Original Article

Molecular Phylogenetic Study of Strains Morphologically Identified as *Exophiala dermatitidis* from Clinical and Environmental Specimens in Japan

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ABSTRACT

In this study, we aimed to clarify the phylogenetic distribution of *Exophiala dermatitidis* in Japan and describe the characteristics of genotypes. We examined 67 clinical and environmental isolates that were morphologically identified and preserved as *E. dermatitidis* and we confirmed the identification on the basis of the ribosomal DNA internal transcribed spacer region. Genotype sequences were aligned and compared using maximum likelihood phylogenetic tree analyses of the ITS1 region. Additionally, the strains of each genotype were tested for mycological characteristics, such as growth temperature, growth rate, and drug sensitivity. The 67 strains examined were isolated from Japan, the United States, Brazil, Venezuela, and China. In accordance with the establishment of a phylogenetic tree for the ITS1 region, 45 of the 49 Japanese strains were classified as genotype A, two as genotype B, and two as genotype D (A2 according to the method of Matos et al. (2003)). Chinese strains were divided into genotypes A and D (A2), and South American strains were classified into genotypes A, B, B2, and C2, while all strains from the U.S. belonged to genotype A. New genotype groups B2 and C2 were identified in Brazilian and Venezuelan strains, respectively. There were no specific differences among the genotypes or isolated regions in the antifungal susceptibility test for all *E. dermatitidis* isolates. However, genotypes B2 and D (A2) exhibited growth at higher temperatures than the other genotypes.

Key words : antifungal susceptibility, *exophiala dermatitidis*, genotype, growth temperature, phylogenetic distribution

Introduction

The genus *Exophiala*, an opportunistic pathogenic black yeast, is spread throughout the environment globally, particularly in plants and soil, and is responsible for subcutaneous and invasive human infections. *Exophiala* also includes numerous potential pathogens that affect warm-blooded¹⁾ and cold-blooded animals²⁾. The pathogenicity to humans depends on the capacity to grow at 37-40°C and the presence of melanin on the cell wall and lipids on the cell membrane³⁾.

Exophiala species cause a large spectrum of human diseases, including chromoblastomycosis, mycetoma, and phaeohyphomycosis. Infections are typically observed in immunocompromised patients, including organ recipients exhibiting subcutaneous or systemic involvement⁴⁾. Disseminated fatal human infections with *Exophiala dermatitidis*,

Exophiala spinifera, and *Exophiala asiatica* have been reported in immunocompetent and immunocompromised hosts⁵⁻⁸⁾, and *E. dermatitidis*, *Exophiala lecanii-corni*, *Exophiala oligosperma*, *Exophiala phaeomuriformis*, and *Exophiala xenobiotica* were identified as the species most commonly associated with systemic infections^{9, 10)}. Moreover, *E. dermatitidis* is recognized as an agent of neurotropic infections in humans^{1, 7)}. Formerly, the identification of *Exophiala* species at the species level was conducted through morphological observation, but recently it has been complemented by molecular methods based on sequencing of the internal transcribed spacer (ITS) region of rDNA^{8, 9, 11, 12)}.

E. dermatitidis has been reported to have a molecular phylogenetic polymorphism in strains from Thailand⁷⁾, Iran¹³⁾, Turkey^{14, 15)}, and Slovenia¹⁶⁾. However, the distribution in Japan has not been investigated. Hence, here we examined the phylogenetic distribution of *E. dermatitidis* in Japan.

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Materials and methods

Fungal isolates and strains

In this study, 67 isolates from clinical and environmental strains in Japan, the U.S., Brazil, Venezuela, and China (listed in Table 1) were preserved at the Medical Mycology Research Center, Chiba University. Out of these, a few strains were identified as *E. dermatitidis* on the basis of morphological observations.

Growth test and morphological observation

The strains were cultivated on potato dextrose agar (PDA-Difco™ Becton, Dickinson and Company, Sparks, MD, USA) at 30, 37, 40, 42, and 44°C for 14 days. The inoculated conidial suspension was made from a 7-day culture on PDA and adjusted to 10⁴ cells/mL using a hemocytometer. The strains were cultured on Czapek solution agar (CZA-Difco™ Becton, Dickinson and Company/Sparks, MD, USA) to determine whether *E. dermatitidis* can assimilate nitrates or nitrites as the sole source of nitrogen. The diameter of the colonies was measured, and the hyphae and conidia were observed with a scanning electron microscope (SEM; Hitachi S3400, Tokyo, Japan).

DNA extraction, PCR, sequencing, and phylogenetic analysis

Genomic DNA was extracted and purified from *E. dermatitidis* mycelia cultivated on PDA using the PrepMan Ultra Sample Preparation Reagent (Applied Biosystems™, Foster City, CA, USA). The ITS region of the ribosomal RNA gene was sequenced using the universal fungal primer sets ITS1 and ITS4, as described by White et al.¹⁷⁾. Samples were amplified using Illustra PuReTaq Ready-To-Go PCR Beads (GE Healthcare UK Limited, Little Chalfont, Buckinghamshire, UK) with the following cycling parameters: an initial cycle of 10 min at 95°C followed by 35 cycles of 1 min at 95°C, 1 min at 55°C, and 1 min at 72°C, and a single extension cycle at 72°C for 10 min. DNA fragments from the sequencing reactions were purified using Illustra ExoProStar (GE Healthcare UK Limited) and sequenced using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) on an ABI PRISM® 3130 Genetic Analyzer (Applied Biosystems). Sequence alignments were conducted using Clustal W¹⁸⁾. Phylogenetic analysis was conducted using Molecular Evolutionary Genetics Analysis software (MEGA, version 10.1.8) software^{19, 20)}. The phylogenetic tree was calculated by MEGA using the maximum likelihood (ML) method with the Jukes-Cantor model algorithm²¹⁾, which yielded the tree with the highest likelihood for this dataset. Bootstrap values were obtained from 1,000 replications²²⁾. All positions containing gaps and missing data were eliminated (complete deletion option). Furthermore, *Exophiala heteromorpha* CBS 232.33 was used as an outgroup in this study.

The ITS rDNA sequences obtained in this study were deposited in the GenBank database (Table 1).

Antifungal susceptibility test

The minimal inhibitory concentration (MIC) and minimal effective concentration (MEC) of antifungal agents against the strains were measured using the microdilution method, in accordance with the Standardized Clinical and Laboratory Standards Institute M38 - Ed3 Method (CLSI M38 - Ed3) for filamentous fungi²³⁾ with slight modifications. All strains were pre-cultured on PDA for 7 days at 35°C.

Results

Phylogenetic analysis

All 67 isolates were identified as *E. dermatitidis* by sequencing of the ITS region. The genotypes of *E. dermatitidis* were defined according to the method of Matos et al.²⁴⁾ on the basis of polymorphisms in ITS1. In the current study, *E. dermatitidis* strains were divided into five clades (Fig. 1) using phylogenetic analysis. The differences in the ITS1 region among the genotypes are shown in Table 2. Out of the 49 strains from Japan, 4 from China, 5 from Venezuela, and 4 from the U.S., 45, 2, 2, and 4 strains were respectively classified as genotype A. Only two strains from Japan and China belonged to genotype D (A2). This genotype is related to genotype A from neighbor-joining (NJ) analysis results and is described as genotype A2²⁵⁾. However, it was separated from genotype A in our analysis and, therefore, reclassified as genotype D because the ML method is more robust than the NJ method. The NJ method produces a single tree without any means to compare it with other potential trees, while the ML method investigates the optimum criterion of all possible phylogenetic trees to identify the best trees. Two strains from Japan and four out of five from Brazil belonged to genotype B, and the remaining one belonged to the new clade named genotype B2. Furthermore, most strains from Venezuela (3 of 5) were located in another new clade named genotype C2. In this study, we did not find any strains belonging to genotypes C and D2 (A3). The genotype D2 in the current study was described as genotype A3 in a previous study²⁵⁾. The above results revealed that most of the Japanese strains belonged to genotype A. Only a small number of the Japanese strains belonged to genotype B and genotype D. Furthermore, except for two strains, the remaining Japanese clinical strains belonged to genotype A. These results suggest that the genotypes of Japanese strains are relatively uniform. Furthermore, results for Chinese and North American strains were similar in the present study. Notably, the South American strains had diverse genotype distribution. Additionally, the new genotype B2 consisted of one strain from Brazil, while the new genotype C2 consisted of three strains from Venezuela.

Table 1. Clinical and environmental strains used in this study

IFM no.	Scientific name	Other institute no.	Source	Country	Genotype	GenBank accession no. (ITS rDNA)
4826	<i>E. dermatitidis</i>	CBS100338	Humidifier	Japan	A	LC627209
4829	<i>E. dermatitidis</i>		Chromomycosis of the larynx	Japan	A	LC627210
4830	<i>E. dermatitidis</i>		Chromomycosis	Japan	A	LC627211
4831	<i>E. dermatitidis</i>		Chromomycosis	Japan	A	LC627212
4832	<i>E. dermatitidis</i>		Chromomycosis	Japan	A	LC627213
4833	<i>E. dermatitidis</i>	KUM1182	Unknown	Japan	A	LC627238
4834	<i>E. dermatitidis</i>	KUM1183	Unknown	Japan	A	LC627239
4835	<i>E. dermatitidis</i>	KUM1168	Unknown	Japan	A	LC627240
4836	<i>E. dermatitidis</i>	KUM1308, CBS153.94	Chromomycosis of the liver	Japan	A	LC627214
4837	<i>E. dermatitidis</i>		Generalized chromoblastomycosis	Japan	A	LC627215
4838	<i>E. dermatitidis</i>		Chromomycosis	Japan	A	LC627216
4839	<i>E. dermatitidis</i>		Chromomycosis	Japan	A	LC627217
4840	<i>E. dermatitidis</i>		Phaeohyphomycosis of the brain and liver	Japan	A	LC627218
4841	<i>E. dermatitidis</i>		Chromomycosis	Japan	A	LC627219
4842	<i>E. dermatitidis</i>		Generalized chromomycosis	Japan	A	LC627220
4958	<i>E. dermatitidis</i>	CBS100342	Medicated bath water in a public bathhouse	Japan	A	LC627221
4959	<i>E. dermatitidis</i>		The same water with IFM4958	Japan	A	LC627222
4960	<i>E. dermatitidis</i>		Drainpipe sludge in a bathroom	Japan	A	LC627223
4961	<i>E. dermatitidis</i>		Drainpipe sludge in a bathroom	Japan	A	LC627224
4962	<i>E. dermatitidis</i>		Bath water in a household	Japan	A	LC627225
4963	<i>E. dermatitidis</i>		Bath water in a household	Japan	D (A2)	LC627264
4964	<i>E. dermatitidis</i>		The same bath water with IFM4963	Japan	A	LC627226
4965	<i>E. dermatitidis</i>		Bath water in a household	Japan	D (A2)	LC627265
40902	<i>E. dermatitidis</i>		Unknown	Japan	A	LC627241
40903	<i>E. dermatitidis</i>		Unknown	Japan	A	LC627242
40918	<i>E. dermatitidis</i>		Unknown	Japan	A	LC627243
41480	<i>E. dermatitidis</i>		Humidifier	Japan	A	LC627244
41482	<i>E. dermatitidis</i>	CBS207.35, IMI93967	Facial chromomycosis of man	Japan	A	LC627245
41489	<i>E. dermatitidis</i>		Human skin	Japan	A	LC627227
61098	<i>E. dermatitidis</i>		Lung	Japan	A	LC627228
61450	<i>E. dermatitidis</i>		Blood	Japan	A	LC627229
62264	<i>E. dermatitidis</i>		Humidifier	Japan	A	LC317584
62533	<i>E. dermatitidis</i>		Skin of finger	Japan	A	LC317585
62535	<i>E. dermatitidis</i>		Blood	Japan	A	LC317586
62553	<i>E. dermatitidis</i>		Trahea	Japan	A	LC317587
63808	<i>E. dermatitidis</i>		Resected lung	Japan	A	LC317588
64172	<i>E. dermatitidis</i>		Mucus plug	Japan	A	LC317589
64327	<i>E. dermatitidis</i>		Blood	Japan	A	LC317590
65114	<i>E. dermatitidis</i>		Venous blood	Japan	A	LC627230
65294	<i>E. dermatitidis</i>		Sputum	Japan	A	LC627231
65295	<i>E. dermatitidis</i>		Bronchoscope specimen	Japan	A	LC627232
65296	<i>E. dermatitidis</i>		Sputum	Japan	A	LC627233
65313	<i>E. dermatitidis</i>		Axillary lymph node biopsy	Japan	A	LC627234
65529	<i>E. dermatitidis</i>		Ear discharge	Japan	A	LC627235
65683	<i>E. dermatitidis</i>		Tracheal suction phlegm	Japan	A	LC627236
65684	<i>E. dermatitidis</i>		Sputum	Japan	A	LC627237
67276	<i>E. dermatitidis</i>		Bronchoscopic specimen	Japan	A	LC636195
57160	<i>E. dermatitidis</i>		Sputum	Japan	B	LC636194
67277	<i>E. dermatitidis</i>		Bronchoscopic specimen	Japan	B	LC636196
4847	<i>E. dermatitidis</i>		Bat	Brazil	B	LC627254
4848	<i>E. dermatitidis</i>	CBS100340	Bat	Brazil	B	LC627255
4849	<i>E. dermatitidis</i>		Bat	Brazil	B	LC627256
4850	<i>E. dermatitidis</i>		Bat	Brazil	B	LC627257
41827	<i>E. dermatitidis</i>		Soil	Brazil	B2	LC627258
41820	<i>E. dermatitidis</i>		Cactus	Venezuela	C2	LC627259
41821	<i>E. dermatitidis</i>		Wood	Venezuela	C2	LC627260
41822	<i>E. dermatitidis</i>		Rotten wood	Venezuela	C2	LC627261
41824	<i>E. dermatitidis</i>		Rotten wood	Venezuela	A	LC627252
41825	<i>E. dermatitidis</i>		Rotten wood	Venezuela	A	LC627253
4844	<i>E. dermatitidis</i>		Unknown	United States	A	LC627249
4845	<i>E. dermatitidis</i>		Unknown	United States	A	LC627250
4846	<i>E. dermatitidis</i>		Unknown	United States	A	LC627251
41506	<i>E. dermatitidis</i>		Human skin	United States	A	LC627248
41686	<i>E. dermatitidis</i>		Rotten wood	China	A	LC627246
41688	<i>E. dermatitidis</i>		Human skin	China	A	LC627247
41689	<i>E. dermatitidis</i>		Human skin	China	D (A2)	LC627262
41690	<i>E. dermatitidis</i>		Human skin	China	D (A2)	LC627263
Reference strains						
	<i>E. dermatitidis</i>	EXF-6276	Dishwasher's rubber	Brazil	A	JF766651
	<i>E. dermatitidis</i>	EXF-6118	Dishwasher's rubber	South Africa	A	JF766656
	<i>E. dermatitidis</i>	EXF-6114	Dishwasher's rubber	South Africa	B	JF766669
	<i>E. dermatitidis</i>	EXF-6275	Dishwasher's rubber	Brazil	B	JF766670
	<i>E. dermatitidis</i>	EXF-5567	Dishwasher's rubber	Slovenia	C	JF766671
	<i>E. dermatitidis</i>	EXF-5586	Dishwasher's rubber	Slovenia	C	JF766672
	<i>E. dermatitidis</i>	EXF-5761	Dishwasher's rubber	Slovenia	D (A2)	JF766659
	<i>E. dermatitidis</i>	EXF-5768	Dishwasher's rubber	Slovenia	D (A2)	JF766660
	<i>E. dermatitidis</i>	EXF-5647	Dishwasher's rubber	Slovenia	D2 (A3)	JF766664
	<i>E. dermatitidis</i>	EXF-5654	Dishwasher's rubber	United States	D2 (A3)	JF766665

Fig. 1. The phylogenetic tree calculated by maximum likelihood method using the sequences of the ITS1 region from 78 members of *Exophiala dermatitidis* and relatives.

Exophiala heteromorpha CBS 232.33 was taken as an outgroup. Bootstrap values over 50% are shown. There were a total of 176 bp positions in the final dataset. BRA: Brazil, ZA: South Africa, JPN: Japan, CHN: China, USA: United States, VEN: Venezuela, SI: Slovenia.

Table 2. Genotypes within *Exophiala dermatitidis* based on polymorphisms in rDNA ITS1

Total 211 bp (<i>E. dermatitidis</i> rDNA ITS1)	63 bp	115 bp	124 bp	152 bp	153 bp	163 bp	181 bp	185 bp	195 bp	197 bp	203 bp
Genotype A:	A	C	T	A	-	T	A	-	T	A	-
Genotype B:	A	C	T	A	-	C ⁽²⁴⁾	A	T ⁽²⁴⁾	T	C ⁽²⁴⁾	-
Genotype B2:	A	C	T	A	-	T	A	-	T	C	-
Genotype C: (Deletion of 80~105 bp)	A	C	T	C	-	T	T	-	G	A	A
Genotype C2:	A	G	T	A	-	T	T	-	T	A	-
Genotype D (A2):	G	C	C	A	-	T	A	-	T	A	-
Genotype D2 (A3):	G	C	T	A	A	T	A	-	T	A	-

Table 3. Growth characterization in different media and temperature (10⁴ cells/ml (10µl), 14 days)

n = 19	PDA					CZA
	30°C	37°C	40°C	42°C	44°C	30°C
Genotype A (n = 10)	+	+	-	-	-	Poor growth
Genotype B (n = 2)	+	+	-	-	-	Poor growth
Genotype B2 (n = 1)	+	+	+	-	-	Poor growth
Genotype C2 (n = 2)	+	+	-	-	-	Poor growth
Genotype D (A2) (n = 4)	+	+	+	-	-	Poor growth

Growth test and morphology

In the growth test, genotypes A, B, and C2 exhibited similar growth patterns (Table 3). These strains showed optimal growth up to 37°C, but growth was inhibited at 40°C. We also did not find any differences with other groups in morphological observations (Fig. 2). At 40°C, we found that genotype B2 and most of the genotype D (A2) strains (3 of 4) showed growth on PDA (Table 3). From this observation, we concluded that growth at 40°C is the distinguishing feature of genotypes B2 and D (A2). The genotypes A, B, and C2 incubated at 37°C did not show any differences as observed via SEM (Fig. 2). However, for genotype B2, it was observed that hyphal formation increased at 37°C, whereas it decreased at 40°C.

Antifungal susceptibility

All strains showed high antifungal susceptibility to amphotericin B, itraconazole, voriconazole, miconazole, and posaconazole, and no differences among the genotypes or isolated regions were found (Table 4).

Discussion

Black yeasts have very few stable morphological or physiological parameters for recognition; therefore, molecular parameters are important for their recognition. Zeng et al.^{9, 26)} proved that the rDNA ITS region is a reliable marker for distinguishing black yeast entities. The rDNA ITS genotypes

of *E. dermatitidis* were defined according to Matos et al.²⁴⁾ as nuclear rDNA ITS sequencing of numerous reference strains confirmed the existence of two significant genotypes, genotype A and B differing in three positions in ITS1 (162 bp, 184 bp, and 196 bp). Moreover, in a previous study by Zalar et al.²⁷⁾, the genotype A of *E. dermatitidis* was prevalent, comprising 67% of the strains isolated in this species. However, these studies focused on the genotype distribution of *E. dermatitidis* from the environment, and that of *E. dermatitidis* in Japan has not been reported so far. In particular, it is necessary to determine the distribution characteristics of clinical strains of *E. dermatitidis* in Japan.

In previous studies (summarized in Table 5), there was only a single genotype, genotype A, in East Asian strains, including eight Japanese strains; while strains in Thailand, Turkey, and Iran exhibited variable genotypes. In the present study, we focused on describing the genotype distribution of the Japanese *E. dermatitidis* isolates based on their rDNA ITS1 region using the ML method. For further comparison, Chinese, North American (U. S.), and South American (Brazil and Venezuela) strains were also analyzed (Table 5). It should be noted that most of the previous studies used the NJ method to determine the genotypes of *E. dermatitidis*. Because the ML method is more stable compared to the NJ methods, we decided to use ML as the inference method for this study.

In a study by Döğen et al.¹⁵⁾, in colder climates at high altitudes, railways were primarily contaminated with clinically

Fig. 2. Growth characterization on PDA medium.

10^4 cells/ml (10 μ l), incubated in 30, 37, 40, 42 and 44°C on PDA, 14 days. The hyphal and the conidia were observed by the SEM.

Table 4. MEC and MIC* results for all of the genotypes and isolated regions

	MCFG	CPFG	AMPH	5-FC	FLCZ	ITCZ	VRCZ	MCZ	PSCZ
Genotype									
A (n = 18)	4-> 16	2-16	0.25-1	1-4	4-16	0.12-0.5	0.12-1	0.12-0.25	0.5-1 (n = 5)
B (n = 4)	> 16	8-16	1	4-8	4	0.25	0.12	0.12-0.25	0.5 (n = 2)
B2 (n = 1)	> 16	8	1	2	4	0.25	0.12	0.25	1 (n = 1)
C2 (n = 3)	> 16	2-8	0.5-1	2-4	4-8	0.25	0.12-0.25	0.12-0.25	0.25-0.5 (n = 2)
D (A2) (n = 4)	> 16	2-8	0.25-1	2-4	4-8	0.25-0.5	0.12-0.25	0.25-0.5	0.5 (n = 3)
Country									
Japan (n = 12)	8-> 16	2-16	0.25-1	1-4	4-8	0.25-0.5	0.12-1	0.12-0.5	0.5-1 (n = 4)
China (n = 4)	8-> 16	2-8	0.25-0.5	2-4	4-8	0.25-0.5	0.12-0.25	0.12-0.25	0.5 (n = 2)
USA (n = 4)	4-> 16	4-16	0.5-1	1-4	8-16	0.25-0.5	0.12-0.5	0.12-0.25	0.5-1 (n = 2)
Brazil (n = 5)	> 16	8-16	1	2-8	4	0.25	0.12	0.12-0.25	0.5-1 (n = 3)
Venezuela (n = 5)	> 16	2-8	0.5-1	2-4	4-8	0.25	0.12-0.25	0.12-0.25	0.25-0.5 (n = 2)

MCFG: Micafungin, CPFG: Caspofungin, AMPH: Amphotericin B, 5-FC: 5-flucytosine, FLCZ: Fluconazole, ITCZ: Itraconazole, VRCZ: Voriconazole, MCZ: Miconazole, PSCZ: Posaconazole.

*: CLSI M38-Ed3, 35°C, 10⁴ cells/ml, MEC: 48 h, MIC: 72 h, Antibiotic concentration: µg/ml.

Table 5. Ratios of genotypes of *Exophiala dermatitidis*

Country, region	Source	Ratios of genotypes	Reference
Eupore	Clinical, environment, and unknown	<i>E. dermatitidis</i> (n = 28) Genotypes A, B and C (D)* (19:8:1)	Motos et al., 2003
East Asia (Japan, Taiwan and Korea)	Clinical and environment	<i>E. dermatitidis</i> (n = 12) Genotype A (12)	
Africa and Middle east (Mauritius and Iraq)	Clinical and unknown	<i>E. dermatitidis</i> (n = 2) Genotype A and B (1:1)	
South America (Brazil)	Bat	<i>E. dermatitidis</i> (n = 1) Genotypes B (1)	
Thailand	Environment	<i>E. dermatitidis</i> (n = 413) Genotypes A, B and C (376:34:3)	Sudhatham et al., 2008
Turkey	Environment	<i>E. dermatitidis</i> (n = 56) Genotypes A, B, C, D (A2)* and D2 (A3)* (15:5:6:13:17)	Dogen et al., 2013
Turkey	Environment	<i>E. dermatitidis</i> (n = 24) Genotypes A and B (23:1)	Gümral et al., 2014
Slovenia	Environment	<i>E. dermatitidis</i> (n = 40) Genotypes A, B, C and D (A2)* (30:6:3:1)	Zupančič et al., 2016
Iran	Environment	<i>E. dermatitidis</i> (n = 20) Genotypes B and D (A2)* (2:18)	Yazdanparast et al., 2017
Japan	Clinical, environment, and unknown	<i>E. dermatitidis</i> (n = 49) Genotypes A, B and D (A2)* (45:2:2)	Alimu et al., 2021 (present study)
China	Clinical, environment, and unknown	<i>E. dermatitidis</i> (n = 4) Genotypes A and D (A2)* (2:2)	
North America (USA)	Clinical and unknown	<i>E. dermatitidis</i> (n = 4) Genotype A (4)	
South America (Brazil and Venezuela)	Environment and bat	<i>E. dermatitidis</i> (n = 10) Genotypes A, B, B2 and C2 (2:4:1:3)	

*: (A2 or A3) Genotypes described in previous research.

insignificant human opportunists (i.e., *Exophiala crusticola* and *E. xenobiotica*), whereas in regions close to the sea level with warmer climates, *E. dermatitidis* was predominant on railways. Furthermore, in a study by Zhao et al.²⁸⁾, instead of *E. dermatitidis*, *E. xenobiotica* and *Exophiala bergeri* were isolated from railways in regions with temperate climate in the Netherlands. They speculated that the absence of *E. dermatitidis* in temperate climates can be attributed to the tropical origin of this fungus. *E. dermatitidis* seems to be consistently predominant in subtropical climates, suggesting the effect of weather conditions on the species composition in creosoted wood²⁵⁾. *Exophiala* species are also prevalent in high water temperatures. Matos et al.^{24, 29)} and Sudhadham et al.⁷⁾ noted consistent presence of *E. dermatitidis* and *E. phaeomuriformis* in steam baths. Matos et al.²⁹⁾ noticed small differences in the ecological preferences of the two species: *E. dermatitidis* occurred in steam rooms, whereas *E. phaeomuriformis* was found in the warm but less extreme environments of adjacent halls and bathrooms. This is consistent with their physiological differences: *E. dermatitidis* tolerates higher temperatures, and the production of extracellular capsules³⁰⁾ also enhances its adhesion to smooth plastic surfaces and tiles of steam rooms. In the black yeast genus *Exophiala*, *E. dermatitidis* and *E. phaeomuriformis* show a surprising combination of meristematic morphology, thermotolerance, and opportunistic pathogenic behavior. Their human association has been documented in the colonization of the lungs of patients with cystic fibrosis and in their occurrence as causative agents of neurotropic disseminated infections³¹⁾, particularly in East Asia⁷⁾.

In our study, on the basis of the above results, we used *E. dermatitidis* strains to conduct a thermotolerance test. The thermotolerance test results indicated that, in all 19 *E. dermatitidis* strains tested, 40°C was the distinguishing temperature of A2 and B2 from other groups in PDA. Unlike in previous studies, we did not observe high thermotolerance in strains grown above 40°C. One of the primary reasons for this is that our sub-culture condition was different from previous studies. We used a low sub-cultural conidia concentration (10² cells) in our study. Furthermore, all of the genotypes showed poor growth in Czapek's agar because *E. dermatitidis* cannot assimilate nitrates as the sole source of nitrogen, which is one of the growth characteristics of *E. dermatitidis*. In SEM observation, genotypes A, B, and C2 did not show any morphological differences in conidiophores, phialides, and conidia at 37°C. In genotype B2, strains grown at 37°C showed increased hyphal formation. However, at 40°C, the strains showed reduced hyphal and conidial formation, and artificial conidia were observed. Genotype D (A2) strains grown at 40°C showed changes similar to genotype B2.

Additionally, severe clinical cases of *E. dermatitidis* are difficult to treat despite surgery and antifungal therapy.

Although *E. dermatitidis* is susceptible to widely used antifungal drugs, such as voriconazole, itraconazole, and posaconazole *in vitro*, treatment remains difficult because of frequent relapses. In a study by Yazdanparast et al.¹³⁾, amphotericin B and itraconazole had low MICs against *E. dermatitidis*, but a study by Badali et al.³²⁾ suggested that the outcome of most patients treated with these drugs is poor. The treatment failures may be due to less than optimal penetration into the central nervous system. In contrast, voriconazole and posaconazole have good cerebral penetration and, thus, may provide additional therapeutic options for disseminated mycoses by *E. dermatitidis*¹³⁾.

In the current study, the MIC results showed that all of the strains have high sensitivity to amphotericin B, itraconazole, voriconazole, miconazole, and posaconazole. Because itraconazole and voriconazole are mainly used for clinical therapy in Japan, on the basis of the results of the above studies, we recommend that itraconazole and voriconazole continue to be the first choice for clinical treatment in Japan.

To the best of our knowledge, our study is the first to investigate the phylogenetic diversity of *E. dermatitidis* in Japan. Furthermore, compared with previous research, this is a rare case where phylogenetic diversity was investigated using both clinical and environmental *E. dermatitidis* strains in the same study (Table 5).

Conclusion

In this study, we determined that the molecular phylogenetic diversity of the isolates in Japan is relatively uniform. Molecular polymorphisms were found within species in South American strains. We further found new *E. dermatitidis* genotypes B2 and C2 from South American strains, and reclassified genotypes A2 and A3 to D and D2 by changing the phylogenetic analysis method. In the antifungal susceptibility test for all *E. dermatitidis* isolates, we did not find any specific differences between the different genotypes or isolated regions.

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Conflicts of interest

None.

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