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RESEARCH ARTICLE

Acrophialophora ellipsoidea, an Undescribed Species Isolated from Soil in Korea

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ABSTRACT

A designated fungal isolate, KNU-US-1802E was isolated from the soil in Uiseong, Korea. To identify characteristics of the isolate, it was cultured on PDA media for 6 days at 35°C. Colonies on PDA are flat, light gray, dense, with entire margins; reverse dark gray to black, with white margins. Aerial mycelia were smooth-walled, hyaline and 40~42 mm diameter after 6 days at 35°C. Conidia were hyaline, one-celled, ellipsoidal to fusiform, forming long chains with average length x width of $5.0 \pm 0.3 \times 2.9 \pm 0.2$ μm. Molecular analysis indicates that the internal transcribed spacer (ITS) region and partial beta-tubulin (tub2) gene sequence showed 100% and 99% similarities, respectively with *Acrophialophora ellipsoidea* CGMCC 3.15255 collected from China. Phylogenetic analysis by the neighbor-joining (NJ) method shows that the KNU-US-1802E was clustered with *A. ellipsoidea* CGMCC 3.15255 in a phylogenetic tree constructed using the concatenated sequences of ITS region and tub2 gene sequences with a high bootstrap value. Based on these findings, the isolate KNU-US-1802E was identified as *Acrophialophora ellipsoidea*, and this is the first report of this isolate in Korea.

Keywords: *Acrophialophora ellipsoidea*, Morphological characteristic, Phylogenetic analysis, Soil-inhabitant

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INTRODUCTION

The genus *Acrophialophora* is monophyletic and belongs to the family Chaetomiaceae [1]. Edward (1959) established *Acrophialophora* genus with *A. nainiana* Edward as the type [1]. The genus *Taifanglania* was considered synonymous to *Acrophialophora*, and accordingly, all *Taifanglania* species were transferred to genus *Acrophialophora* [2]. The species of *Acrophialophora* are saprophytic, thermotolerant, and have characteristics that may play an essential role in the degradation of cellulose [3-5]. *A. ellipsoidea* is characterized by solitary phialides tapering into thin necks and long chains of ellipsoidal to fusiform conidia. *A. ellipsoidea* is found most frequently in soil, and it is widely distributed in temperate and tropical regions. It is also commonly isolated as a decomposer of compost and other self-heating substrates [6, 7]. During a survey, the strain KNU-US-1802E was isolated and identified as *A. ellipsoidea*, which until now was an unreported fungal species in Korea. In this study, we describe morphological features and cultural characteristics of the *A. ellipsoidea* KNU-US-1802E strain and its phylogenetic relationship with allied species.

MATERIALS AND METHODS

Soil sample collection and fungal isolation

In 2018, the soil sample was collected from a field in Uiseong, Korea (N 36°25'12.8", E 128°45'33.6") at a depth of 15 to 30 cm. The sample was transferred to polyethylene zipper bags after air drying, and then stored at 4°C until use. KNU-US-1802E was isolated by a conventional dilution plating technique [8]. Briefly, 1 g of soil was suspended in 10 mL of sterile distilled water and gently vortexed. The suspension was serially diluted, and a defined volume spread on potato dextrose agar (PDA; Difco, Detroit, USA) plates.

Morphological characterization

The isolate, KNU-US-1802E, was cultured on PDA media and incubated at 35°C. Colony characteristics such as color, shape, and size were identified and recorded, and the morphological description was made from colonies on PDA media after 6 days in culture [2]. The model BX-50 light microscope (Olympus, Tokyo, Japan) was used to observe the morphological structures of the isolate.

Genomic DNA extraction, PCR amplification and sequencing

Using a HiGene Genomic DNA prep kit (Biofact, Daejeon, Korea) and following the instructions of the manufacturer, we extracted genomic DNA for molecular identification based on multiple genes. Polymerase chain reaction (PCR) amplification was performed to amplify two gene markers; ITS1F and ITS4 [9, 10] were used to amplify the internal transcribed spacer (ITS) regions, and Bt2a and Bt2b [11] were used to amplify a portion of the beta-tubulin (tub2) gene. Then, the amplified PCR products were purified with ExoSAP-IT (Thermo Fisher Scientific, Waltham, USA) and sequenced (Macrogen, Daejeon, Korea).

Phylogenetic analyses

The DNA sequences obtained from our isolate were compared with reference sequences from the GenBank database of the National Center for Biotechnology Information (NCBI), using the basic local alignment search tool (BLAST). The isolates used to construct the phylogenetic tree are summarized in Table 1 with their strain and GenBank accession numbers. Based on Kimura's neighbor-joining (NJ) algorithm, the evolutionary distance matrices were generated [12]. Phylogenetic analyses were performed using the program MEGA 7 [13] with bootstrap values based on 1,000 replications. The analyzed sequences were deposited at the collection facility of NCBI GenBank, with accession numbers of LC485179 and LC485180 for the ITS region and partial of tub2 gene sequences, respectively.

Table 1. Comparison of morphological characteristics of isolate KNU-US-1802E with the reference strain *Acrophialophora ellipsoidea*

Characteristics		*Acrophialophora ellipsoidea* KNU-US-1802E[a]	*Acrophialophora ellipsoidea* CGMCC 3.15255[b]
Colony	Color and shape	Flat, light gray with entire margins, has velvet like aerial-mycelia, reverse dark gray to black with white margin	Flat, light gray, margin entire, velvet-like aerial mycelia, reverse dark gray to black, with no pigmentation in agar
	Growth (Diam.)	42 mm after 6 days at 35°C on PDA	63 mm after 6 days at 35°C on PDA
Conidia	Conidial size	$5.0 \pm 0.3 \times 2.9 \pm 0.2$ μm (n=50)	$4.9 \pm 0.7 \times 2.8 \pm 0.2$ μm (n=50)
	Shape and position	Hyaline, one-celled, ellipsoidal to fusiform, forming long chains of more than 10 conidia	One-celled, ellipsoidal or fusiform, hyaline, smooth-walled, forming chains of more than 10 conidia
Phialide	Shape	Cylindrical, tapering at one end, solitary and lateral	Solitary and lateral on somatic hyphae, Cylindrical or ellipsoidal swollen bases, tapering into thin necks

[a] Fungal isolate studied in this paper.

[b] Source of description (Zhang et al., 2015)

RESULTS AND DISCUSSION

Morphology of the KNU-US-1802E isolate

Colony diameters were 42 mm at 6 days of culture at 35°C on PDA. Colonies on PDA were flat, light gray, dense, with entire margins; reverse dark gray to black, with white margins (Fig. 1). Conidia were hyaline, one-celled, ellipsoidal to fusiform, forming long chains of more than ten conidia with an average length × width of $5.0 \pm 0.3 \times 2.9 \pm 0.2$ μm (n=50). Although the isolate showed small differences in culture characteristics, most of morphological characteristics were like those previously reported for *A. ellipsoidea* CGMCC 3.15255 (Table 1). This result suggests that the fungal isolate KNU-US-1802E was closely related to *A. ellipsoidea*.

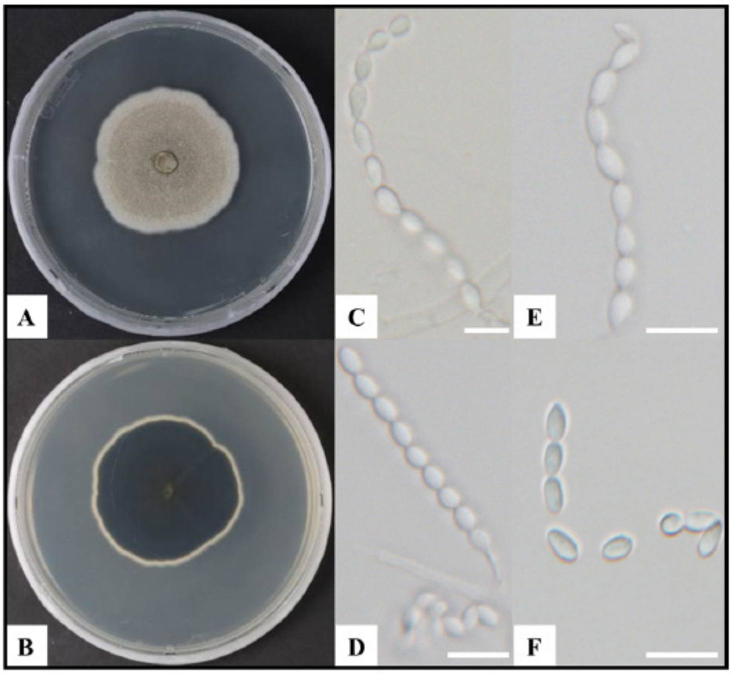

Fig. 1. Cultural and morphological characteristics of *Acrophialophora ellipsoidea* KNU-US-1802E. A, B, Colony on potato dextrose agar (Front and reverse sides, respectively) after 6 days at 35°C; C, D and E, Chains of conidia on phialides; F, conidia. (Scale bars = 10 μm).

Molecular phylogeny of the KNU-US-1802E isolate

In studying the phylogenetic relationship between isolate KNU-US-1802E and the previously reported *A. ellipsoidea*, their ITS regions and a portion of their tub2 gene sequences were compared and analyzed. After the sequencing analysis, sequences of 507 bp and 488 bp were obtained from the ITS region and tub2 gene, respectively. BLAST search results indicated that the ITS region and the partial tub2 gene sequence showed 100% and 99% similarities, respectively, with *A. ellipsoidea* CGMCC 3.15255 collected from China. The concatenated sequences of the ITS region and tub2 gene were used to determine the molecular relationships between the sequences of *A. ellipsoidea* retrieved from GenBank (Table 2). A neighbor-joining method generated a phylogenetic tree showing that the strain KNU-US-1802E clustered in the same clade as other *Acrophialophora* strains, indicating that KNU-US-1802E is a strain of *A. ellipsoidea* (Fig. 2). Thus, fungal strain KNU-US-1802E was identified as *A. ellipsoidea*, and the fungal isolate KNU-US-1802E was deposited in the National Institute of Biological Resources (NIBRFGC000502237).

Genus *Acrophialophora* has been known to be a monophyletic and belongs to the family Chaetomiaceae [1]. Some species of these fungi can produce highly active laccase and cellulose as well as useful thermostable enzymes [14], while other species have been predicted to be emerging opportunistic pathogens in humans, associated with keratitis pulmonary colonization and infection [15] and devastating cerebral infections requiring intensive antifungal therapy [16]. According to previous reports, *A. ellipsoidea* is thermotolerant, having ideal growth temperatures of 37~40°C and maximum growth temperatures near 50°C, which are key to its acceptance into the *Acrophialophora* genus [6]. Therefore, the fungi can produce thermostable enzymes that help the food and paper industries and enhance agriculture, notwithstanding the fact that the species can cause infections in humans. Further studies are required to provide in-depth knowledge about this species. In this study, we report *Acrophialophora ellipsoidea* for the first time in Korea.

Table 2. List of the sequences used in this study

Species name	Strain No.	GenBank assession numbers	
		ITS	tub2
Acrophialophora acuticonidiata ^{T}	CGMCC 3.17245	KJ026975	KJ147441
A. angustiphialis	CGMCC 3.15257	KJ026971	KJ147437
A. angustiphialis ^{T}	CGMCC 3.15258	KJ026972	KJ147438
A. cinerea ^{T}	GZDXIFR-H-57-1	DQ243694	KP143110
A. ellipsoidea	LC1842	KJ026970	KJ147436
A. ellipsoidea ^{T}	CGMCC 3.15256	KJ026969	KJ147435
A. ellipsoidea	CGMCC 3.15255	KJ026967	KJ147433
A. ellipsoidea	**NIBRFGC000502237**	LC485179	LC485180
A. furcate ^{T}	GZDXIFR-H-104-1	DQ243695	KP143113
A. fusispora ^{T}	CBS 380.55	KP233037	KP233043
A. jiangsuensis ^{T}	HC48-1	KF719171	KP143112
A. liboensis ^{T}	CGMCC 3.18309	KP192127	KP999978
A. levis	CBS 484.70	KP233038	KP233044
A. major ^{T}	GZDXIFR-H-57-2	DQ243696	KP143116
A. nainiana ^{T}	CBS 100.60	KP233036	KP233042
Chaetomium thermophilum var. coprophilum ^{T}	ATCC 16451	JF412013	KP336893

^{T} Type strain; bold letters: the isolates used in this study

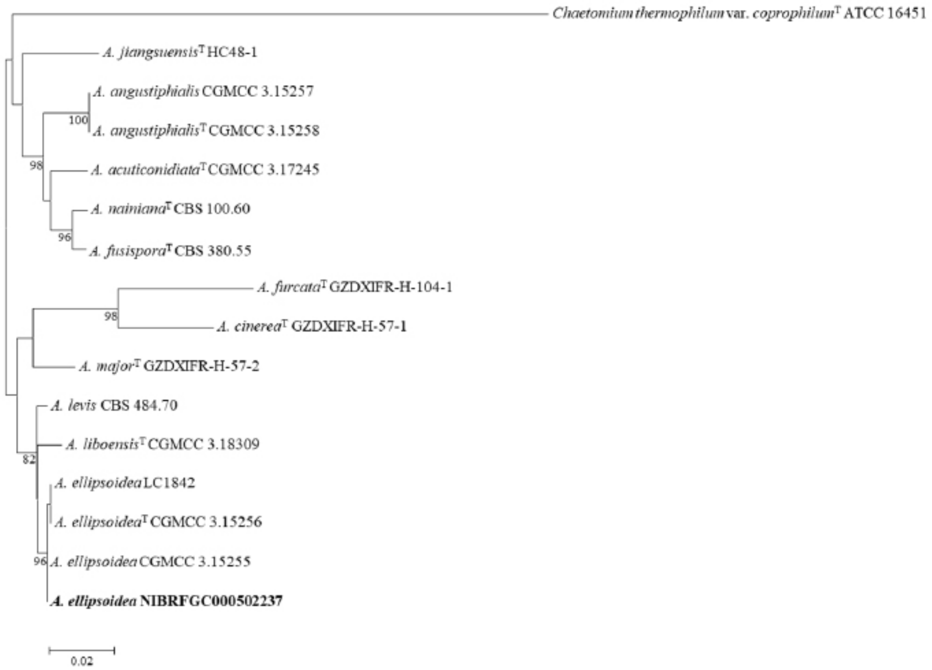

Fig. 2. Phylogenetic tree constructed with neighbor-joining (NJ) method, based on the concatenated ITS and tub2 gene sequences, shows the phylogenetic position of *Acrophialophora ellipsoidea* KNU-US-1802E among members of the genus *Acrophialophora*. The strain isolated in this study is shown in boldface. Bootstrap values (based on 1,000 replications) are shown at the branch points. *Chaetomium thermophilum* var. *coprophilum* ^{T} ATCC 16451 was used as an outgroup. Bar means 0.02 substitutions per nucleotide position.

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