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Original Article

Epidemiological Study of *Fusarium* Species Causing Invasive and Superficial Fusariosis in Japan

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ABSTRACT

In Japan, *Fusarium* species are known etiological agents of human fungal infection; however, there has been no report of a large-scale epidemiological study on the etiological agents of fusariosis. A total of 73 *Fusarium* isolates from patients with invasive fusariosis (IF, n = 36) or superficial fusariosis (SF, n = 37), which were obtained at hospitals located in 28 prefectures in Japan between 1998 and 2015, were used for this study. *Fusarium* isolates were identified using *Fusarium*- and *Fusarium solani* species complex (FSSC) -specific real-time PCR and partial DNA sequences of the elongation factor-1 alpha (EF-1α) gene and the nuclear ribosomal internal transcribed spacer (ITS) region. FSSC was predominately isolated from both patients with IF and SF (IF, 77.8% and SF, 67.6%). Distribution of the phylogenetic species of FSSC isolates from patients with IF and SF exhibited different spectra; specifically, *F. keratoplasticum* (FSSC 2) (25.0%) was the most frequent isolate from patients with IF, whereas *F. falciforme* (FSSC 3 + 4) (32.4%) was the most frequent isolate from patients with SF. *Fusarium* sp. (FSSC 5) was the second most frequent isolate from both patients with IF and SF (IF, 22.2% and SF, 24.3%). Notably, *F. petroliphilum* (FSSC 1) was isolated only from patients with IF. Each species was isolated from a broad geographic area, and an epidemic was not observed. This is the first epidemiological study of *Fusarium* species causing IF and SF in Japan.

Key words: *Fusarium solani* species complex, invasive fusariosis, Japan, superficial fusariosis

Introduction

Fusarium species are well known as major plant pathogens and soil habitants with a worldwide distribution[1−5]. In humans, *Fusarium* species are also common pathogenic fungi that cause fusariosis. Superficial fusariosis (SF), including keratitis and onychomycosis, occurs mainly in immunocompetent individuals[6−9], whereas invasive fusariosis (IF), including locally invasive and disseminated fusariosis, occur mainly in immunocompromised patients[10−16]. In particular, disseminated fusariosis is a life-threatening infection with a 90-day survival of 42%–44%[17, 18] and 12-week survival of 53.3%[19].

The type of fusariosis largely depends on the immune status of the host and route of entry[14]. *Fusarium* keratitis occurs mainly in farmers and other workers with agricultural occupations, and a major risk factor is corneal trauma caused by plant material (e.g., branches and thorns)[7]. The predisposing factors for *Fusarium* onychomycosis are mostly trauma and contact with soil, and the most often affected occupations are gardeners and farmers[20, 21]. On the contrary, several routes of entry for disseminated fusariosis are suspected. The most well-known route is airborne, in which water distribution systems of hospitals represent a reservoir and infection source[22−24]. Skin is another route of entry, and primary skin lesions or onychomycosis lead to

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disseminated fusariosis in immunocompromised patients[25–27].

Over the past few decades, the number of case reports on human fusariosis has steadily increased; in particular, approximately 40% of these reports were due to *F. solani* and 20% were due to *F. oxysporum*[28]. However, the distribution of etiological agents varies for different types of fusariosis, for example, three studies on *Fusarium* keratitis illustrated that the isolation rate of the *F. oxysporum* species complex (FOSC) was 1.68%–6.5%[9, 29, 30], whereas three studies on *Fusarium* onychomycosis reported an isolation rate for FOSC of 32.8%–53.5%[8, 31, 32].

In Japan, *Fusarium* is known as one of the etiological agents of human fungal infection[33, 34]; however, a large-scale epidemiological survey on the etiological agents of fusariosis has not been reported. The main goal of the present study was to determin the distribution of *Fusarium* species causing IF and SF in Japan.

Materials and methods

1. Clinical strains

A total of 73 *Fusarium* clinical isolates (IF, n = 36; SF, n = 37) deposited in the Medical Mycology Research Center (MMRC), Chiba University, Japan were used for this study. All isolates were obtained from hospitals located in 28 prefectures in Japan between 1998 and 2015 and were sent to the MMRC for fungal identification. A summary of the isolates is shown in Table 1.

2. DNA extraction

Genomic DNA was extracted from 7–14-day-old mycelia cultured on potato dextrose agar using PrepMan™ Ultra sample preparation reagent (Thermo Fisher Scientific, CA, USA) according to the manufacturer's instructions.

3. *Fusarium*- and FSSC-specific real-time PCR

All isolates were initially identified by *Fusarium*-specific real-time PCR using the primer pair Fusa28sF1 and Fusa28sR1 and probe Fprobe1[35]. Subsequently, *Fusarium* isolates were classified as *Fusarium solani* species complex (FSSC) or non-FSSC *Fusarium* by FSSC-specific real-time PCR using the primer pair Fusa28sF1 and Fusa28sR1 and probe FSprobe1[35]. Real-time PCR conditions were described in our previous study[35].

4. Molecular identification

For fungal identification based on DNA sequencing analysis, a partial the elongation factor-1 alpha (EF-1α) gene was amplified by PCR using the primers HS392 and HS393, as described in our previous study[35]. The nuclear ribosomal internal transcribed spacer (ITS) region, including ITS1, 5.8S, and ITS2, was amplified by PCR using the primers ITS5 and ITS4[36]. PCR mixtures were prepared as follows. The total 25 µl reaction volume included 10 ng genomic DNA, 12.5 µl 2 × PCR buffer for KOD FX neo (Toyobo, Tokyo, Japan), 5 µl 2 mM dNTPs (Toyobo), 0.3 µM each primer, and 0.5 U KOD FX neo (Toyobo). PCR was performed using the iCycler (Bio-Rad, Tokyo, Japan) under the following conditions: 95℃ for 2 min, followed by 30 cycles of 95℃ for 30 s, 55℃ for 30 s, 72℃ for 1 min, with a final extension at 72℃ for 5 min. PCR products were purified using a FastGene Gel/PCR Extraction Kit (NIPPON Genetics) and sequenced by the direct sequencing method using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific, Tokyo, Japan) and the ABI 3130xl Genetic Analyzer (Thermo Fisher Scientific) according to the manufacturer's instructions. Primers HS392, HS393, EF11[37], EF21[37], ITS5, and ITS4 were used for the cycle sequencing. Sequences were assembled using ATGC v.6.0 (Genetyx, Tokyo, Japan) and Genetyx®-win v12 (Genetyx). BLAST searches of the sequence data were performed using the *Fusarium* MLST database (http://www.cbs.knaw.nl/fusarium/)[38].

5. Molecular phylogeny

For phylogenetic analysis, the two-locus dataset comprising partial EF-1α gene and ITS region sequences of 53 FSSC isolates obtained in this study and 20 *Fusarium* spp. isolates obtained from the *Fusarium* MLST database were aligned using ClustalX v. 2.1[39], followed by manual adjustments with Genetyx®-win v12 (Genetyx). The best-fit model was selected by hLRT in MrModeltest 2.3[40] and PAUP v.4.0 β 8 (Sinauer Associates, MA, USA). The phylogenetic tree was constructed using Bayesian inference with MrBayes v. 3.2[41]. Markov chain Monte Carlo iterations were performed to 500,000 generations, when the average standard deviations of split frequencies were less than 0.01, indicating convergence of the iterations. Phylogenetic trees were edited using FigTree v1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/).

Table 1. *Fusarium* isolates isolated from invasive and superficial fusariosis in Japan from 1998 to 2015

IFM No.	Species complex	Species	Type of fusariosis	Underlying disease	Source	Year	Geographic origin	GenBank accession No.	
								EF-1α	ITS
49277	FSSC	*F. petroliphilum*(FSSC 1)	IF(disseminated)	ALL	blood	1999	Ibaraki	LC177260	LC184196
58043	FSSC	*F. petroliphilum*(FSSC 1)	IF(disseminated)	ALL	blood	2008	Fukuoka	LC177271	LC184207
59480	FSSC	*F. petroliphilum*(FSSC 1)	IF(disseminated)	AML	blood	2010	Chiba	LC177277	LC184213
61165	FSSC	*F. petroliphilum*(FSSC 1)	IF(disseminated)	AML	blood	2012	Saitama	LC177307	LC184243
61380	FSSC	*F. petroliphilum*(FSSC 1)	IF(disseminated)	ML	blood	2012	Shizuoka	LC177308	LC184244
49272	FSSC	*F. keratoplasticum*(FSSC 2)	IF(disseminated)	ML	urine	1998	Tokyo	LC177263	LC184199
49274	FSSC	*F. keratoplasticum*(FSSC 2)	IF(disseminated)	leukemia	blood	1999	Chiba	LC177302	LC184238
50956	FSSC	*F. keratoplasticum*(FSSC 2)	IF(disseminated)	MM	blood	2001	Chiba	LC177300	LC184236
58015	FSSC	*F. keratoplasticum*(FSSC 2)	IF(disseminated)	MDS	urine	2008	Kyoto	LC177267	LC184203
59778	FSSC	*F. keratoplasticum*(FSSC 2)	IF(disseminated)	AML	blood	2010	Tokyo	LC177281	LC184217
62236	FSSC	*F. keratoplasticum*(FSSC 2)	IF(disseminated)	AML	blood	2013	Tokyo	LC177297	LC184233
62550	FSSC	*F. keratoplasticum*(FSSC 2)	IF(disseminated)	CML	blood	2012	Kumamoto	LC177316	LC184252
62701	FSSC	*F. keratoplasticum*(FSSC 2)	IF(disseminated)	gastric cancer	blood	2013	Chiba	LC177319	LC184255
63624	FSSC	*F. keratoplasticum*(FSSC 2)	IF(disseminated)	ALL	skin	2015	Hyogo	LC177331	LC184267
57371	FSSC	*F. keratoplasticum*(FSSC 2)	SF(keratitis)	none	cornea	2008	Kagoshima	LC177305	LC184241
58307	FSSC	*F. keratoplasticum*(FSSC 2)	SF(keratitis)	none	cornea	2009	Tokyo	LC177273	LC184209
57129	FSSC	*F. keratoplasticum*(FSSC 2)	SF(onychomycosis)	none	nail	2007	Mie	LC177324	LC184260
58017	FSSC	*F. falciforme*(FSSC 3+4)	IF(disseminated)	MDS	skin	2008	Aichi	LC177268	LC184204
62232	FSSC	*F. falciforme*(FSSC 3+4)	IF(disseminated)	AML	skin	2013	Nagasaki	LC177314	LC184250
57115	FSSC	*F. falciforme*(FSSC 3+4)	IF(locally invasive)	MDS	extremity injuries	2006	Hiroshima	LC177323	LC184259
62614	FSSC	*F. falciforme*(FSSC 3+4)	IF(locally invasive)	AML	conjunctiva	2013	Chiba	LC177318	LC184254
51945	FSSC	*F. falciforme*(FSSC 3+4)	SF(keratitis)	none	cornea	2002	Chiba	LC177301	LC184237
55596	FSSC	*F. falciforme*(FSSC 3+4)	SF(keratitis)	none	cornea	2006	Gifu	LC177304	LC184240
57139	FSSC	*F. falciforme*(FSSC 3+4)	SF(keratitis)	none	cornea	2007	Shizuoka	LC177326	LC184262
58106	FSSC	*F. falciforme*(FSSC 3+4)	SF(keratitis)	none	cornea	2009	Gifu	LC177272	LC184208
61623	FSSC	*F. falciforme*(FSSC 3+4)	SF(keratitis)	none	cornea	2012	Gifu	LC177311	LC184247
61944	FSSC	*F. falciforme*(FSSC 3+4)	SF(keratitis)	none	cornea	2012	Ehime	LC177287	LC184223
61945	FSSC	*F. falciforme*(FSSC 3+4)	SF(keratitis)	none	cornea	2013	Tokyo	LC177288	LC184224
62108	FSSC	*F. falciforme*(FSSC 3+4)	SF(keratitis)	none	cornea	2013	Tokyo	LC177290	LC184226
62125	FSSC	*F. falciforme*(FSSC 3+4)	SF(keratitis)	none	cornea	2013	Tokushima	LC177291	LC184227
62226	FSSC	*F. falciforme*(FSSC 3+4)	SF(keratitis)	none	cornea	2013	Ehime	LC177295	LC184231
62228	FSSC	*F. falciforme*(FSSC 3+4)	SF(keratitis)	none	cornea	2013	Tokushima	LC177296	LC184232
62911	FSSC	*F. falciforme*(FSSC 3+4)	SF(keratitis)	none	cornea	2014	Tokushima	LC177299	LC184235
49271	FSSC	*Fusarium* sp.(FSSC 5)	IF(disseminated)	AML	skin	1999	Nara	LC177261	LC184197
58331	FSSC	*Fusarium* sp.(FSSC 5)	IF(disseminated)	AML	skin	2009	Oita	LC177274	LC184210
61484	FSSC	*Fusarium* sp.(FSSC 5)	IF(disseminated)	AML	skin	2012	Osaka	LC177309	LC184245
61568	FSSC	*Fusarium* sp.(FSSC 5)	IF(disseminated)	AA	blood	2012	Hokkaido	LC177310	LC184246
62309	FSSC	*Fusarium* sp.(FSSC 5)	IF(disseminated)	ALL	skin	2013	Tokyo	LC177315	LC184251
62720	FSSC	*Fusarium* sp.(FSSC 5)	IF(disseminated)	ALL	blood	2014	Tokyo	LC177320	LC184256
63303	FSSC	*Fusarium* sp.(FSSC 5)	IF(disseminated)	AML	skin	2015	Nagasaki	LC177329	LC184265
63504	FSSC	*Fusarium* sp.(FSSC 5)	IF(locally invasive)	ALL	vitreous humor	2015	Chiba	LC177330	LC184266
49275	FSSC	*Fusarium* sp.(FSSC 5)	SF(keratitis)	none	cornea	1999	Hokkaido	LC177303	LC184239
57138	FSSC	*Fusarium* sp.(FSSC 5)	SF(keratitis)	none	cornea	2007	Ehime	LC177325	LC184261
58332	FSSC	*Fusarium* sp.(FSSC 5)	SF(keratitis)	none	cornea	2009	Chiba	LC177275	LC184211
59509	FSSC	*Fusarium* sp.(FSSC 5)	SF(keratitis)	none	cornea	2010	Gifu	LC177278	LC184214
61910	FSSC	*Fusarium* sp.(FSSC 5)	SF(keratitis)	none	cornea	2012	Ibaraki	LC177285	LC184221
61912	FSSC	*Fusarium* sp.(FSSC 5)	SF(keratitis)	none	cornea	2012	Hokkaido	LC177286	LC184222
62053	FSSC	*Fusarium* sp.(FSSC 5)	SF(keratitis)	none	cornea	2012	Gifu	LC177313	LC184249
62107	FSSC	*Fusarium* sp.(FSSC 5)	SF(keratitis)	none	cornea	2013	Shizuoka	LC177289	LC184225
59445	FSSC	*Fusarium* sp.(FSSC 5)	SF(onychomycosis)	none	nail	2010	Kochi	LC177276	LC184212
62551	FSSC	*Fusarium* sp.(FSSC 6)	IF(locally invasive)	diabetes	aqueous humor	2014	Tokyo	LC177317	LC184253
62225	FSSC	*Fusarium* sp.(FSSC 9)	SF(keratitis)	none	cornea	2013	Ehime	LC177294	LC184230
60607	FSSC	*Fusarium* sp.(FSSC 18)	IF (disseminated)	ALL	blood	2011	Tokyo	LC177306	LC184242
62174	FOSC	*Fusarium* sp.(FOSC 18)	SF (keratitis)	none	cornea	2013	Ehime	LC177292	LC184228
62224	FOSC	*Fusarium* sp.(FOSC 18)	SF (keratitis)	none	cornea	2012	Osaka	LC177293	LC184229
60522	FOSC	*Fusarium* sp.(FOSC 18)	SF (onychomycosis)	none	nail	2011	Okayama	LC177282	LC184218
61765	FOSC	*Fusarium* sp.(FOSC 48)	SF (keratitis)	none	cornea	2012	Aichi	LC177312	LC184248
54796	FOSC	*Fusarium* sp.(FOSC 48)	SF (onychomycosis)	none	nail	2005	Gifu	LC177264	LC184200
55588	FOSC	*Fusarium* sp.(FOSC 53)	IF (locally invasive)	uveitis	aqueous humor	2006	Wakayama	LC177322	LC184258
58003	FOSC	*Fusarium* sp.(FOSC 53)	SF (keratitis)	none	cornea	2008	Fukuoka	LC177266	LC184202
58042	FOSC	*Fusarium* sp.(FOSC 53)	SF (keratitis)	none	cornea	2008	Fukuoka	LC177270	LC184206
49276	FFSC	*F. fujikuroi*	IF (disseminated)	AML	blood	1998	Kagawa	LC177262	LC184198
59558	FFSC	*F. fujikuroi*	IF (disseminated)	ALL	skin	2010	Fukui	LC177280	LC184216
62831	FFSC	*F. fujikuroi*	IF (locally invasive)	prostate cancer	paranasal sinus tissue	2014	Aichi	LC177298	LC184234
62896	FFSC	*F. sacchari*	IF (disseminated)	AA	skin	2013	Miyagi	LC177321	LC184257
61844	FFSC	*F. acutatum*	SF (keratitis)	none	cornea	2013	Tokushima	LC177284	LC184220
52350	FFSC	*F. acutatum*	SF (onychomycosis)	none	nail	2003	Tokyo	LC177259	LC184195
58002	FFSC	*F. napiforme*	SF (keratitis)	none	cornea	2007	Gifu	LC177265	LC184201
59549	FDSC	*F. dimerum*	IF (disseminated)	ML	urine	2010	Chiba	LC177279	LC184215
63175	FDSC	*F. dimerum*	IF (locally invasive)	AA	ileocecal tissue	2014	Osaka	LC177328	LC184264
58035	FDSC	*F. delphinoides*	IF (disseminated)	leukemia	blood	2008	Ehime	LC177269	LC184205
58306	FIESC	*Fusarium* sp.(FIESC 1)	SF (keratitis)	none	cornea	2009	Gifu	LC177327	LC184263
61624	FIESC	*Fusarium* sp.(FIESC 18)	SF (keratitis)	none	cornea	2012	Tottori	LC177283	LC184219

Fusarium solani species complex, FSSC; *Fusarium oxysporum* species complex, FOSC; *Fusarium fujikuroi* species complex, FFSC; *Fusarium dimerum* species complex, FDSC; *Fusarium incarnatum-Fusarium equiseti* species complex, FIESC; invasive fusariosis, IF; superficial fusariosis, SF; acute lymphocytic leukemia, ALL; acute myeloid leukemia, AML; chronic myeloid leukemia, CML; multiple myeloma, MM; myelodysplastic syndromes, MDS; aplastic anemia, AA; malignant lymphoma, ML.

Results

A total of 73 *Fusarium* clinical isolates were initially identified mainly on the basis of fungal morphological observation under the microscope. To support the morphological identification, we identified all the isolates using *Fusarium*-specific real-time PCR. The results from the two identification methods indicated that all isolates belong to the genus *Fusarium*. Subsequently, all isolates were classified as FSSC or non-FSSC *Fusarium* via FSSC-specific real-time PCR. As a result, 72.6% (53/73) of *Fusarium* isolates were categorized as FSSC, and 27.4% (20/73) were identified as non-FSSC *Fusarium*. Furthermore, non-FSSC *Fusarium* isolates were identified through DNA sequencing of the partial EF-1α gene and the nuclear ribosomal ITS region. The DNA sequence data were deposited in GenBank with accession numbers LC177259–LC177331 for EF-1 α, LC184195–LC184267 for ITS. FOSC (11.0%, 8/73) was found to be the most frequent species complex of non-FSSC *Fusarium*, followed by *F. fujikuroi* species complex (FFSC) (9.6%, 7/73), *F. dimerum* species complex (FDSC) (4.1%, 3/73), and *F. incarnatum-F. equiseti* species complex (FIESC) (2.7%, 2/73) (Table 2).

To determine the relationship between the etiological species and the type of fusariosis, we classified all the isolates according to whether they were obtained from patients with IF or SF. Among the IF isolates, the most frequent species complex was FSSC (77.8%, 28/36), followed by FFSC (11.1%, 4/36), FDSC (8.3%, 3/36), and FOSC (2.8%, 1/36). Among the SF isolates, the most frequent species complex was FSSC (67.6%, 25/37), followed by FOSC (18.9%, 7/37), FFSC (8.1%, 3/37), and FIESC (5.4%, 2/37).

Next, we performed molecular phylogenetic analyses of the FSSC isolates based on the two-locus dataset comprising the partial EF-1α gene and ITS region sequences (1137–1157 bases). Phylogenetic taxa in FSSC were categorized according to O'Donnell *et al.*[42, 43], Zhang *et al.*[44] and Short *et al.*[45]. Our phylogenetic tree showed that all FSSC isolates were nested within clade 3, and these isolates were divided into seven phylogenetic species (Fig. 1). Among IF isolates, the most frequent etiological species was *F. keratoplasticum* (FSSC 2) (25.0%, 9/36), followed by *Fusarium* sp. (FSSC 5) (22.2%, 8/36), *F. petroliphilum* (FSSC 1) (13.9%, 5/36), *F. falciforme* (FSSC 3+4) (11.1%, 4/36), *Fusarium*. sp. (FSSC 6) (2.8%, 1/36), and *Fusarium* sp. (FSSC 18) (2.8%, 1/36) (Table 2). Among SF isolates, the most frequent causative species was *F. falciforme* (FSSC 3+4) (32.4%, 12/37) followed by FSSC 5 (24.3%, 9/37), *F. keratoplasticum* (FSSC 2) (8.1%, 3/37), and *Fusarium* sp. (FSSC 9) (2.7%, 1/37) (Table 2).

Discussion

This is the first epidemiological report on the investigation of etiological species of human fusariosis in Japan.

FSSC accounted for the majority of isolates responsible for fusariosis (72.6%), and four species complexes, FSSC, FOSC, FFSC, and FDSC, accounted for 97.3% of the isolates, consistent with data reported by O'Donnell *et al.*; in their study, they reported that FSSC accounted for 60% of isolates from patients with fusariosis, and four species complexes, FSSC, FOSC, FFSC, and FDSC, accounted for approximately 85% of such species within the United States[46]. Dalyan Cilo *et al.* reported that FFSC accounted for 51.5% of isolates from patients with fusariosis, followed by FSSC (42.4%) in Turkey[47]. These data suggested that regional differences exist in the distribution of etiological agents of fusariosis.

In the present study, most cases of IF (80.6%) were disseminated infection, and most cases of SF (86.5%) were keratitis. A study by Scheel *et al.* indicated that 98.8% of *Fusarium* isolates from patients with IF were classified as FSSC[48], whereas two studies illustrated that both FFSC (35.5%–40%) and FSSC (33%–33.5%) were predominantly isolated from patients with IF[17, 19]. In the present study, FSSC was the most frequent isolate in patients with IF (77.8%), whereas the isolation rate of FFSC (11.1%) was lower than that in the two aforementioned studies[17, 19]. Three studies focusing on *Fusarium* keratitis reported an isolation rate of 59.1%–76.3% for FSSC[9, 29, 30], which agrees with the isolation rate of FSSC from patients with SF (72.6%) in the present study.

Recent molecular phylogenetic studies revealed that *F. solani* harbors closely related multispecies. O'Donnell reported that FSSC was divided into three major clades; namely, clades 1, 2, and 3, using phylogenetic analysis based on the DNA sequences of three genes (28s rRNA, ITS region, and EF-1α)[42]. In addition, all FSSC isolates

Table 2. Identification of *Fusarium* isolates causing invasive and superficial fusariosis

Species complex Species	% of isolates		
	Invasive fusariosis (n = 36)	Superficial fusariosis (n = 37)	Total (n = 73)
FSSC	77.8 (28/36)	67.6 (25/37)	72.6 (53/73)
F. petroliphilum (FSSC 1)	13.9 (5/36)		6.8 (5/73)
F. keratoplasticum (FSSC 2)	25.0 (9/36)	8.1 (3/37)	16.4 (12/73)
F. falciforme (FSSC 3 + 4)	11.1 (4/36)	32.4 (12/37)	21.9 (16/73)
Fusarium sp. (FSSC 5)	22.2 (8/36)	24.3 (9/37)	23.3 (17/73)
Fusarium sp. (FSSC 6)	2.8 (1/36)		1.4 (1/73)
Fusarium sp. (FSSC 9)		2.7 (1/37)	1.4 (1/73)
Fusarium sp. (FSSC 18)	2.8 (1/36)		1.4 (1/73)
FOSC	2.8 (1/36)	18.9 (7/37)	10.9 (8/73)
Fusarium sp. (FOSC 18)		8.1 (3/37)	4.1 (3/73)
Fusarium sp. (FOSC 48)		5.4 (2/37)	2.7 (2/73)
Fusarium sp. (FOSC 53)	2.8 (1/36)	5.4 (2/37)	4.1 (3/73)
FFSC	11.1 (4/36)	8.1 (3/37)	9.6 (7/73)
F. fujikuroi	8.3 (3/36)		4.1 (3/73)
F. acutatum		5.4 (2/37)	2.7 (2/73)
F. sacchari	2.8 (1/36)		1.4 (1/73)
F. napiforme		2.7 (1/37)	1.4 (1/73)
FDSC	8.3 (3/36)	0 (0/37)	4.1 (3/73)
F. dimerum	5.5 (2/36)		2.7 (2/73)
F. delphinoides	2.8 (1/36)		1.4 (1/73)
FIESC	0 (0/36)	5.4 (2/37)	2.7 (2/73)
Fusarium sp. (FIESC 1)		2.7 (1/37)	1.4 (1/73)
Fusarium sp. (FIESC 18)		2.7 (1/37)	1.4 (1/73)

FSSC, *Fusarium solani* species complex; FOSC, *Fusarium oxysporum* species complex; FFSC, *Fusarium fujikuroi* species complex; FDSC, *Fusarium dimerum* species complex; FIESC, *Fusarium incarnatum-Fusarium equiseti* species complex.

from humans and animals were nested within clades 3, and *F. falciforme* (FSSC 3 + 4), *F. keratoplasticum* (FSSC 2), *Fusarium* sp. (FSSC 5), *F. petroliphilum* (FSSC 1), and *Fusarium* sp. (FSSC 6) were the most important clinical species[43–45, 49]. We performed a phylogenetic analysis based on the two-locus dataset and found that all FSSC isolates were nested within clade 3, and 96.2% of the FSSC isolates were classified as the five aforementioned phylogenetic species. The etiological species responsible for IF and SF displayed different distribution spectra in the present study; specifically, *F. keratoplasticum* (FSSC 2) was the most frequent isolate from patients with IF, whereas *F. falciforme* (FSSC 3 + 4) was the most frequent isolate from patients with SF. *Fusarium* sp. (FSSC 5) was the second most frequent isolate from both patients with IF and SF. Notably, *F. petroliphilum* (FSSC 1) was isolated only from patients with IF. Each species was isolated from a broad geographic area, and an epidemic was not observed. The principal route of entry for disseminated fusariosis appears to be the inhalation of fungal elements (hyphae or spores) in the environment. Some studies revealed that *F. petroliphilum* (FSSC 1) and *F. keratoplasticum* (FSSC 2)

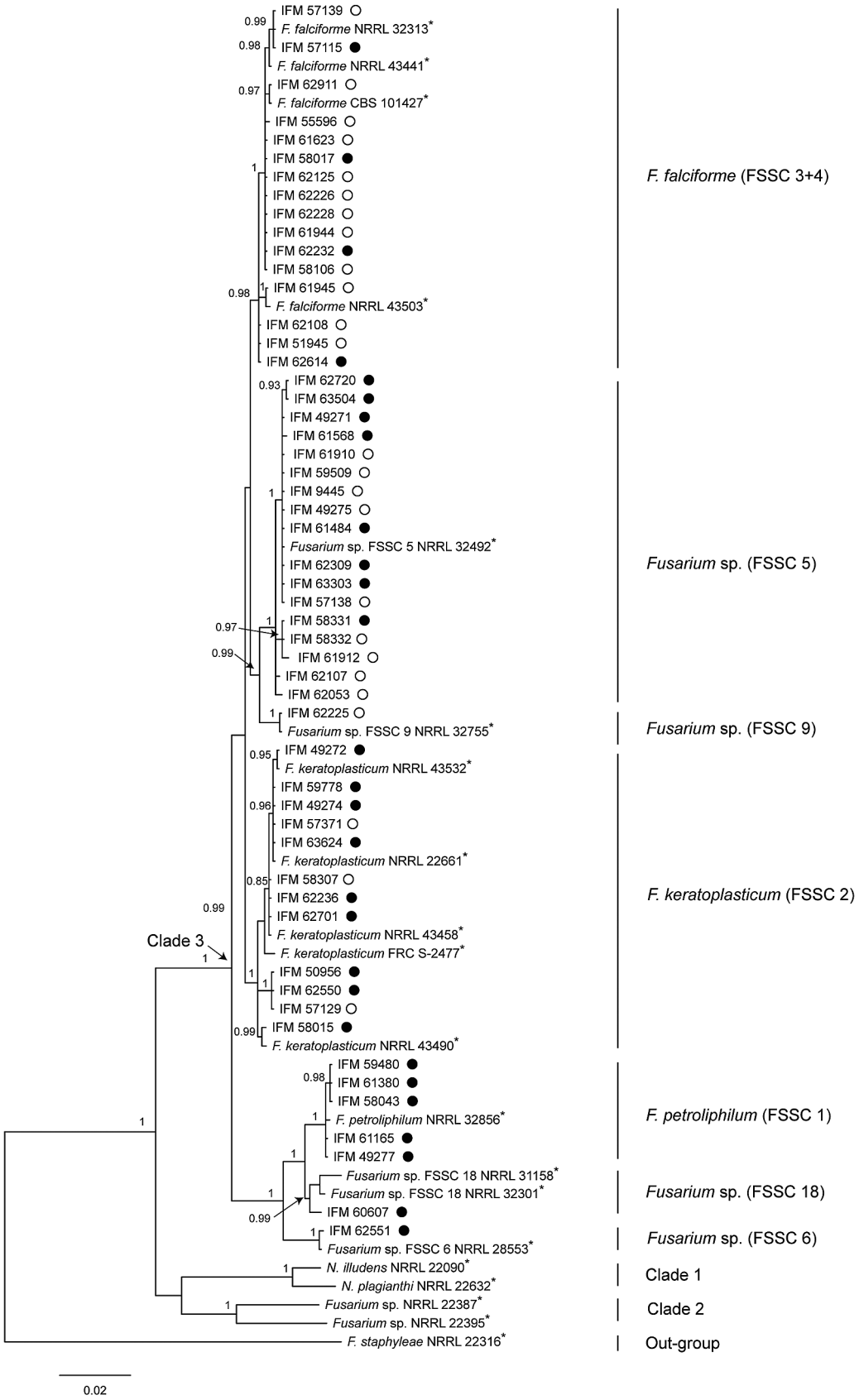

Fig. 1. Bayesian inference tree based on the two-locus dataset comprising partial EF-1α gene and ITS region sequences from 53 *Fusarium solani* species complex (FSSC) isolates from this study and 20 reference strains from the *Fusarium* MLST database. The numbers on each node indicate the Bayesian posterior probabilities (only those with values ≥ 0.85 are shown). ●, invasive fusariosis; ○, superficial fusariosis; *, reference strains.

were frequently isolated from indoor environments including water systems in hospitals[43, 45, 48, 49], and it was suspected to be a reservoir and infection source of fusariosis. On the contrary, the principal route of entry for *Fusarium* keratitis is corneal trauma caused by plant material (e.g., branches and thorn)[7]. In a recent study, *Fusarium* sp. (FSSC 5) and *F. falciforme* (FSSC 3 + 4) were isolated from soybean roots[50]. In a case of *Fusarium* keratitis outbreak associated with contact lens use in the United States between 2004 and 2005, O'Donnell *et al*. concluded that patients' bathroom sink drains represented a likely source of *Fusarium* infection[51]. In the present study, predominant species from patients with SF, *F. falciforme* (FSSC 3 + 4) and *Fusarium* sp. (FSSC 5), were minor isolates from both corneal and patients' environment samples in their study[51]. These data support the view that etiological species of fusariosis are associated with the route of entry and distribution of *Fusarium* species in the environment.

In conclusion, our study indicated that FSSC was the most common etiological agent of both IF and SF in Japan, and the distribution of phylogenetic species in FSSC isolates from patients with IF and SF had different spectra. Our data will help reveal the infection source of fusariosis, which will be helpful for appropriate prevention and treatment to achieve better prognosis.

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Conflict of interest

Self-declared COI content: none

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