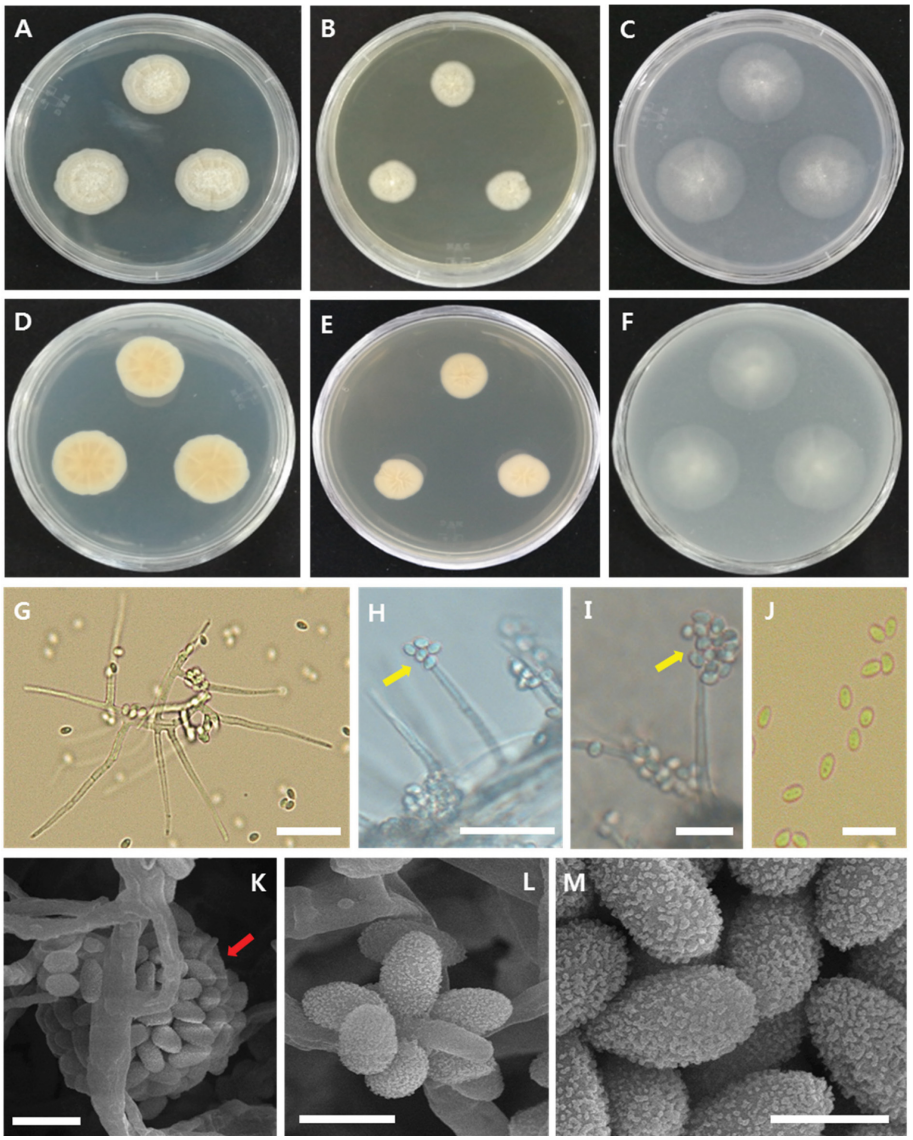

Fig. 4. Morphological characteristics of *Acremonium persicinum* CNUFC-GSNPF3-1-1. A–F, Colonies on potato dextrose agar (A, D), malt extract agar (B, E), or oatmeal agar (C, F) (A–C, obverse view; D–F, reverse view); G, A long and sharp phialide with conidia; H, I, Oval conidia arranged in slimy heads (arrows) on the phialide; J, Conidia; K, Conidia clustered in the slimy head (arrow); L, Arranged conidia with rough walls in the slimy head; M, Oval conidia with rough walls (scale bars: G, H = 20 μm, I, J = 10 μm, K, L = 2.5 μm, M = 2 μm).

the fastest on the OA medium.

DISCUSSION

Recent molecular studies [19] showed that the genus *Acremonium* is polyphyletic, whose species split into different orders of Sordariomycetes. As a result, significant taxonomic changes for *Acremonium* and *Acremonium*-like anamorphs were recently made. To date, many *Acremonium* species have been reported, but there are no trustworthy classification systems and little sequence data are available in GenBank for multigene analyses. Herein, the phylogenetic positions of *A. variecolor* and *A. persicinum* were confirmed based only on a combination of ITS region and large subunit rDNA sequence analysis.

Giraldo *et al.* [19] used only several loci of ITS, 28S, and actin gene sequences that are available in GenBank to create a phylogenetic tree. Nevertheless, because the act-1 sequences for *A. variecolor* are lacking in GenBank, it was impossible to apply this type of analysis to the samples obtained here.

Multilocus sequence analyses employed herein revealed that the 2 species undescribed in Korea were well placed within the related clades without the use of act-1 data. This is because there are still unknown and unconfirmed species related to these *A. variecolor* and *A. persicinum* species [19].

The *A. persicinum* strain identified in this study was placed into the persicinum clade within the *Gliomastix*/*Bionectria* clade. Moreover, the *A. variecolor* strain identified herein completely matched the type strain of *A. variecolor* CBS130360 described by Giraldo *et al.* [19].

Most *Acremonium* are saprophytes isolated from diverse dead plant materials and soils. The role of *Acremonium* species in human infections has long been known. The high prevalence of these species in soil or air may lead to superficial infections [26]. Since Guarro *et al.* [27] provided an overview of opportunistic fungal infections caused by *Acremonium* (*Cephalosporium*) species and discussed the classification of these species along with the diagnosis and treatment of the relevant diseases, much progress in *Acremonium* research has been made.

The 2 *Acremonium* species, *A. variecolor* and *A. persicinum*, for the first time recorded in Korea in this study, were isolated from soil samples. *A. variecolor* and *A. persicinum* were first isolated from forest soil collected at Garganta de Escuaín, Huesca, in Northern Spain and from soil collected at Koyna Valley in Chiplun, India, respectively [19, 24].

Acremonium species are generally resistant to antifungal agents. According to Guarro *et al.* [27], clinical and environmental isolates of *Acremonium* are also resistant to antifungal agents including azole compounds, but are sensitive to amphotericin B in *in vitro* antibiotic sensitivity assays.

In this study, the 2 species *A. variecolor* and *A. persicinum* produced phialides with conidia arranged in slimy heads, but only *A. persicinum* produced single conidia or conidia in a chain. By contrast, *A. persicinum* CNUFC-GSNPF3-1-1 did not produce them.

Although some species of *Acremonium* have been found previously in Korea [9, 13-16], there is little information regarding descriptions and phylogenetic analyses. Especially, the sequence data on *Acremonium cellulolyticus* Y-94 (AB474749) retrieved from GenBank reveals that the species name is wrong and was placed into *Talaromyces cellulolyticus* as mentioned by Fujii and colleagues [15, 17].

Further studies are needed, especially regarding the growth of these strains under different environmental conditions and resistance to antifungal agents.

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