The Korean Journal of Mycology www.kjmycology.or.kr

RESEARCH ARTICLE

Aphanoascus fulvescens: A New Record from Crop Field Soil in Korea

Sun Kumar Gurung[1], Mahesh Adhikari[1], Setu Bazie[1], Hyun Seung Kim[1], Hyun Gu Lee[1], Hyang Burm Lee[2], Youn Su Lee[1*]

[1]Division of Biological Resources Sciences, Kangwon National University, Chuncheon 24341, Korea
[2]Division of Food Technology, Biotechnology and Agrochemistry, College of Agriculture and Life Sciences, Chonnam National University, Gwangju 61186, Korea

*Corresponding author: younslee@kangwon.ac.kr

Abstract

Aphanoascus fulvescens KNU16-343 was isolated from crop field soil and identified based on morphological and molecular characteristics. The shape and size of conidia and conidiophores, as well as the internal transcribed spacer region of rDNA, confirmed that the isolate was *A. fulvescens*. This is the first report of this fungal species in Korea.

Keywords: *Aphanoascus fulvescens*, Internal transcribed spacer, Micromorphology

Introduction

The ascomycete genus *Aphanoascus* consists a large number of species characterized by spherical, lenticular ascospores, pale to dark brown ascomata either discoid or oblate, with or without an equatorial rim, pale to dark brown with a reticulate, pitted or verrucose wall [1]. The genus *Aphanoascus* was first described in 1890 by Zukal [1, 3]. *Aphanoascus fulvescens* is mostly found in soil and dung, living as a keratinophilic saprotroph [2]. Although this fungus is a pseudodermatophyte, it is opportunistic and has been reported to cause infection in humans and other mammals [3, 4]. In this study, to assess fungal diversity in Korea, we collected fungal strains from the soil of different crop fields. Among the fungal isolates, we encountered *A. fulvescens*, which has not been reported before in Korea. The purpose of the study was to confirm the newly identified isolate as *A. fulvescens,* by studying its morphology and phylogenetic status, based on rDNA sequence analysis.

Materials and Methods

Soil sampling and isolation of fungi

Soil samples were collected in 2013 from crop fields at various locations in Gyeongnam

OPEN ACCESS

Kor. J. Mycol. 2017 June, 45(2): 107-113
https://doi.org/10.4489/KJM.20170013

pISSN : 0253-651X
eISSN : 2383-5249

Received: 11 May, 2017
Revised: 26 May, 2017
Accepted: 29 May, 2017

city (N 35.081337°, E 128.273832°), Gyeongsangnam-do, Korea. Samples were collected from a depth of 0~15 cm, air-dried, and stored in plastic bags at 4°C. Fungi were isolated using a conventional dilution technique [5] and cultured on potato dextrose agar (PDA; Difco, Detroit, MI, USA) supplemented with 100 mg/L chloramphenicol (a bacteriostatic agent) for 5~7 days at 25°C until fungal colonies were observed. The pure cultures were maintained on PDA slants at 4°C for further use.

Morphological characterization

Five different types of media were used for the morphological characterization of the study isolate (KNU16-343): PDA, oatmeal agar (OMA), yeast extract sucrose agar (YESA), malt extract agar (MEA), and Czapek yeast extract agar (CYEA). All growth media were prepared according to Samson [6]. The isolate KNU16-343 was cultured in 9 cm petri dishes with three-point inoculation and incubated in the dark at 25°C for 7 days. Obverse and reverse colony colors, as well as the degree of speculation, were determined. An HK 3.1 CMOS digital camera (KOPTIC, Seoul, Korea), attached to an Olympus BX50F-3 microscope (Olympus, Tokyo, Japan), was used to capture microscopic images of the fungal isolate. Scanning electron microscopy on a LEO Model 1450VP Variable Pressure Scanning Electron Microscope (Carl Zeiss, Oberkochen, Germany) was used to observe and capture the micro-morphological features of the fungal isolate.

DNA extraction, PCR amplification, sequencing, and data analysis

Genomic DNA was extracted from 1-week-old colonies grown on PDA media, using a DNeasy Plant Mini Kit (Qiagen, Germantown, MD, USA) following the manufacturer's instructions. The internal transcribed spacer region (ITS) was amplified using ITS1 (5'-TCCGTAGGTGAACCTGCG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') primers [7]. The amplified PCR products were sequenced on an Applied Biosystems 3730 DNA analyzer (Foster City, CA, USA).

Phylogenetic analysis

Sequences were compared with reference ITS sequences retrieved from GenBank (National Center for Biotechnology Information), using basic local alignment search tool (BLAST) software [8]. This newly identified fungal isolate was deposited in the National Institute of Biological Resources (NIBR) under the deposition number NIBRFG0000499481. The annotated nucleotide sequence of the KNU16-343 isolate was deposited in GenBank with the accession number KY906225. Molecular Evolutionary Genetics Analysis (MEGA 6.0) software [9] was used for the alignment of all sequences. The phylogenetic tree was generated by the neighbor-joining method, utilizing the Kimura 2-parameter model with a bootstrap analysis of 1,000 replications for each clade.

Results and Discussion

Aphanoascus fulvescens (Cooke) Apinis, Mycopathologia et Mycologia Applicata 35: 99 (1968)

Morphology of the KNU16-343 isolate

The KNU16-343 isolate attained a diameter of 21~24 mm within 7 days when grown on YESA media at 25°C. Detailed morphological features of the fungal isolate KNU16-343 are shown in Fig. 1. The front side of the mycelium was white, while the rear was black (Fig. 1A, 1F). Sporulation was moderate to dense and the conidia were present in mass, with irregular form and smooth surface. On CYEA media, the isolate attained a diameter of 24~26 mm within 7 days at 25°C. The dorsal side of the mycelium was white, whereas the ventral side was hairy and green in the middle (Fig. 1B, 1G). Sporulation was moderately dense, and the conidia were seen in mass, with irregular form and smooth surface. The KNU16-343 isolate attained a diameter of 18~21 mm within 7 days when grown on PDA media at 25°C. The dorsal side of the mycelium was white, whereas the ventral side was pale brown in the middle (Fig. 1C, 1H). Sporulation was moderately dense, and the conidia were seen in mass, with irregular form and smooth surface. On MEA media, the KNU16-343 isolate attained a diameter of 22~23 mm within 7 days at 25°C. The front and rear sides of the mycelium were black (Fig. 1D, 1I). On OMA media, the isolate attained a diameter of 35~37 mm within 7 days at 25°C. The front and rear sides of the mycelium were dark black

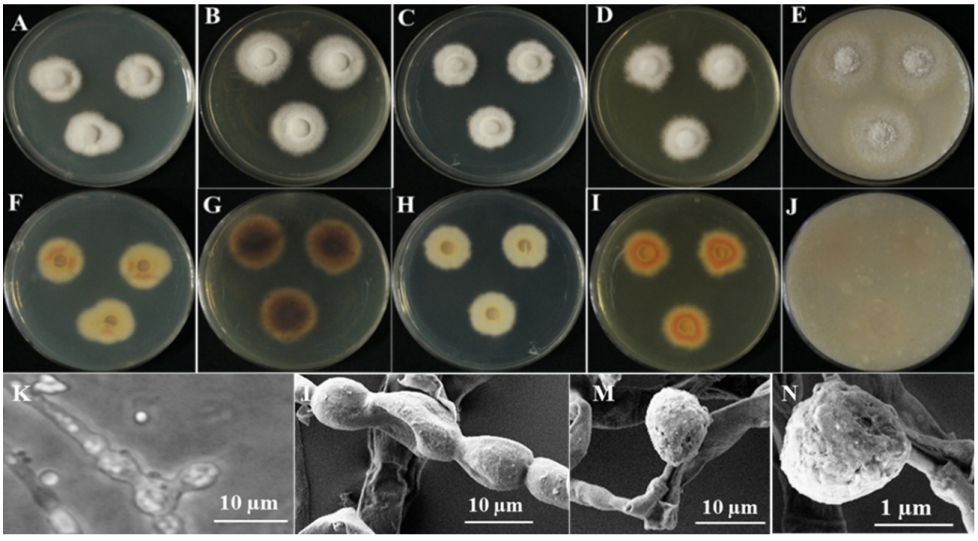

Fig. 1. Morphology of the isolate *Aphanoascus fulvescens* KNU16-343, grown for 7 days on YESA, CYEA, PDA, MEA, and OMA at 25°C. Obverse colonies (A~E) and reverse colonies (G~J) grown on YESA, PDA, OMA, CYEA and MEA, from left to right. Microscopic images of ascospores (K, L), and scanning electron microscopic images of ascospores (M, N). YESA, yeast extract sucrose agar; CYEA, Czapek yeast extract agar; PDA, potato dextrose agar; MEA, malt extract agar; OMA, oatmeal agar.

(Fig. 1E, 1J). Sporulation was moderate, and the conidia were present in mass, with irregular form and rough surface.

The isolate displayed club-shaped conidia. Hyphae were hyaline, branched, and 1.6~2.7 μm wide. Ascospores were ellipsoidal and lens or disc shaped. Ascomata were smooth and thick walled and asci were ellipsoidal (Fig. 1K, 1N). Detailed comparisons of the morphological characteristics of the KNU16-343 isolate with those previously reported for *A. fulvescens* [1, 10] are described in Table 1.

Molecular phylogeny

The KNU16-343 isolate was obtained from the crop field soil of Gyeongnam city, Gyeongsangnam-do, Korea. The isolates used to construct the phylogenetic tree are shown in Table 2, along with their GenBank accession numbers. The KNU16-343 isolate was most closely related to *A. fulvescens* (NBRC 8390) and formed a monophyletic group, supported by a bootstrap value of 99% (Fig. 2). Phylogenetic analysis revealed that the isolate was *A. fulvescens*.

The KNU16-343 isolated from the crop field soil was presumed to be *Aphanoascus*

Table 1. Morphological comparison between the studied isolate KNU16-343 and a previously described isolate of *Aphanoascus fulvescens*

Characteristics		Study isolate *A. fulvescens*	Previously reported *A. fulvescens* [a]
Colony	Diameter (mm)	PDA = 21～24, MEA = 22～23, YESA = 21～24, CYEA = 24～26 and OMA = 35～37	NA
Ascomata	Size	288~497 μm	290~500 μm
	Shape	smooth and thick walled, spherical	superficial, solitary or in clusters, spherical, glabrous,
Asci	Size	9.4~10 × 6.9~9 μm	9.5~11 × 7~9.0 μm
	Shape	ellipsoidal, 8 spored	numerous, subglobose to ellipsoidal, 8-spored, evanescent
Ascospores	Structure	lenticular, oblate pale to dark brown	lenticular, discoid or oblate, pale to dark-brown, one-celled, with reticulate, pitted or verrucose wall
	Size	3.4~3.9 × 2.2~3.4 μm	3.5~4 × 2.5~3.5 μm
Conidia	Structure	hyaline, smooth intercalary conidia	terminal and lateral, sessile, solitary, hyaline, smooth, intercalary conidia frequent, cylindrical
	Size	10.9~14.8 × 3.9~5.4 μm	11~15 × 4.0~5.5 μm
Hyphae	Structure	hayaline, smooth, forming globose mass	hyaline, branched, septate, thick-walled, sometimes forming globose mass
	size	14.9~16.8 × 3.6~5.7 μm	15~17.5 × 3.7~6.0 μm

PDA, potato dextrose agar; MEA, malt extract agar; OMA, oatmeal agar.
[a]Source of description [1, 10, 12]; NA= Not available

Table 2. *Aphanoascus fulvescens* and related species used in phylogenetic tree generation in this study

Serial No.	Species	Isolate No.	GenBank Accession No.
1	*Aphanoascus arxii*	CBS 466.88	AJ315843
2	*Aphanoascus canadensis*	UAMH 4574	AJ439435
3	*Aphanoascus clatratus*	IMI 329400	AJ439436
4	*Aphanoascus duras*	FMR 5651	AJ439434
5	*Aphanoascus cubensis*	FMR 4220	AJ439432
6	*Aphanoascus foetidis*	CBS 452.75	AJ439448
7	*Aphanoascus fulvescens*	NBRC 8390	JN943431
8	*Aphanoascus hipanicus*	IMI 335379	AJ439438
9	*Aphanoascus keratinophilus*	IMI 319010	AJ133436
10	*Aphanoascus mephitalis*	IMI 151084	AJ439439
11	*Aphanoascus pinarensis*	FMR 4221	AJ439433
12	*Aphanoascus punsolae*	IMI 334818	AJ439440
13	*Aphanoascus reticulisporus*	NBRC 32373	JN943435
14	*Aphanoascus saturnoideus*	IMI 318416	AJ439442
15	*Aphanoascus terreus*	NBRC 32655	JN943438
16	*Aphanoascus verrucosus*	NBRC 32382	JN943439
17	*Ctenomyces serratus*	CBS 187.61	AY176733
18	*Aphanoascus fulvescens*	KNU16-343	KY906225

based on its morphological characteristics (colony, hyphae, conidia, and ascospores), and it reasonably fit the description of *A. fulvescens* [10]. Moreover, the shape and size of the ascospores were similar to those of *A. fulvescens*, as described by Cano and Guarro [1]. We also observed colony characteristics using different media and found differences in growth and color (Fig. 1A~1J). In addition, neighbor-joining phylogenetic ITS sequence analysis of different species of Aphanoascus NBRC 8390 was in close proximity (99% similarity) with that of our study isolate KNU16-343 as *A. fulvescens*. In conclusion, based on the ITS sequence analysis, the present isolate was found to be closely related with *A. fulvescens* (Fig. 2), which was also strongly supported by morphological comparison (Table 1). *A. fulvescens* has been reported as a human pathogen by different authors [3, 4, 11].

Acknowledgements

This work was supported by a grant from the NIBR, funded by the Ministry of Environment of the Republic of Korea, and the University-Industry Cooperation Foundation of Kangwon National University.

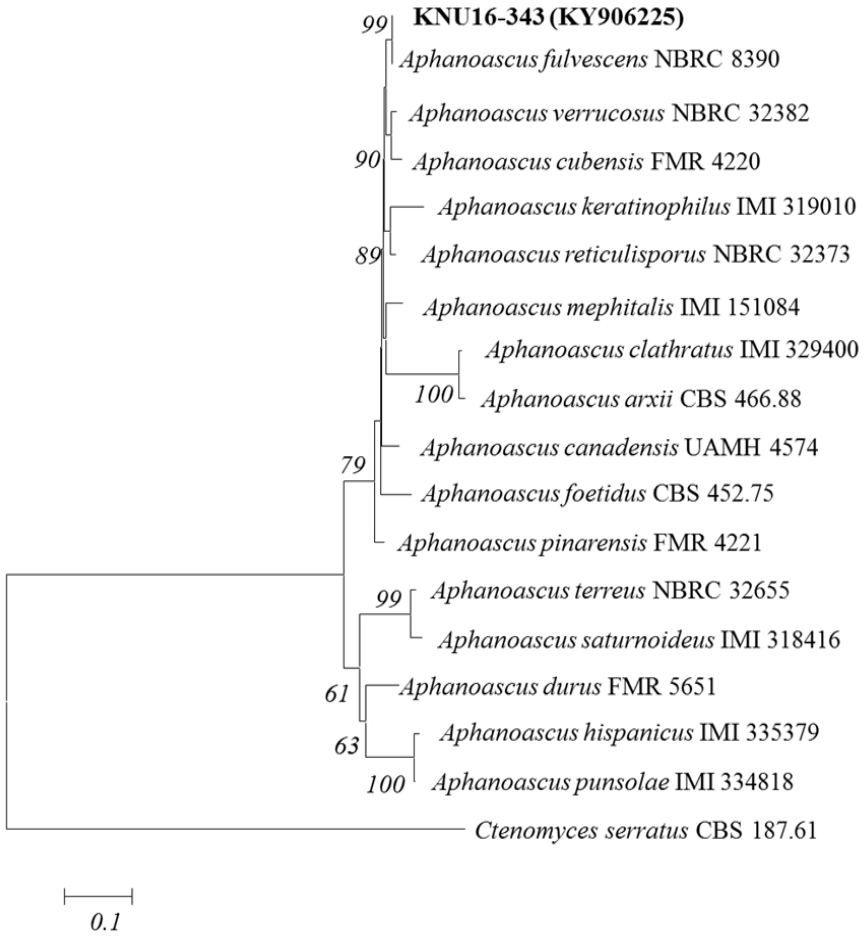

Fig. 2. Neighbor-joining phylogenetic analysis of partial 18S-ITS1-5.8S-ITS2-28S rDNA sequences of KNU16-343. The phylogenetic tree was constructed using the MEGA 6 program. The sequence obtained in the study in shown in bold. Numerical values (>60) on branches are the bootstrap values as percentages of bootstrap replication from a 1,000 replicate analysis. The scale bar represents the number of substitutions per site.

REFERENCES

1. Cano J, Guarro J. The genus *Aphanoascus*. Mycol Res 1990;94:355-77.
2. Cano J, Sagues M, Barrio E, Vidal P, Castaneda RF, Gene J, Guarro J. Molecular taxonomy of *Aphanoascus* and description of two new species from soil. Stud Mycol 2002;47:153-64.
3. Rippon JW, Lee FC, McMillen S. Dermatophyte infection caused by *Aphanoascus fulvescens*. Arch Dermatol 1970;102:552-5.
4. Marin G, Campos R. Dermatophytosis caused by *Aphanoascus fulvescens*. Sabouraudia 1984;22:311-14.
5. Davet P, Rouxel F. Detection and isolation of soil fungi. Enfield: Science; 2000.
6. Samson RA. Food and indoor fungi. Utrecht: CBS-KNAW Fungal Biodiversity Centre; 2010.

7. White TJ, Bruns TD, Lee SB, Taylor JW. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, editors. PCR protocols: a guide to methods and applications. San Diego: Academic Press; 1990. p. 315-22.
8. National Center for Biotechnology Information. Basic Local Alignment Search Tool [Internet]. Bethesda (MD): National Center for Biotechnology Information; 2015 [cited 2017 April 21]. Available from: https://blast.ncbi.nlm.nih.gov/Blast.cgi.
9. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Mol Biol Evol 2013;30:2725-9.
10. Apinis AE. Relationships of certain keratinophilic Plectascales. Mycopathol Mycol Appl 1968;35:97-104.
11. Todaro F, Griseo G, Urzi C. A propos d'un cas de *tinea pedis* par *Anixiopsis fulvescens* var. *fulvescens* (Cooke) De Vries. Bull Soc Francaise Mycol Méd 1984;13:239-42.
12. Kidd S, Halliday CL, Alexiou H, Ellis D. Descriptions of medical fungi. 3rd ed. Panorama: David Ellis; 2016.