Ann Dermatol Vol. 24, No. 3, 2012 http://dx.doi.org/10.5021/ad.2012.24.3.287

ORIGINAL ARTICLE

Molecular Phylogenetics of *Exophiala* Species Isolated from Korea

Moo Kyu Suh, M.D., Ho Chung Lee, M.D., Dong Min Kim, M.D., Gyoung Yim Ha, M.D.[1], Jong Soo Choi, M.D.[2]

Departments of Dermatology and [1]Laboratory Medicine, Dongguk University College of Medicine, Gyeongju, [2]Department of Dermatology, Yeungnam University College of Medicine, Daegu, Korea

Background: Recently, identification of fungi have been supplemented by molecular tools, such as ribosomal internal transcribed spacer (ITS) sequence analysis. According to these tools, morphological *Exophiala* species was newly introduced or redefined. **Objective:** This study was designed to investigate the phylogenetics based on ribosomal ITS sequence analysis from clinical *Exophiala* species isolated in Korea. **Methods:** The strains of *Exophiala* species were 4 clinical isolates of phaeohyphomycosis agents kept in the department of dermatology, Dongguk University Medical Center(DUMC), Gyeongju, Korea. The DNAs of total 5 strains of *Exophiala* species were extracted by bead-beating method. Polymerase chain reaction of ITS region using the primer pairs ITS1-ITS4, was done and phylogenetic tree contributed from sequences of ITS region from 5 Korean isolates including *E. dermatitidis* CBS 109154 and comparative related strains deposited in GenBank. **Results:** The strains of *Exophiala* species were 3 strains of *E. dermatitidis*, 1 strain of *E. jeanselmei* and 1 strain of Exophiala new species. Among the 3 subtypes (type A, B, C) of *E. jeanselmei*, *E. jeanselmei* DUMC 9901 belonged to type B. Of the 2 main types of *E. dermatitidis* (type A, B) and 3 subtypes of *E. dermatitidis* type A (A0, A1 and A2), two strains (*E. dermatitidis* CBS 709.95, *E. dermatitidis* CBS 109154) belonged to A0 subtypes, 1 strain (*E. dermatitidis* DUMC 9902) A1 subtype, respectively. **Conclusion:** Phylogenetic analysis of ITS region sequence provided useful information not only for new species identification but for the subtyping and origin of *Exophiala* species. **(Ann Dermatol 24(3) 287～294, 2012)**

-Keywords-
Exophiala, Phylogeny, Ribosomal internal transcribed spacer

INTRODUCTION

Exophiala is the main genus of black yeasts, characterised by annellidic conidiogenesis and mostly isolated from environmental substrates, including soil, wood, and other plant material. The majority of these infections are cutaneous and subcutaneous, but fatal systemic infections can occur[1,2]. Genus *Exophiala* includes *E. jeanselmei* complex, *E. dermatitidis* and *E. spinifera* complex. *E. jeanselmei* complex has darkened rocket-shaped conidiogenous cells without multicellular conidiophores. *E. spinifera*, unlike *E. jeanselmei*, has large multicellular conidiophores and capsular material around budding cells. *E. dermatitidis* has numerous conidiophores and conidiogenous cells either intercalary or free, and flask shaped. This species grows at up to 42°C, shows no growth with nitrate and nitrite and is sometimes called *Wangiella dermatitidis*[1]. With recent advances in molecular biological techniques such as internal transcribed spacer (ITS) sequences analysis and phylogenetic analysis, *Exophiala* species has been further classified and new species have been identified and named[3-15]. As a result, *E. jeanselmei* have recently been molecular biologically re-identified as including *E. jeanselmei*, *E. xenobiotica*, *E.*

Received July 1, 2011, Revised August 24, 2011, Accepted for publication August 30, 2011

Corresponding author: Moo Kyu Suh, M.D., Department of Dermatology, Gyeongju Hospital, College of Medicine, Dongguk University, 87 Dongdae-ro, Gyeongju 780-350, Korea. Tel: 82-54-770-8268, Fax: 82-54-773-1581, E-mail: smg@dongguk.ac.kr

oligosperma, *E. exophialae*, *E. nishimurae*, *E. bergeri* and *E. nigra*[5]. *E. jeanselmei* have been further classified as fifteen subtypes by ITS-restriction fragment length polymorephism (RFLP) analysis[8]. *E. dermatitidis* also have been subgrouped as A, B, C or D. Most of the subgroup A contains clinical strains, whereas subgroup B contains environmental strains[11]. For this reason ITS sequences analysis and phylogenetic analysis have remained a very useful distinguishing parameter for *Exophiala* species.

This study was designed to determine the molecular phylogenetics of *Exophilala* species isolated from phaeohyphomycosis patients in Korea.

MATERIALS AND METHODS

MATERIALS

In this study five clinical strains, which had been isolated from the phaeohyphomycosis patients, were included: two strains of *E. jeanselmei* (*E. jeanselmei* Dongguk University Medical Center [DUMC] 9901 and *E. jeanselmei* DUMC 0501) and two strains of *E. dermatitidis* (*E. dermatitidis* DUMC 9902 and *E. dermatitidis* CBS 709.95), both isolated from four phaeohyphomycosis patients and preserved in our hospital, and one strain of *E. dermatitidis* CBS 109154 isolated from a patient with a cerebral infection which was obtained from GenBank (Table 1). The identification and typing of the Korean isolates were confirmed by colony morphology, microscopy, sugar assimilation test, heat tolerance test, and the ITS sequence analysis.

METHODS

1) DNA extraction

Fungi grown at 25°C in Sabouraud's dextrose agar for 2 weeks were mixed with glass beads (0.5 mm diameter) in distilled water and shaken for 5 minutes. To purify DNA, the mixture was extracted with phenol/chloroform/isoamyl alcohol (25 : 24 : 1) (Sigma, St. Louis, MO, USA) and centrifuged at 12,000 rpm at room temperature for 10 minutes. A tenth volume of 3 M sodium acetate (Sigma) and 3 volumes of absolute ethanol were added to the supernatant for DNA precipitation at −20°C for 12 hours. DNA was then centrifuged, rinsed with 70% ethanol, dried, and stored at −20°C in distilled water.

2) Polymerase chain reaction (PCR)

To amplify the ITS 1-5.8S rDNA-ITS 2 region of rDNA according to White et al.[16], universal primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTC CGCTTATTGATATGC-3') were prepared by Bioneer Corp. (Daejeon, Korea). Fungal DNA was mixed with 10× PCR buffer, 1.6 ml of 2.5 mM dNTP, 0.4 ml of primers and 5 units of Taq polymerase (Takara, Otsu, Japan). After heating at 95°C for 3 minutes, 30 cycles of denaturation at 95°C for 30 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 1 minute were performed and then followed by the final extension at 72°C for 10 minutes using a thermal cycler, PTC-100 (MJ Research Inc., Watertown, MA, USA). The amplified DNA was electrophoresed on a 1% agarose gel containing ethidium bromide at 100 volt for 20～30 minutes using a Mupid-2 Mini Gel System (Cosmo Bio Co., Seoul, Korea). The amplified DNA fragments were observed under ultraviolet light, cut out of the gel, purified using AccuPrep® PCR Purification Kit (Bioneer Corp.), and then subjected to DNA sequencing by Macrogen Inc. (Seoul, Korea).

3) Phylogenetic analysis

After the ITS sequences of the four strains of *Exophiala* species isolated from Korea were determined, GenBank was searched using the Blast program to find identical or similar sequences. Thereafter, the ITS sequences of *E. dermatitidis* CBS 109154 and relative strains that represent each subgroup of *Exophiala* species were obtained from GenBank for the alignment of ITS sequences by using Clustal X version 2.0[17]. The ITS sequences were aligned by Neighbor-Jointing (NJ) analysis using MEGA4 software[18] with Close-Neighbor-Interchange algorithm with an option of search level 7 and 1,000 booststrap replicates[19]. All alignment gaps that were introduced to maximize the

Table 1. Korean isolates of *Exophiala* species

Species and strain number	Source	Locality
E. jeanselmei DUMC 9901	Subcutaneous phaeohyphomycosis	Daegu
E. jeanselmei DUMC 0501	Subcutaneous phaeohyphomycosis	Jinju
E. dermatitidis DUMC 9902	Subcutaneous phaeohyphomycosis	Daegu
E. dermatitidis CBS 709.95	Subcutaneous phaeohyphomycosis	Chonnam
E. dermatitidis CBS 109154	Cerebral phaeohyphomycosis	Busan

E.: *Exophiala*, DUMC: Dongguk University Medical Center.

homology were considered missing data, and the branch distances were calculated by using the average pathway method[19].

RESULTS

PCR and sequence analysis

The ITS regions of four Korean isolates of *Exophiala* species preserved in our hospital were amplified to produce approximately 643-bp fragments. After the ITS sequences of *E. dermatitidis* CBS 109154 and relative strains that represent each subgroup of *Exophiala* species were obtained, the length of ITS nucleotides was compared by multiple alignment of ITS sequences. The number of ITS nucleotides was around 552 in *E. jeanselmei* and around 584 in *E. dermatitidis*. There were almost no significant differences in length.

Phylogenetic analysis

A phylogenetic tree was constructed by NJ analysis, and the evolutionary disturbances between individual strains were described as horizontal branches. The ITS sequences of twenty five *Exophiala* strains including the five Korean isolates and twenty representative strains were compared. The five Korean isolates did not show morphological diversity and only three species, including one strain of *E. jeanselmei*, three strains of *E. dermatitidis* and one strain of other *Exophiala* species were identified. *E. jeanselmei*

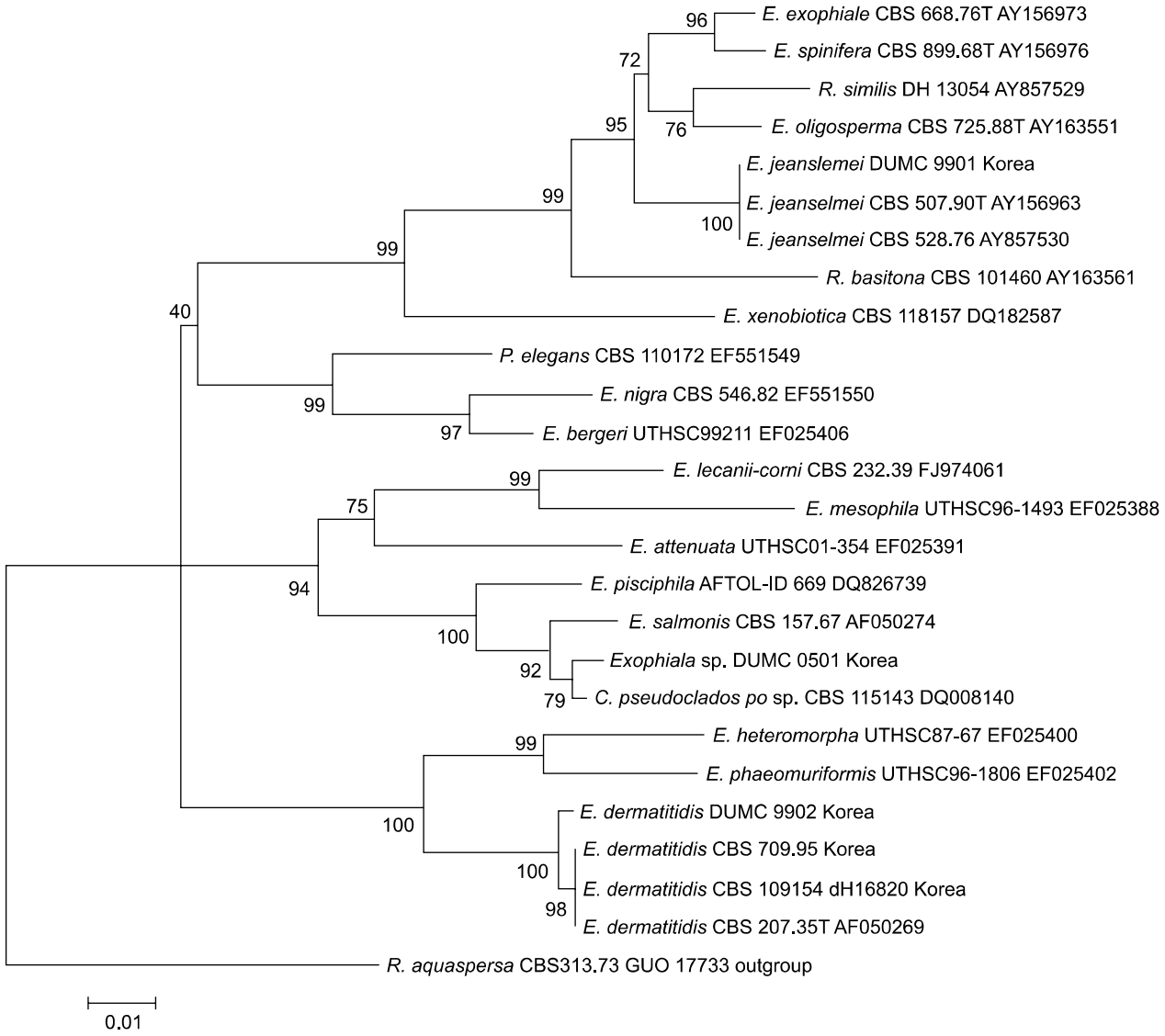

Fig. 1. Neighbor-joining tree based on sequences of the ITS region from the 25 members of *Exophiala* species and relatives; neighbor-joining algorithm with 1,000 bootstrap replicates. *R. aquaspersa* CBS 313.73 is taken as an outgroup. ITS: internal transcribed spacer, *E.*: *Exophiala*, *R.*: *Rhinocladiella*, sp.: species, DUMC: Dongguk University Medical Center.

DUMC O501 did not show any identical ITS sequences to *Exophiala* species, which was regarded as a new species (Fig. 1).

E. jeanselmei was classified into three subtypes: type A, which showed identical ITS sequneces to the type strain, *E. jeanselmei* CBS 507.90, and type B and C which were not. Kawasaki et al.[8] have reported intraspecies variation of the genotypes of *E. jeanselmei* isolated from patients. To compare with subtypes of this study, we treated with suppositive restriction enzyme. It is presumed that types A and B identified by this study would be identical with E5 identified by Kawasaki et al.[8] and that type C identified by this study would be identical with E2 or E3. *E. jeanselmei* DUMC 9901 belonged to the type B reported in Japan and United States, and showed identical ITS sequences with the Japanese species *E. jeanselmei* CBS 116.86. Korean strains and Japanese strains caused only skin infections, although there is a lack of information on human infections (Table 2, Fig. 2).

Matos et al.[11] subclassified *E. dermatitidis* as group A (clinical strain), group B (environmental strain), group C or group D. In this study, we also subclassified the isolates as groups A, B and D. Since the ITS sequences of group C was not available, group C was excluded. Group A was further divided into 3 subgroups: group A0 which was identical with *E. dermatitidis* CBS 207.35, and groups A1 and A2 which were similar to *E. dermatitidis* CBS 207.35. All three Korean isolates were in group A. *E. dermatitidis* CBS 709.95 and *E. dermatitidis* CBS 10915 belonged to group A0, and *E. dermatitidis* DUMC 9902 belonged to

Table 2. Strains of *Exophiala jeanselmei* grouped by similarities in ITS region sequences

Strain	GenBank	Nation	Source	Genotype	Other name
CBS 507.90 T	AY156963	Uruguay	Man, mycetoma	A	dH15933; ATCC 34123; CBS 664.76; IP 71.52
CBS 528.76	AY857530	-	Man, skin	A	dH15968; dH3058; IP 1792.88
IP 70.52	DQ836793	-	-	A	
UTMB 2670	AF549447	-	Man, arm	A	UTHSC86-72; UTMB2674
BMU 00014	EU910261	China	-	A	
CBS 116. 86	AY163556	Japan	Man, chromomycosis	B	dH15309
DUMC 9901		Korea, Daegu	Man, subcutaneous infection	B	
UTHSCR-3338	EF025411	USA	-	B	
UTHSC94-28	EF025410	USA	-	B	
CBS 677.76	AY163553	UK, England	Man, skin, abscess	C	dH16163;ATCC 34123;IHM 1586
UTHSC93-2459	EF025412	USA	-	C	

ITS: internal transcribed spacer, DUMC: Dongguk University Medical Center.

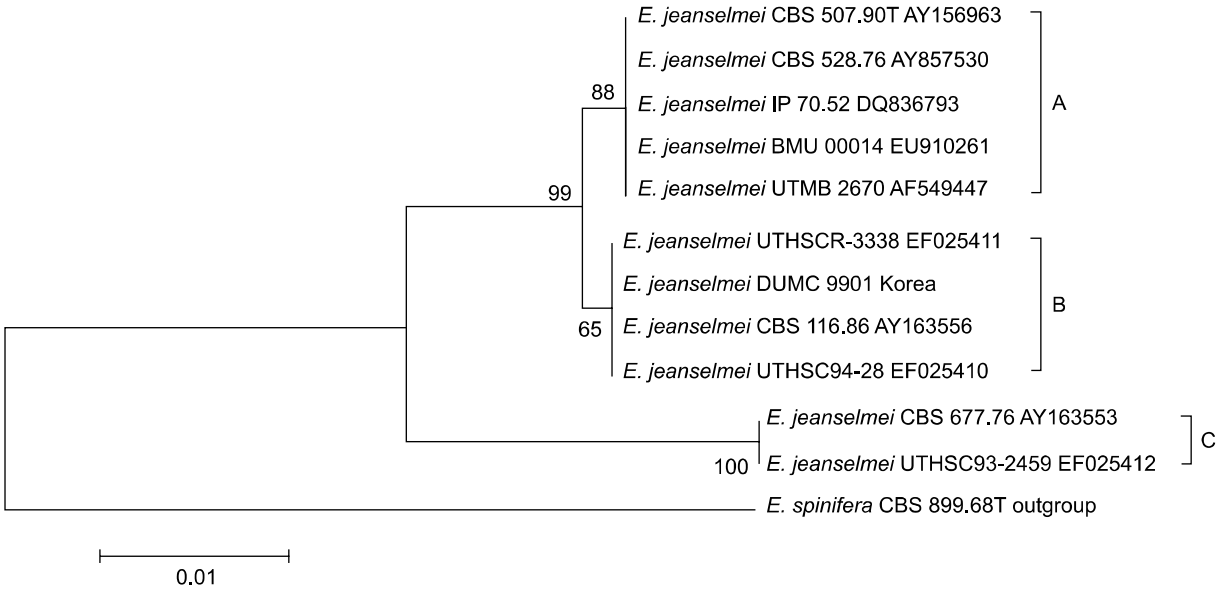

Fig. 2. Neighbor-joining tree based on sequences of the ITS region from the 11 members of *E. jeanselmei* and relatives; neighbor-joining algorithm with 1,000 bootstrap replicates. *E. spinifera* CBS 899.68T is taken as an outgroup. ITS: internal transcribed spacer, *E.*: *Exophiala*.

A1. Since groups A0 and A1 have been isolated in many countries, including Japan, China, United States and Germany, there is no significant regional difference in isolated strains between countries. Human infections ranged in virulence from skin infections to fatal systemic infections (Table 3, Fig. 3).

A new *Exophilala* species DUMC 0501 was similar to *Pseudocladosporium* species and *E. salmonis* CBS 157.67, but no identical ITS sequences were found. This new species was presumed to originate from soil by the ITS sequences analysis with GenBank (Table 4, Fig. 1, 4).

DISCUSSION

Exophiala species invades the human body, mainly causing phaeohyphomycosis which shows brown hyphae or

Table 3. Strains of *Exophiala dermatitidis* grouped by similarities in ITS region sequences

Strain	GenBank	Nation	Source	Genotype
CBS 207.35T	AF050269	Japan; Osaka University	Man, facial chromomycosis	A0
CBS 109154		Korea, Busan	Man, fatal brain infection	A0
CBS 709.95		Korea, Chonnam	Man, subcuatenous abscess	A0
BMU 00037	EU910260	China	Fatal case	A0
UTHSCR-1002	EF025399	USA	-	A0
dH9883		Germany	Dried fruit	A1
DUMC 9902		Korea, Daegu	Man, subcutaneous infection	A1
UWFP 985	AY213651	USA	-	A1
BMU 00040	EU910264	China	Fatal case	A1
BMU 00039	EU910263	China	Fatal case	A1
CBS 149.90	AF050268	Germany	Sputum, cystic fibrosis	A2
CBS 115663	AY663828	Qatar	Endotracheal aspirate of cancer patient	B
NIOCC F50	EF568099	India	Deep sea sediments	B
WM 05.13	EF568099	China		B
CBS_150.90		Netherlands; Amsterdam	Man, sputum of patient with broncho-pneumoniae	D

ITS: internal transcribed spacer, DUMC: Dongguk University Medical Center.

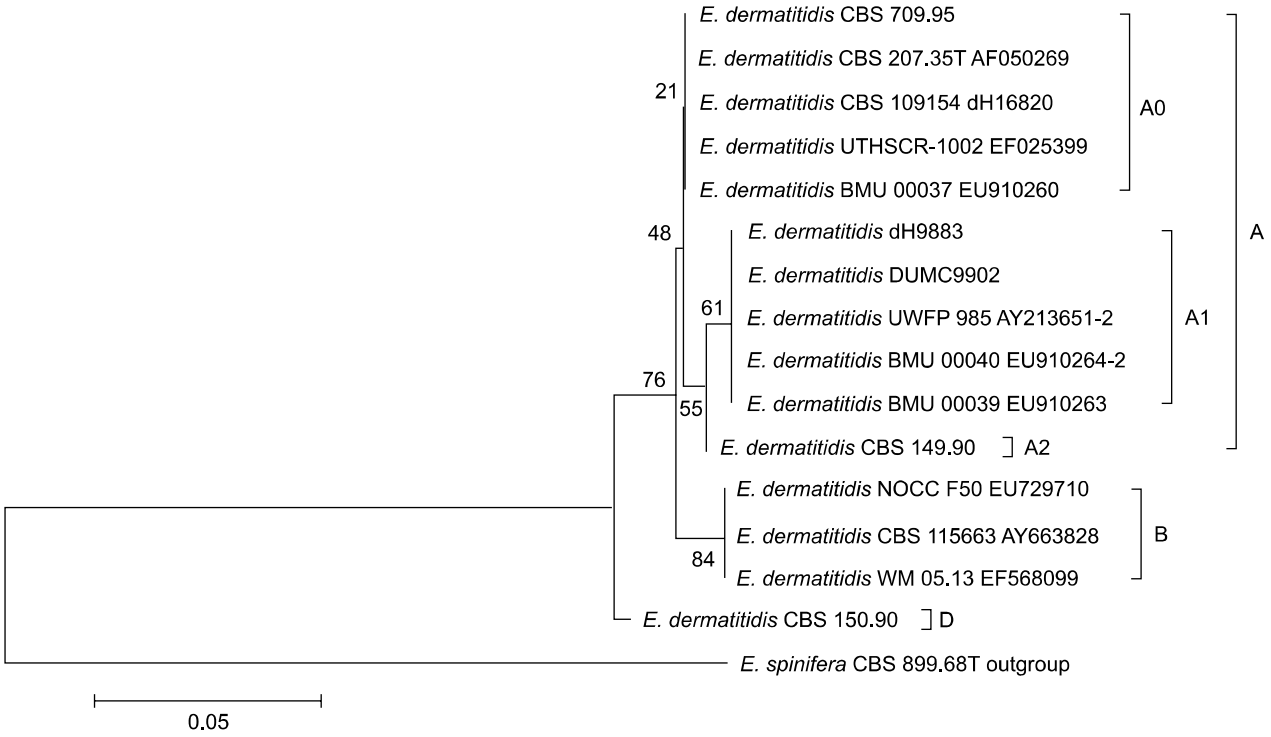

Fig. 3. Neighbor-joining tree based on sequences of the ITS region from the 15 members of *E. dermatitidis* and relatives; neighbor-joining algorithm with 1,000 bootstrap relatives *E. spinifera* CBS 899.68T is taken as an outgroup. ITS: internal transcribed spacer, *E.*: *Exophiala*.

Table 4. Strains of *Exophiala* sp. DUMC 0501 (new species) and relatives according to similarities in the sequences of the ITS region

Strain	GenBank	Nation	Source	
Exophiala sp. DUMC 0501		Korea, Jinju	Man, subcutaneous infection	
Uncultured soil fungus clone 167-1	DQ420723	USA, Minesota	Soil	553/557
Uncultured ascomycete	AM901745	Finland	House dust	552/555
Pseudocladosporium sp. CBS 115143	DQ008140	Australia	Bottled spring water	541/545
Fungal sp. GMG_C6	FJ439580	UK; Drigg	Coast soil	549/556
Uncultured soil fungus clone T1-A12 FL	GU083143	USA; Alaska	Raw soil	550/555
Exophiala sp. WW-2009a MDL-15-44h	FJ665274	Canada	Roots of aspen	550/555
Uncultured *Herpotrichiellaceae* clone LTSP_EUKA_P6P13	FJ554453	Canada	Forest soil	550/555
Exophiala salmonis CBS 157.67	AF050274	Canada	Man, mycetoma	538/561

DUMC: Dongguk University Medical Center, ITS: internal transcribed spacer, sp: species.

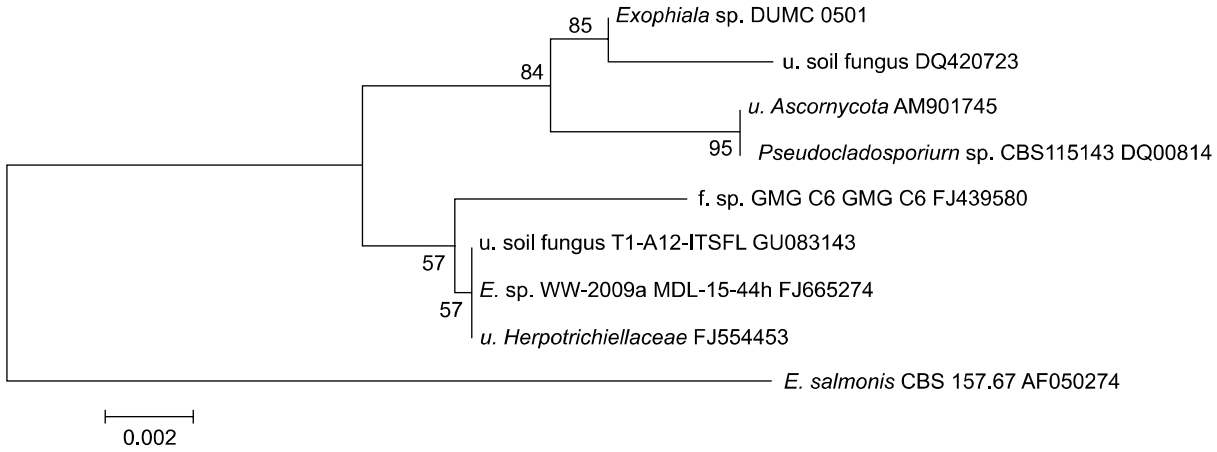

Fig. 4. Neighbor-joining tree based on sequences of the ITS region from the 9 members of *Exophiala* species DUMC 0501 (new species) and relatives; neighbor-joining algorithm with 1,000 bootstrap replicates. ITS: internal transcribed spacer, DUMC: Dongguk University Medical Center, *E.*: *Exophiala,* u.: uncultured, f.: fungal.

yeast-like spores in involved tissue. The species rarely causes either chromoblastomycosis in which round-shaped sclerotic cells or muriform cells with a thick wall are observed or eumycotic mycetoma which is characterized by the development of abscesses, draining sinuses, and the formation of fistulae discharging granules[1,20,21]. Phaeohyphomycosis usually occurs in the skin or subcutaneous tissue. The skin lesion appears as pustules or verrucous plaques. It is rarely disseminated to the internal organs[22-24]. In Korea, three strains of *E. jeanselmei* species and three strains of *E. dermatitidis* were isolated from patients with phaeohyphomycosis[25-30]. Most of these strains caused subcutaneous infections, but only one strain of *E. dermatitidis* species involved the brain[30]. More strains of *Exophiala* species were isolated in many countries: 188 strains in United States[6], 76 strains in Japan[9] and 20 strains in China[3]. It is expected that more strains will appear in Korea. In this study, we used two strains of *E. jeanselmei* and two strains of *E. dermatitidis* which were isolated and preserved in our hospital as well as one strain of *E. dermatitidis* CBS 109154 obtained from GenBank. One strain of *E. jeanselmei* isolated by Kim et al.[27] was excluded from the study because no molecular biological information was available. Of these five strains, one was identified as *E. jeanselmei* by Suh et al.[25] through morphological analysis. However, this strain was regarded as a new strain because it had no identical ITS sequences with *Exophiala* species. Therefore, molecular biological analysis is recommended as a supplementary method if morphological analysis is inadequate.

The classification and identification of *Exophiala* species have been performed by morphological examination, including colony morphology and light microscopy, as well as a physiological examination, including sugar assimilation tests and heat tolerance[1]. Molecular biological analysis has recently been used as a supplementary method[3-15]. *E. jeanselmei* species is morphologically and molecular biologically heterogeneous[4-10,13,14], whereas *E. dermatitidis* species is homogeneous[6,8,9,14].

In the past, *E. jeanselmei* species were re-identified as *E.*

jeanselmei var. jeanselmei, *E. jeanselmei* var. *heteromorpha* and *E. jeanselmei* var. *lecanii-corni* by morphological and cultural features[31]. After then, this species were further classified as *E. jeanselmei* var. *jeanselmei*, *E. jeanselmei* var. *heteromorpha* and *E. jeanselmei* var. *lecanii-corni* and *E. lecanii-corni* by mitochondrial DNA analysis[14]. Furthermore, *E. jeanselmei* was newly subclassified *E. jeanselmei* as *E. jeanselmei*, *E. xenobiotica*, *E. oligosperma*, *E. lecanii-corni* and *E. heteromorpha* by sequence analysis of the ITS regions, elongation factor 1-α (EF1-α) and β-tubulin (β-TUB)[7]. Currently, *E. jeanselmei* is much more subdivided into seven species, including *E. jeanselmei*, *E. xenobiotica*, *E. oligosperma*, *E. exophialae*, *E. nishimurae*, *E. bergeri* and *E. nigra* by sequence analysis of the ITS regions[5]. The above-mentioned species was confirmed as fifteen subtypes by ITS- RFLP analysis[8].

In this study, we classified *E. jeanselmei* as types A, B and C by ITS-RFLP analysis, and matched with fifteen subtypes suggested by Kawasaki et al.[8]; types A and B are considered to be subtype E5, whereas type C is considered to be subtype E2 or E3. In addition, since *E. jeanselmei* DUMC 9901 corresponds to type B, and subtype E5 is the most commonly isolated subtype in Japan, there is a possibility that more type B strains will be identified in Korea.

Although the DUMC O501 strain isolated in Korea was identified as *E. jeanselmei* by morphological and physiological analyses, it had a 84% (432/512) homology to *E. jeanselmei* by ITS sequences analysis. While this strain was close to *Pseudocladosporium* species and *E. salmonis*, this is regarded as a new strain because no identical ITS sequences were detected from GenBank. Since most of the strains with similar ITS sequences originate from soil, this strain is also thought to originate from soil. Because morphological and physiological analyses have some limitations in the identification of strains, molecular biological analysis is recommended as supplementary method. Since one of the five Korean isolates is a new species, further new species will be identified in Korea.

Since *E. dermatitidis* species is very homogeneous[6,8,9,14], it had not been subclassified by morphological or molecular biological analysis before the studies by Uijthof et al.[15] which reported that of the five subgroups, group I is most common and the number of nucleotides in groups II to V is different by 1 to 4 from that in group I. In 2003, Matos et al.[11] further classified *E. dermatitidis* species as groups A, B, C and D by ITS sequence analysis and M-13 fingerprinting. They also stated that most strains belong to groups A and B, and that group A strains are clinical isolates, whereas group B strains are environmental isolates. In this study, it was found that the 3 strains isolated in Korea were classified as A0, A1 and A2, although there were no significant differences in locations and clinical features between A0, A1 and A2.

In conclusion, the five Korean isolates did not show more diversity than western isolates and only three species, including one strain of *E. jeanselmei*, three strains of *E. dermatitidis* and one strain of other *Exophiala* species were identified. All these strains were isolated from patients with phaeohyphomycosis. Despite the low number of the strains included in this study, *E. dermatitidis* is the most commonly isolated strains from phaeohyphomycosis patients in Korea. In the Western world, however, *E. jeanselmei* is the main causative agent of phaeohyphomycosis.

Taken together, ITS sequence analysis and phylogenetic analysis can supplement traditional morphological and physiological analyses in the identification of *Exophiala* species as well as the evaluation of its distribution, determination of subtypes and detection of new species.

REFERENCES

1. De Hoog GS, Guarro J, Gene J, Figueras MJ. Atlas of clinical fungi. 2nd ed. Virgili: Centraalbureau voor Schimmelcultures, 2000:645-668.
2. Ellis D, Davis S, Alexiou H, Handke R, Bartley R. Descriptions of medical fungi. 2nd ed. Adelaide: Nexus Print Solutions, 2007:62-65.
3. Li DM, Li RY, De Hoog GS, Wang YX, Wang DL. *Exophiala* asiatica, a new species from a fatal case in China. Med Mycol 2009;47:101-109.
4. Aoyama Y, Nomura M, Yamanaka S, Ogawa Y, Kitajima Y. Subcutaneous phaeohyphomycosis caused by *Exophiala xenobiotica* in a non-Hodgkin lymphoma patient. Med Mycol 2009;47:95-99.
5. Zeng JS, De Hoog GS. *Exophiala spinifera* and its allies: diagnostics from morphology to DNA barcoding. Med Mycol 2008;46:193-208.
6. Zeng JS, Sutton DA, Fothergill AW, Rinaldi MG, Harrak MJ, de Hoog GS. Spectrum of clinically relevant *Exophiala* species in the United States. J Clin Microbiol 2007;45: 3713-3720.
7. De Hoog GS, Zeng JS, Harrak MJ, Sutton DA. *Exophiala xenobiotica* sp. nov., an opportunistic black yeast inhabiting environments rich in hydrocarbons. Antonie Van Leeuwenhoek 2006;90:257-268.
8. Kawasaki M, Anzawa K, Tanabe H, Mochizuki T, Ishizaki H, Nishimura K. Intra-species variation of genotypes of *Exophiala jeanselmei* isolated from patients in Japan. Nihon Ishinkin Gakkai Zasshi 2005;46:261-265.
9. Kawasaki M, Ishizaki H, Nishimura K, Miyaji M. Mitochondrial DNA analysis of *Exophiala jeanselmei* and *Exophiala dermatitidis*. Mycopathologia 1990;110:107-112.
10. de Hoog GS, Vicente V, Caligiorne RB, Kantarcioglu S, Tintelnot K, Gerrits van den Ende AH, et al. Species diversity

and polymorphism in the *Exophiala spinifera* clade containing opportunistic black yeast-like fungi. J Clin Microbiol 2003;41:4767-4778.
11. Matos T, Haase G, Gerrits van den Ende AH, de Hoog GS. Molecular diversity of oligotrophic and neurotropic members of the black yeast genus *Exophiala*, with accent on *E. dermatitidis*. Antonie Van Leeuwenhoek 2003;83:293-303.
12. Vitale RG, de Hoog GS. Molecular diversity, new species and antifungal susceptibilities in the *Exophiala spinifera* clade. Med Mycol 2002;40:545-556.
13. Wang L, Yokoyama K, Miyaji M, Nishimura K. Identification, classification, and phylogeny of the pathogenic species *Exophiala jeanselmei* and related species by mitochondrial cytochrome b gene analysis. J Clin Microbiol 2001;39:4462-4467.
14. Kawasaki M, Ishizaki H, Matsumoto T, Matsuda T, Nishimura K, Miyaji M. Mitochondrial DNA analysis of *Exophiala jeanselmei var. lecanii-corni* and *Exophiala castellanii*. Mycopathologia 1999;146:75-77.
15. Uijthof JM, Van Belkum A, De Hoog GS, Haase G. *Exophiala dermatitidis* and *Sarcinomyces phaeomuriformis*: ITS1-sequencing and nutritional physiology. Med Mycol 1998;36:143-151.
16. White TJ, Burns T, Lee S, Taylor J. Amplication and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, editors. PCR protocols: a guide to methods and applications. San Diego: Academic Press, 1990:315-322.
17. Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 1997;25:4876-4882.
18. Tamura K, Dudley J, Nei M, Kumar S. MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol Biol Evol 2007;24:1596-1599.
19. Nei M, Kumar S. Molecular evolution and phylogenetics. New York: Oxford University Press, 2000.
20. Queiroz-Telles F, Esterre P, Perez-Blanco M, Vitale RG, Salgado CG, Bonifaz A. Chromoblastomycosis: an overview of clinical manifestations, diagnosis and treatment. Med Mycol 2009;47:3-15.
21. Al-Tawfiq JA, Amr SS. Madura leg due to *Exophiala jeanselmei* successfully treated with surgery and itraconazole therapy. Med Mycol 2009;47:648-652.
22. Revankar SG. Dematiaceous fungi. Mycoses 2007;50:91-101.
23. Suh MK. Phaeohyphomycosis in Korea. Nihon Ishinkin Gakkai Zasshi 2005;46:67-70.
24. Suh MK, Lee YH. Infectious caused by dematiaceous fungi. Korean J Med Mycol 2005;10:77-82.
25. Suh MK, Kwon SW, Kim TH, Sun YW, Lim JW, Ha GY, et al. A case of subcutaneous phaeohyphomycosis caused by *exophiala jeanselmei*. Korean J Dermatol 2005;43:124-127.
26. Suh MK, Suh JC, Seo SK, Na GY, Kim YJ, Bang JS, et al. A case of subcutaneous phaeohyphomycosis caused by *exophiala jeanselmei*. Korean J Dermatol 1999;37:395-399.
27. Kim HU, Kang SH, Matsumoto T. Subcutaneous phaeohyphomycosis caused by *Exophiala jeanselmei* in a patient with advanced tuberculosis. Br J Dermatol 1998;138:351-353.
28. Kim DS, Yoon YM, Kim SW. Phaeohyphomycosis due to. *Exophiala dermatitidis* successfully treated with itraconazole. Korean J Med Mycol 1999;4:79-83.
29. Lee SC, Chun IK, Kim YP. A case of phaeomycotic subcutaneous abscess caused by *Wangiella Dermatitidis*. Korean J Dermatol 1986;24:692-696.
30. Chang CL, Kim DS, Park DJ, Kim HJ, Lee CH, Shin JH. Acute cerebral phaeohyphomycosis due to *Wangiella dermatitidis* accompanied by cerebrospinal fluid eosinophilia. J Clin Microbiol 2000;38:1965-1966.
31. De Hoog GS. *Rhinocladiella* and allied genera. Stud Mycol 1977;15:141-144.