

Characterization of Two New Records of Zygomycete Species Belonging to Undiscovered Taxa in Korea

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Abstract During a biodiversity survey of undiscovered taxa in Korea, two zygomycetous fungal strains were isolated. The first strain, EML-FSDY6-1 was isolated from a soil sample collected at Dokdo Island in the East Sea of Korea in 2013, and the second strain, EML-DG-NH3-1 was isolated from a rat dung sample collected at Chonnam National University garden, Gwangju, Korea in 2014. Based on the morphological characteristics and phylogenetic analysis of the internal transcribed spacer, 18S and 28S rDNA, actin and translation elongation factor-1 α genes. EML-FSDY6-1 and EML-DG-NH3-1 isolates were confirmed as zygomycete species, *Absidia pseudocylindrospora* and *Absidia glauca*, respectively. Neither species has previously been described in Korea.

Keywords *Absidia glauca*, *A. pseudocylindrospora*, Dokdo, Undiscovered taxa, Zygomycete fungi

The Zygomycota is a group of related basal clades comprising the subphyla Mucoromycotina, Entomophthoromycotina, Mortierellomycotina, Zoopagomycotina, and Kickxellomycotina [1]. Members of the Zygomycota generally reproduce asexually via sporangiospores and sexually via the formation of zygospores. The Mucorales is the largest order of the Zygomycota and include 56 genera and approximately 300 species. Most mucoralean species (called pin moulds) live as saprophytes growing on different organic substrates such as fruit, dung, plant and soil. Some species are parasites or pathogens of animals, plants and fungi. A few species cause human and animal disease zygomycosis, as well as allergic reactions. Zygomycete fungi belong to undiscovered taxa in Korea, due to lack of the species diversity information

about it. According to the Korean species list of fungi [2], approximately 25 species of zygomycetes were known in Korea.

The genus *Absidia* belongs to the order Mucorales within the Cunninghamellaceae family. This genus was originally described by van Tieghem [3] with the type species *Absidia reflexa* Tieghem. To date, 21 species of *Absidia* have been recognized [4]. *Absidia* species are characterized by the production of stolons and sporangiophores bearing pyriform columellate sporangia with deliquescent walls and a septum below the apophysis and by zygospores enveloped in appendages from the suspensors. *Absidia* is closely related to *Rhizopus*; however, unlike the sporangiophores of *Rhizopus*, those of *Absidia* never arise opposite the rhizoids. *Absidia* also resembles *Phycomyces* with regard to its manner of sexual reproduction. However, in *Phycomyces*, large rough-walled zygospores are formed between tong-like suspensors [5, 6].

Subsequent to van Tieghem's original description of the genus *Absidia*, some species of this genus were transferred to other genera such as *Tieghemella* Berl. & De Toni, *Mycocladius* Beauverie, *Pseudoabsidia* Bainier, and *Proabsidia* Vuill. However, with the exception of *Lichtheimia*, all are regarded as synonyms of *Absidia* [4, 5].

Absidia species typically exhibit rapid growth at temperatures ranging from 25°C to 34°C. They are frequently isolated from soil, dung, and dead or dying plant tissue [7-9]. Several species of *Absidia* are implicated in diseases affecting humans and animals, such as mucormycosis [10, 11]. Recently, Hoffmann *et al.* [7] revised the classification of *Absidia*

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based on physiological, phylogenetic, and morphological characteristics. They observed temperature-dependent growth differences and divided the species into three groups—thermotolerant (optimal growth between 37°C and 45°C), mesophilic (optimal growth between 25°C and 34°C), and mycoparasitic (species that are potentially parasitic to other fungi within the order Mucorales and which exhibit optimal growth between 16°C and 20°C).

Twenty five zygomycetous fungi which have been described in Korea were assigned in 12 genera and 8 families. Within the Mucoraceae, only 8 species have been described [2]. Data regarding the Mucoraceae of Korea, especially with respect to *Absidia* species, are lacking. A new species, *Absidia koreana*, was isolated from a soil sample collected at Dokdo Island in 2013 [12]. Almost all species belonging to the Mucoraceae in Korea have been discovered from soil, and to our knowledge, there are no previous published literature records of this family from animal dung.

Recently, several studies have evaluated the phylogenetic relationships between species of mucoralean fungi and other zygomycetes, using combined sequences of internal transcribed spacer (ITS) region, 18S and 28S rDNA, actin (*act-1*), and translation elongation factor-1 α (*EF-1 α*) genes [13-17].

The objective of the present study was to characterize two unrecorded zygomycete species, *Absidia pseudocylindrospora* and *Absidia glauca*, based on the morphological characteristics and sequence analyses.

MATERIALS AND METHODS

Isolation of fungal strains from soil and rat dung sample. Soil samples were collected from Dongdo (eastern islet) of Dokdo (37°14'21.3" N, 131°52'04.4" E) Island in the East Sea of Korea in 2013. Samples were obtained at a depth of 10~15 cm, transported in sterile 50-mL conical tubes, and stored at 4°C until examination. Fungi were isolated using 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 10⁻¹ to 10⁻⁴ were then made. An aliquot of 0.1 mL from each dilution was transferred to potato dextrose agar (39 g PDA in 1 L of deionized water; Becton, Dickinson and Co., Sparks, MD, USA) and incubated at 27°C for 3~7 days.

Rat dung sample was collected from the garden of Chonnam National University, Gwangju, Korea in 2014 using sterile forceps. The samples were transferred to the laboratory in plastic bags, placed onto sterile moist Whatman's filter paper in a Petri dish, and incubated in a moist chamber at 25°C for 7 days. Hyphal tips were transferred to PDA plates using a stereomicroscope.

To isolate pure cultures, individual colonies of varied morphologies were transferred to PDA plates. Pure isolates such as EML-FSDY6-1 and -2, EML-DG-NH3-1 and -2 were maintained in PDA slant tubes and stored in 20% glycerol at -80°C at the Environmental Microbiology Laboratory

Fungarium, Chonnam National University, Gwangju, Korea. The dry cultures (EML-FSDY6-1 and EML-DG-NH3-1) were preserved at Chonnam National University Fungal Collection (CNUFC), Division of Food Technology, Biotechnology and Agrochemistry, College of Agriculture and Life Sciences, Gwangju, Korea. The ex-type (EML-FSDY6-1 and EML-DG-NH3-1) strains were also deposited as glycerol stock at -80°C at the Culture Collection of National Institute of Biological Resources (NIBR), Incheon, Korea as KOSPFGC000002026 and KOSPFGC000002025, respectively.

Morphological studies. To obtain samples for microscopic examination and growth rate determination, EML-FSDY6-1 and EML-DG-NH3-1 were cultured on each of three different media. The media used were PDA, synthetic mucor agar (SMA; 40 g dextrose, 2 g asparagine, 0.5 g KH₂PO₄, 0.25 g MgSO₄·7H₂O, 0.5 g thiamine hydrochloride, and 15 g agar, in 1 L of deionized water), and malt extract agar (33.6 g MEA in 1 L of deionized water; Becton, Dickinson and Co.). The plates were incubated at 20°C, 23°C, 25°C, 27°C, 30°C, 35°C, and 37°C in the dark for 7 days. Samples were mounted in lactophenol solution (Junsei Chemical Co. Ltd., Tokyo, Japan) and examined under a light microscope (DFC290; Leica Microsystems, Wetzlar, Germany). Fine fungal structures were observed using scanning electron microscopy (Hitachi S4700; Hitachi, Tokyo, Japan). Samples were cultured on PDA medium in the dark at 27°C for 7 days, fixed in 2.5% paraformaldehyde-glutaraldehyde in 0.05 M phosphate buffer (pH 7.2) for 2 hr, and then washed with cacodylate buffer (Junsei Chemical Co. Ltd.). Cellular membranes were preserved by fixing the samples in 1% osmium tetroxide (Electron Microscopy Sciences, Hatfield, PA, USA) diluted in cacodylate buffer for 1 hr, washing again in cacodylate buffer, dehydrating in graded ethanol (Emsure, Darmstadt, Germany) and isoamyl acetate (Junsei Chemical Co. Ltd.), and drying in a fume hood. Finally, samples were sputter-coated with gold and observed under a Hitachi S4700 field emission scanning electron microscope at the Korea Basic Science Institute, Gwangju, Korea.

DNA extraction, PCR, and sequencing. Total genomic DNA was extracted directly from the mycelia of fungal isolates using the HiGene Genomic DNA Prep Kit (BIOFACT Co., Daejeon, Korea). The ITS region; small subunit of 18S rDNA, large subunit of 28S rDNA, actin (*act-1*), and elongation factor 1 α (*EF-1 α*) sequences were amplified with the primer pairs ITS1, ITS4 [18]; NL1, NS4 [14]; LROR, LR5F [19, 20]; Act-1, Act-4R [21]; and MEF-11, MEF-4 [14], respectively. The PCR amplification mixture (total volume, 20 μ L) contained 10 ng of fungal gDNA template, 5 pmol/ μ L of each primer, and Accupower PCR Premix (*Taq* DNA polymerase, dNTPs, buffer, and a tracking dye; Bioneer Co., Daejeon, Korea). PCR products were purified using the Accuprep PCR Purification Kit (Bioneer Co.) according to the manufacturer's instructions. DNA sequencing was performed on an ABI 3700 Automated DNA sequencer

Table 1. Sequences used in this study, including GenBank accession numbers

Taxon name	Collection No. (Isolate No.)	GenBank accession No.				
		ITS	18S	28S	act-1	EF-1 α
<i>Absidia anomala</i>	CBS125.68 (T)	EF030523	-	-	-	-
<i>A. californica</i>	CBS314.78	JN205816	-	-	-	-
<i>A. californica</i>	CBS126.68 (T)	AY944872	-	-	-	-
<i>A. coerulea</i>	CBS101.28	JN205811	-	-	-	-
<i>A. coerulea</i>	CBS104.08	JN205811	-	-	-	-
<i>A. coerulea</i>	FSU1608	AY944869	-	-	-	-
<i>A. cuneospora</i>	CBS101.59 (T)	EF030524	-	-	-	-
<i>A. cuneospora</i>	CBS102.59	JN205819	-	-	-	-
<i>A. cylindrospora</i>	FSU906	AY944889	-	-	-	-
<i>A. cylindrospora</i>	CBS100.08	JN205822	-	-	-	-
<i>A. fusca</i>	CBS102.35 (T)	JN205814	-	-	-	-
<i>A. glauca</i>	CBS101.08 (T)	JN205810	-	-	-	-
<i>A. glauca</i>	CBS100.48	JN205820	-	-	-	-
<i>A. glauca</i>	NRRL2799	AY944876	AF157118	AF157172	AJ287135	X54730
<i>A. glauca</i>	EML-DG-NH3-1	KU923829	KU923820	KU923827	KU923823	KU923821
<i>A. glauca</i>	EML-DG-NH3-2	KU923822	KU923826	KU923828	KU923825	KU923824
<i>A. heterospora</i>	SHTH021	JN942683	-	-	-	-
<i>A. koreana</i>	IFS45-1	-	KR030063	KT321298	KR030056	KR030058
<i>A. koreana</i>	IFS45-2	-	KR030062	KT321299	KR030057	KR030059
<i>A. macrospora</i>	CBS697.68 (T)	AY944882	-	-	-	-
<i>A. macrospora</i>	FSU4746	AY944882	EU736276	EU736303	AY944760	EU736249
<i>A. pseudocylindrospora</i>	CBS100.62 (T)	EF030526	JN206591	-	-	-
<i>A. pseudocylindrospora</i>	CBS480.66	EF030525	-	-	-	-
<i>A. pseudocylindrospora</i>	EML-FSDY6-1	KU923816	KU923818	KU923813	KU923812	-
<i>A. pseudocylindrospora</i>	EML-FSDY6-2	KU923817	KU923819	KU923814	KU923815	-
<i>A. psychrophilia</i>	CBS172.68	AY944874	-	-	-	-
<i>A. psychrophilia</i>	CBS128.68 (T)	-	EU736279	EU736306	AY944762	EU736252
<i>A. spinosa</i> var. <i>spinosa</i>	CBS106.08	AY944888	-	-	-	-
<i>A. spinosa</i>	ATCC22755	AY944887	EU736280	EU736307	EU736227	EU736253
<i>A. repens</i>	CBS102.32	EF030528	-	-	-	-
<i>A. repens</i>	NRRL1336	EF030527	AF113410	AF113448	AJ287136	AF157228
<i>Chlamydoabsidia padenii</i>	NRRL2977	-	AF113415	AF113453	AJ287146	AF157238
<i>Cunninghamella echinulata</i>	NRRL1375	-	EU736286	EU736313	EU736232	EU736259
<i>Cunninghamella echinulata</i>	NRRL1382	-	AF157130	AF157184	AJ287152	AF157244
<i>Dichotomocladium elegans</i>	NRRL6236	-	AF157131	AF157185	AJ287153	AF157245
<i>D. floridanum</i>	FSU8694	-	JQ775462	JQ775491	JX644526	JX644576
<i>D. robustum</i>	NRRL6235	-	JQ775466	JQ775495	JX644529	-
<i>D. sphaerosporum</i>	FSU8697	-	JQ775467	JQ775496	JX644530	JX644580
<i>Gongronella butleri</i>	NRRL1340	-	AF157137	AF157191	AJ287160	AF157252
<i>G. koreana</i>	EML-TS2Bp	-	KT321300	KP636530	KP636527	KP636528
<i>G. koreana</i>	EML-TS2Bp-2	-	KT321301	KP835542	KP835543	KP835544
<i>Halteromyces radiatus</i>	NRRL6197	-	AF157138	AF157192	AJ287161	AF157253
<i>Hesseltinella vesiculosa</i>	CBS 197.68	-	AF157140	AF157194	AJ287163	AF157255
<i>Lichtheimia corymbifera</i>	NRRL2982	-	AF113407	FJ719429	AJ287134	AF157227
<i>L. corymbifera</i>	CBS 429.75	-	JQ014052	GQ342903	GQ342831	FJ719483
<i>L. hyalospora</i>	NRRL2916	-	EU826360	EU826368	EF030531	JX644583
<i>L. hyalospora</i>	NRRL1304	-	AF157117	AF157171	AJ287132	AF157225
<i>L. ramosa</i>	FSU6197	-	JX644470	JX644503	JX644533	JX644584
<i>Umbelopsis isabellina</i>	NRRL1757	-	AF157166	AF157220	AJ287209	AF157300
<i>U. isabellina</i>	CBS 560.63	-	JN206400	-	-	-
<i>U. nana</i>	NRRL22420	-	AF157167	AF157221	AJ287210	AF157301
<i>U. nana</i>	CBS 373.67	-	JN206394	-	-	-

Bold letters indicate isolates and accession numbers determined in our study.

ITS, internal transcribed spacer; EF-1 α , elongation factor-1 α ; CBS, Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; T, ex-type strain; FSU, Friedrich Schiller University, Jena, Germany; NRRL, ARS Culture Collection, Peoria, Illinois, USA; EML, Environmental Microbiology Laboratory Fungarium, Chonnam National University, Gwangju, South Korea; ATCC, Biological Resource Center, Manassas, Virginia, USA.

EF-1 α gene sequences indicated that EML-DG-NH3-1 is closely to *A. glauca* FSU660 (GenBank accession No.

EU736225); the identity values for the act-1 and EF-1 α gene sequences were 98.7% (614/622 bp) and 99.6% (725/

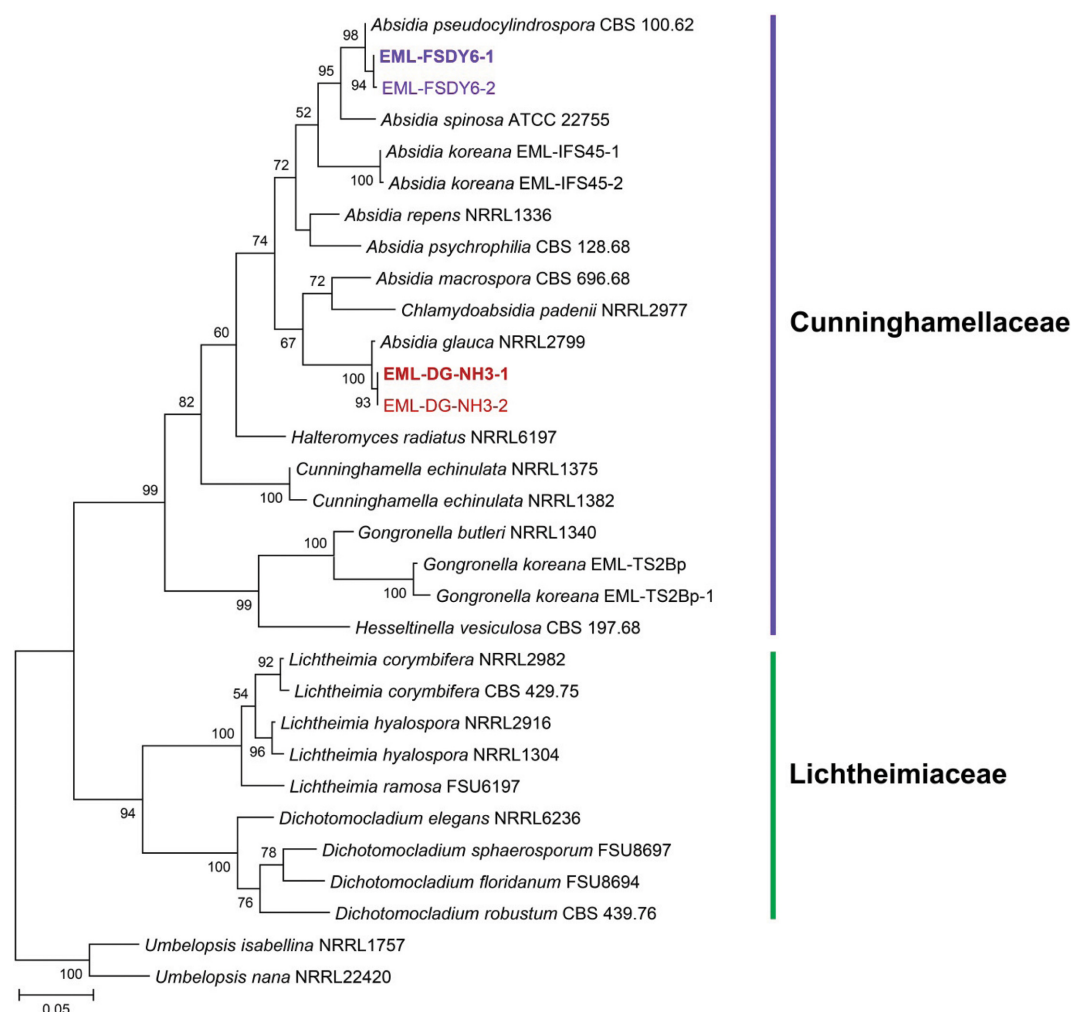


Fig. 2. Phylogenetic tree based on maximum likelihood analysis of multiple loci including 18S and 28S rDNA, actin (act-1), and translation elongation factor-1 α for *Absidia pseudocylindrospora* EML-FSDY6-1, *A. pseudocylindrospora* EML-FSDY6-2, *A. glauca* EML-DG-NH3-1, and *A. glauca* EML-DG-NH3-2. *Umbelopsis isabellina* and *U. nana* were used as outgroups. Bootstrap support values of $\geq 50\%$ are indicated at the nodes. The bar indicates the number of substitutions per position. Ex-type strains are marked as bold purple and red letters.

Table 2. Morphological characteristics of EML-FSDY6-1 and the reference species *Absidia pseudocylindrospora* on synthetic mucor agar medium at 25°C

Character	EML-FSDY6-1	<i>Absidia pseudocylindrospora</i> ^a
Colony color	Rapid growth, mouse gray, and pale gray on colony reverse	Rapid growth, pale olive-gray then mouse gray, and deep olive-buff to pale olive-gray on colony reverse
Odor	None	None
Sporangiophores	3.5~5.5 μm (average, 4.0 μm) in width, variable in length	3.0~6.5 μm in width, 45~172 μm in length
Sporangia	Pyriiform, 15.5~35.5 μm (average, 23.0 μm) in diameter	Pyriiform, 15~35 μm in diameter
Columellae	Globose, hemispherical, 9.5~19.5 μm (average, 15.5 μm) in diameter	Globose, hemispherical, 9~26 μm in diameter
Sporangiospores	Cylindrical, 2.0~3.0 \times 4.0~5.0 μm	Cylindrical, 2.5 \times 3.5~5.0 μm
Zygosporangia	Absent	Unknown
Chlamydospores	Present	Present

^aFrom the description Hesseltine and Ellis [25].

728 bp), respectively. The act-1 gene sequence of isolate EML-FSDY6-1 showed an identity value of 98.5% (599/608 bp) with *A. pseudocylindrospora* FSU5893 (GenBank accession No. EF030534). Based on the ITS sequence analysis, the two isolates were identical to *A. pseudocylindrospora* CBS 100.48 and *A. glauca* CBS 480.66 (Fig. 1). Moreover, analysis of the combined genes placed the two strains within the Cunninghamellaceae family, similar to other *Absidia* species, with well-supported branch values (Fig. 2).

Taxonomy of EML-FSDY6-1.

Absidia pseudocylindrospora C. W. Hesseltine & J. J. Ellis, Mycologia 53: 406 (1961) (Table 2, Fig. 3).

Description: Colonies exhibited rapid growth on SMA,

attaining a diameter of 82~85 mm after 4 days at 25°C. The colony color was initially white, later turning to mouse gray. The colony reverse was pale gray and regularly zonate. Sporangioophores were 3.5~5.5 µm (average, 4.0 µm) wide, appearing upright, in groups of 1~10 (average, 3~5) per whorl from a single point in the stolons, and consistently had a septum under the sporangium. Sporangia measured 15.5~35.5 µm (average, 23.0 µm) in diameter and were pyriform, deep gray, and multispored. Columellae measured 9.5~19.5 µm (average, 15.5 µm) in diameter and were globose and hemispherical. Sporangiospores were mostly cylindrical, 2.0~3.0 µm × 4.0~5.0 µm. Chlamydospores were present in the aerial mycelia. Zygosporangia were not observed on this medium.

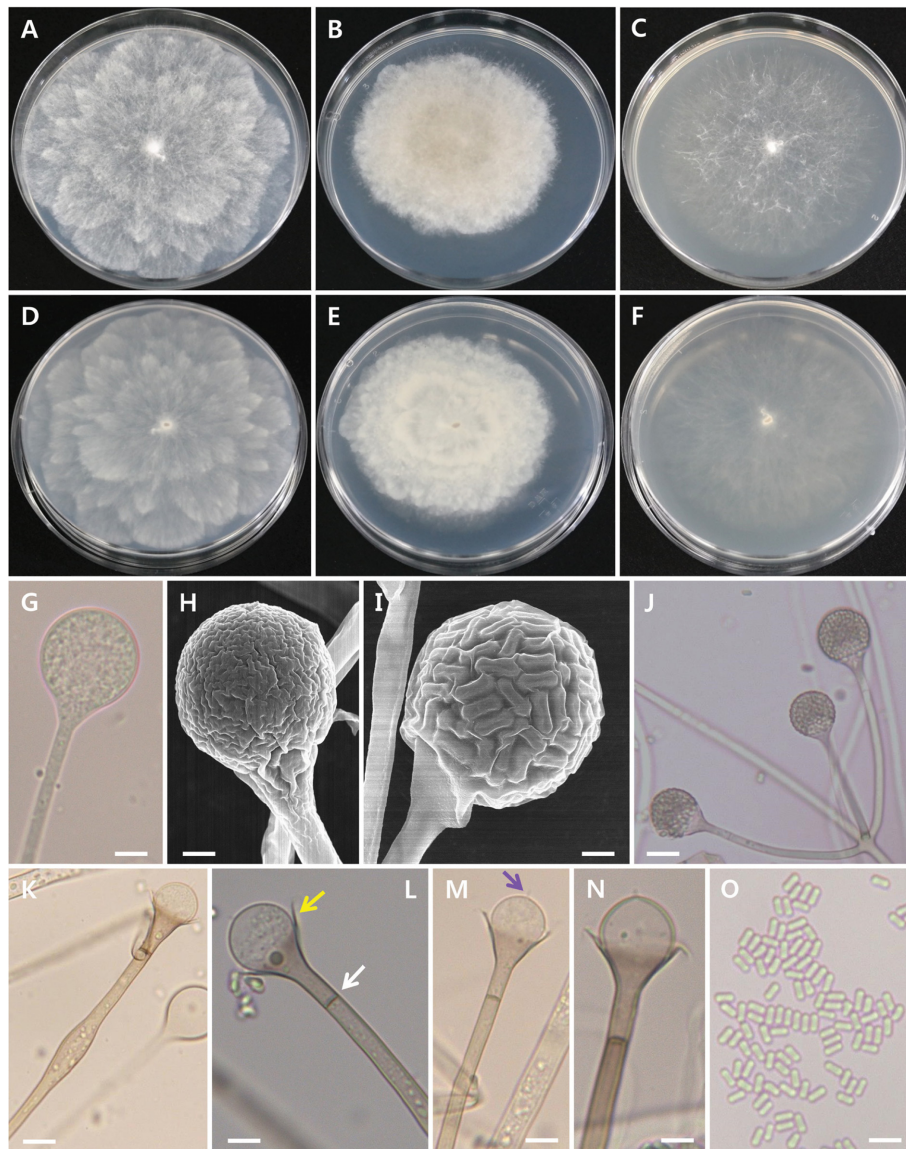


Fig. 3. Morphology of *Absidia pseudocylindrospora* EML-FSDY6-1. Colonies on synthetic mucor agar (A, D), potato dextrose agar (B, E), and malt extract agar (C, F). A~C, Obverse view; D~F, Reverse view; G, Young sporangium; H, I, Young and mature sporangia; J, Attachment of sporangiophores; K~N, Columella with collarette (yellow arrow), sporangial septum (white arrow), and projection (purple arrow); O, Sporangiospores (scale bars: G, K~O = 20 µm, H, I = 10 µm, J = 50 µm).

Table 3. Morphological characteristics of EML-DG-NH3-1 and the reference species *Absidia glauca* on synthetic mucor agar medium at 25°C

Character	EML-DG-NH3-1	<i>Absidia glauca</i> ^a
Colony color	Rapid growth, storm gray	Rapid growth, glaucous to storm gray
Odor	None	None
Sporangiophores	4.0~10.5 µm in width, variable in length	8~12 µm in width, 135~1,000 µm (average, 300~750 µm) in length
Sporangia	Globose, pyriform, 19.0~43.0 × 21.5~52 µm in diameter	Pyriform, 28.5~65.0 µm in diameter
Columellae	Globose or hemispherical, 14.5~33 × 12~27 µm	Hemispherical, 27.5~50 µm in diameter
Sporangiospores	Globose, 2.5~4.5 µm in diameter	Globose, 2.5~5.0 µm in diameter, mostly 3 µm
Zygospores	Absent	125~208 µm in diameter
Chlamydo spores	Present	Present

^aFrom the description by Ellis and Hesseltine [26].

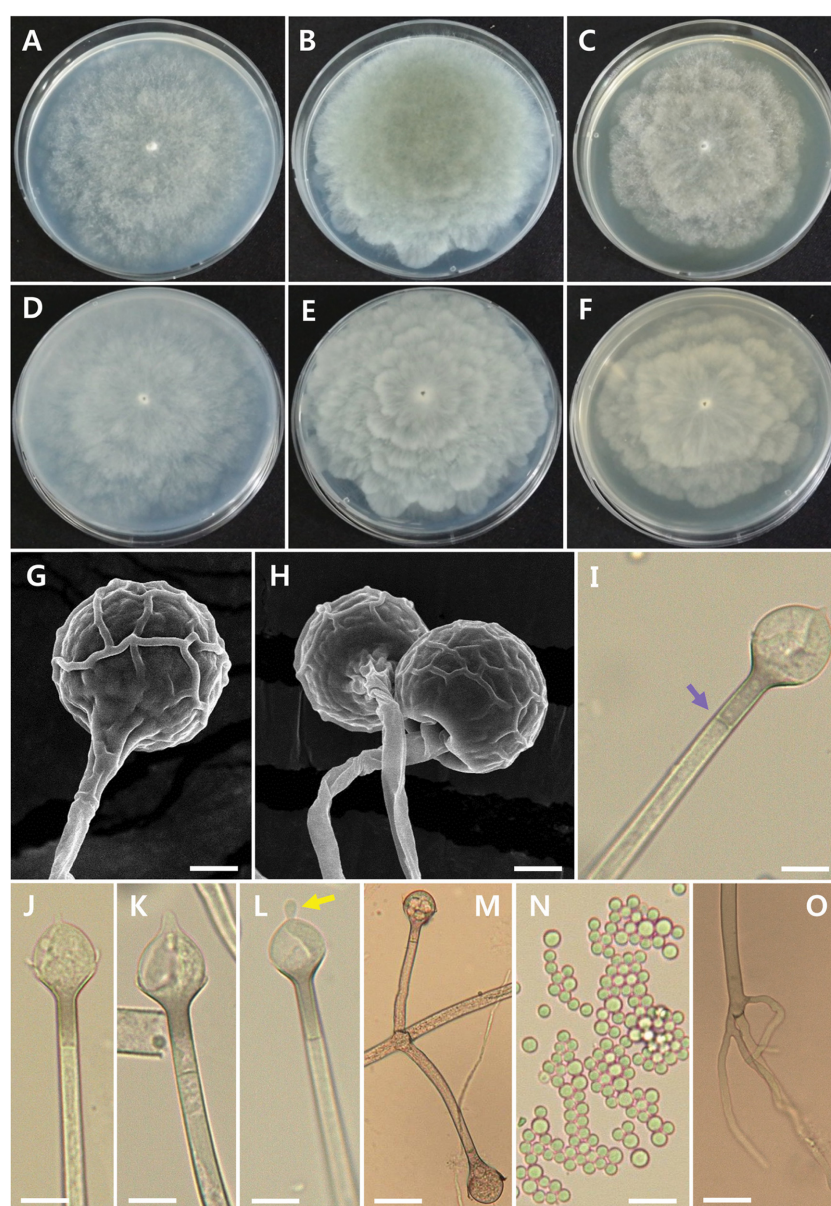


Fig. 4. Morphology of *Absidia glauca* EML-DG-NH3-1. Colonies on synthetic mucor agar (A, D), potato dextrose agar (B, E), and malt extract agar (C, F). A~C, Obverse view; D~F Reverse view; G, H, Sporangia with wall net; I~L, Columella with septum (purple arrow) and projection (yellow arrow); M, Attachment of sporangiophores; N, Sporangiospores; O, Young rhizoid (scale bars: G, I~O = 20 µm, H = 30 µm).

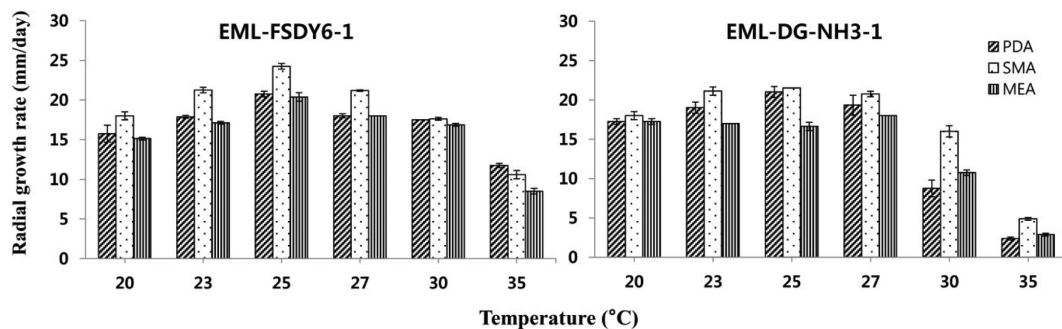


Fig. 5. Effect of temperature and culture medium on mycelial growth of *Absidia pseudocylindrospora* EML-FSDY6-1 and *A. glauca* EML-DG-NH3-1. Mycelia were grown on synthetic mucor agar (SMA), potato dextrose agar (PDA), and malt extract agar (MEA) at different temperatures, as indicated.

Taxonomy of EML-DG-NH3-1.

Absidia glauca Hagem, Skrifter udgivne af Videnskabs-Selskabet I Christiania. Mathematisk-Naturvidenskabelig Klasse 7: 43 (1908) (Table 3, Fig. 4).

≡ *Tieghemella glauca* (Hagem) Naumov, Tab. Opred. Predst. Mucor: 42 (1915)

= *Absidia septata* Tiegh., Annales des Sciences Naturelles Botanique 4: 362 (1878)

= *Absidia sphaerosporangioides* Manka & Truszk., Acta Societatis Botanicorum Poloniae 27: 54 (1958)

Description: Colonies exhibited rapid growth on SMA, attaining a diameter of 80~85 mm after 5 days at 25°C. The colony color was initially white, later turning to storm gray. The colony reverse was light gray and irregularly zonate. Mycelial growth on SMA was sparse, but sporulation was extensive. Sporangioophores were 4.0~10.5 μm wide, appearing upright, in groups of 1~8 (average, 2~4) per whorl from a single point on the stolons, and consistently had a septum under the sporangium. Sporangia were 19.0~43.0 μm × 21.5~52 μm and were globose, pyriform, and brownish yellow. The surface was covered by wall net. Columellae measured 14.5~33 μm × 12~27 μm and were globose to hemispherical. Sporangiospores were globose and measured 2.5~4.5 μm in diameter. Zygosporangia were not observed on this medium. Rhizoids were not well developed.

Mycelial growth. The isolates grew at different rates according to temperature and medium. The average growth rates of EML-FSDY6-1 on PDA, SMA, and MEA were 20.5 mm/day, 24 mm/day, and 20 mm/day, respectively; those of EML-DG-NH3-1 were 21 mm/day, 21.5 mm/day, and 16.5 mm/day, respectively. The optimal growth temperature range was 23~27°C. Slow growth was observed at 35°C and no growth was visible at 37°C (Fig. 5).

DISCUSSION

Until recently, we have reported some kinds of new and undescribed zygomycetous fungi from Dokdo Island of Korea based on current classification system using combined

sequences [12, 27]. This study was also done to discover zygomycetous taxa on that unique island. Thus, the data with regard to such an undiscovered fungal taxa can be considerably meaningful in understanding of geographical distribution of fungal species diversity in Korean Peninsula.

Similar to many other Mucorales, the *Absidia* has been distinguished based on morphological features and growth temperature. With regard to phylogenetic and physiological studies on *Absidia*, Hoffmann *et al.* [7] showed that species of *Absidia* exhibited different growth patterns at different temperatures. The authors distinguished *Absidia* species based on the shape of the sporangiospores including cylindrical, conical, and globose. In our present study, the two investigated strains grew rapidly and were mesophilic (optimum growth temperature range of 23~27°C), but they responded differently at 30°C and 35°C. The strain identified as *A. glauca* showed lower thermotolerance, suggesting that the culture medium influences the maximum growth temperatures of fungi. In addition, the colony morphology and culture characteristics of the two isolates were generally similar to those previously described by Hesselstine and Ellis [25] for *A. pseudocylindrospora* and by Ellis and Hesselstine [26] for *A. glauca*. However, some features differed. Our *A. pseudocylindrospora* isolate presented sporangioophores with swellings, which were not described by Hesselstine and Ellis [25]. Moreover, our *A. glauca* isolate produced columellae with a projection of variable shape and displayed more sporangioophores per whorl than the isolate described by Ellis and Hesselstine [26].

Despite the wide intraspecific variation found among some taxa, the ITS region is the barcode marker for mucoralean species identification. The results of our molecular data analysis of the two investigated species were consistent with the phylogeny presented by Walther *et al.* [17]. Furthermore, in the ITS tree, EML-FSDY6-1 belonged to the cylindrical *Absidia* group, whereas EML-DG-NH3-1 clustered within the globose *Absidia* group as defined by Hoffmann *et al.* [7]. Phylogenetic analyses of four genes showed a close relationship between *A. pseudocylindrospora* and *A. glauca* and other species of the family Cunninghamellaceae. The classification of the two investigated strains within this

family is consistent with similarities in several morphological characters, including rapid growth and production of sporangia with multispores (except in the genus *Hesseltinella*).

Data regarding the diversity of zygomycetous fungi in Korea are lacking. Hence, further studies on the classification of different orders and families within the zygomycete fungi are required to expand our knowledge of undiscovered taxa in Korea.

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