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In vitro antifungal susceptibility and molecular identity of 99 clinical isolates of the opportunistic fungal genus *Curvularia*☆

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ABSTRACT

The in vitro antifungal susceptibility of a set of 99 clinical isolates of *Curvularia* was tested against 9 drugs using a reference microdilution method. The isolates had been identified previously to species level by comparing their ITS rDNA and glyceraldehyde-3-phosphate dehydrogenase gene sequences with those of reference strains. We were able to reliably identify 73.2% of the isolates, the most frequent species being *Curvularia aeria*, *Curvularia geniculata*/*Curvularia senegalensis*, *Curvularia lunata*, *Curvularia inaequalis*, *Curvularia verruculosa*, and *Curvularia borreriae*. Most of these isolates had been recovered from nasal sinus, which is generally considered one of the most frequent sites of infection by these fungi. In addition, at least 3 phylogenetic species that have not yet been formally described were detected. The most active drugs were the echinocandins, amphotericin B, and posaconazole, whereas voriconazole and itraconazole showed poor activity.

1. Introduction

The genus *Curvularia* comprises more than 80 species. Most of them are saprobes in soil or plant pathogens (Manamgoda et al., 2011; Manamgoda et al., 2012; Sivanesan, 1987), but some species have been reported to cause infections in humans and animals (de Hoog et al., 2000).

The identity of most of the clinical isolates of *Curvularia* is confusing. Most literature citations are usually short case reports, in which the *Curvularia* isolates had been identified only by phenotypic criteria. Considering the morphological similarity of *Curvularia* spp. and that differences between them are based on subtle characters that can vary depending on culture conditions, species identification may be incorrect, doubtful, or remain unresolved (Revankar and Sutton, 2010; Vermeire et al., 2010). Recent studies have shown that morphological identification does not correlate with molecular identification (Manamgoda et al., 2012; Yanagihara et al., 2010). In particular, clinical isolates morphologically identified as *Curvularia lunata*, the most commonly reported clinical species (de Hoog et al., 2000; Revankar and Sutton, 2010), were placed in different, unrelated phylogenetic clades (Yanagihara et al., 2010). Other species, such as *Curvularia brachyspora*, *Curvularia clavata*, *Curvularia geniculata*, *Curvularia inaequalis*, *Curvularia pallescens*, *Curvularia senegalensis*, and *Curvularia verruculosa*, have also been reported in clinical cases (de Hoog et al., 2000; Revankar and Sutton, 2010; Wilhelmus and Jones, 2001). These species have been associated with different types of infection, such as keratitis, sinusitis, cutaneous and subcutaneous infections, peritonitis, onychomycosis, endocarditis, endophthalmitis, cerebral phaeohyphomycosis, and allergic bronchopulmonary as well as disseminated disease (Alvarez et al., 2011; Bryan et al., 1993; Ehlers et al., 2011; Forster et al., 1975; Gupta et al., 2007; Moody et al., 2012; Mroueh and Spock, 1992; Sharma et al., 2011; Singh et al., 2008; Tessari et al., 2003; Varughese et al., 2011; Yau et al., 1994). *Curvularia* is able to infect both immunocompetent and immunosuppressed patients, mainly in tropical and subtropical areas. Cases have been reported mainly in India, the United States, Brazil, Japan, and Australia (Agrawal and Singh, 1995; Carter and Boudreaux, 2004; Guarro et al., 1999; Tanabe et al., 2010; Thew and Todd, 2008). However, as mentioned above, the real incidence of the different species of *Curvularia* in human infections is inconclusive. Furthermore, clinical data on the treatment of *Curvularia* infections are scarce, and the most appropriate therapies are unknown.

Species of *Curvularia* are traditionally characterized by dark mycelium, geniculate conidiophores with sympodial, tretic conidiogenous cells and elongated conidia. The conidia are smooth to tuberculate-walled, with several false septa (distosepta) and straight or curved due to an enlarged middle cell that is often more pigmented than the other cells. *Bipolaris*, *Drechslera*, and *Exserohilum* are

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Table 1
Clinical isolates, type, and reference strains of *Curvularia* spp. included in the study.

Species	Strain	Origin	GenBank no.	
			ITS	GPDH
C. aeria	CBS 294.61 (T) (as *C. lunata* var. *aeria*)	Air, Brazil	HE861850	HF565450
	CBS 337.64 (as *C. lunata* var. *lunata*)	*Agropyron repens*, USA		
	CBS 533.70 (as *C. lunata* var. *aeria*)	Seed, *Pennisetum*, Denmark		
	UTHSC 06-3039	Nasal sinus, Arizona		
	UTHSC 07-481	Ethmoid sinus, Arizona		
	UTHSC 07-2859	Leg, Florida	HE861852	HF565451
	UTHSC 07-3183	Nasal sinus, Minnesota		
	UTHSC 08-2	Maxillary sinus, South Carolina		
	UTHSC 08-1226	Maxillary sinus, Vermont		
	UTHSC 08-2586	Nasal sinus, Florida		
	UTHSC 08-2625	Nasal sinus, South Carolina		
	UTHSC 08-2923	Nasal sinus, South Carolina		
	UTHSC 08-3003	Blood, Texas		
	UTHSC 08-3299	Nasal sinus, Washington		
	UTHSC 08-3398	Bronchial wash, Texas		
	UTHSC 08-3490	Frontal sinus, Missouri		
	UTHSC 09-494	Nasal sinus, Texas		
	UTHSC 09-2019	Wound, South Carolina		
	UTHSC 09-2085	Nasal sinus, Minnesota	HE861844	HF565452
	UTHSC 09-2318	Nasal sinus, Texas	HE861851	HF565453
	UTHSC 09-2546	Middle turbinate, Arkansas		
	UTHSC 09-3123	Nasal sinus, South Carolina		
	UTHSC 09-3124	Nasal sinus, South Carolina	HE861843	HF565454
	UTHSC 10-370	Skin, Utah		
	UTHSC 10-543	Peritoneal dialysis fluid, South Carolina		
	UTHSC 10-816	Nasal sinus, Texas		
C. borreriae	CBS 859.73	Volcanic ash soil, Chile	HE861848	HF565455
	UTHSC 08-2957	Corneal ulcer, West Virginia	HE861846	HF565456
	UTHSC 08-3433	Nasal sinus, California	HE861845	HF565457
	UTHSC 09-2408	Sputum, Washington		
	UTHSC 09-3510	Peritoneal dialysis fluid, District of Columbia	HE861847	HF565458
C. cf. *clavata*	UTHSC 10-1041	Bronchial wash, Utah	HE861819	HF565460
C. geniculata/*C. senegalensis*	CBS 220.52	Discolored wood, triplex plank, Suriname		
	CBS 332.64	*Setaria italica*, USA		
	CBS 149.71 (as *C. senegalensis*)	Unknown, Nigeria		
	CBS 431.75 (as *C. senegalensis*)	Sorghum, Fiji		
	UTHSC 07-3111	Eye, Texas	HE861839	HF565461
	UTHSC 07-3044	Nasal sinus, South Carolina		
	UTHSC 07-3620	Skin scraping, Texas		
	UTHSC 07-3740	Toe nail, Minnesota		
	UTHSC 08-2979	Toe nail, Texas		
	UTHSC 08-3314	Bronchial wash, Minnesota	HE861841	HF565462
	UTHSC 08-3531	Eye, Louisiana		
	UTHSC 08-3728	Eye, Missouri		
	UTHSC 09-1824	Knee biopsy, Florida		
	UTHSC 09-2568	Leg, Texas		
	UTHSC 09-2592	Finger wound, Utah		
	UTHSC 09-2753	Corneal ulcer, Tennessee		
	UTHSC 09-3005	Bronchial wash, Tennessee	HE861840	HF565463
	UTHSC 09-3435	Ear, Florida		
C. cf. *inaequalis*	UTHSC 07-3495	Cerebrospinal fluid, Wisconsin		
	UTHSC 08-2346	Foot, Utah		
	UTHSC 08-2860	Ethmoid tissue, Utah	HE861821	HF565464
	UTHSC 08-3472	Nasal sinus, Utah	HE861822	HF565465
	UTHSC 08-3685	Arm, Utah		
	UTHSC 09-1077	Lung biopsy, Utah	HE861820	HF565466
C. intermedia	CBS 334.64	*Avena versicolor*, North Carolina	HE861853	HF565467
	UTHSC 08-1041	Nail, South Carolina	HE861854	HF565468
	UTHSC 09-3240	Tissue, Colorado	HE861855	HF565469
C. lunata	CBS 730.96 (NT)	Lung biopsy, Florida	JX256429[a]	JX256429[a]
	CBS 157.34	Unknown, Indonesia		
	UTHSC 07-3452	Toe nail, Florida		
	UTHSC 08-172	Toe nail, Florida		
	UTHSC 08-959	Nail, South Carolina		
	UTHSC 08-1381	Eye, North Carolina		
	UTHSC 09-31	Wound, Minnesota	HE861816	HF565470
	UTHSC 09-191	Toe nail, South Carolina		
	UTHSC 09-2114	Nail, South Carolina		
	UTHSC 09-2395	Ethmoid sinus, South Carolina	HE861818	HF565471
	UTHSC 09-2719	Wound, South Carolina	HE861817	HF565472
	UTHSC 10-145	Contact lens, Minnesota		
C. protuberata	CBS 376.65 (T)	Leaf, *Deschampsia flexuosa*, Edinburgh	HE861823	HF565473
	UTHSC 08-2588	Left tibia, Minnesota		

(continued on next page)

Table 1 (*continued*)

Species	Strain	Origin	GenBank no.	
			ITS	GPDH
	UTHSC 08-2880	Leg, North Carolina	HE861825	HF565474
	UTHSC 09-1969	Abscess—penis, South Carolina	HE861824	HF565475
C. pseudorobusta	UTHSC 08-3458	Nasal sinus, Texas	HE861838	HF565476
C. cf. *sorghina*	UTHSC 08-809	Sphenoid sinus, South Carolina	HE861826	HF565477
	UTHSC 08-3445	Wound, Minnesota		
	UTHSC 09-868	Nasal sinus, South Carolina	HE861827	HF565478
	UTHSC 09-3575	Leg wound, Texas	HE861828	HF565479
C. verruculosa	CBS 148.63 [(T)]	*Typha* sp., India	HE861829	HF565480
	CBS 149.63	*Elaeis guineensis*, Nigeria		
	UTHSC 07-3093	Cornea, Texas	HE861830	HF565481
	UTHSC 08-827	Corneal ulcer, Texas	HE861831	HF565482
	UTHSC 09-2658	Inferior turbinate, Texas		
	UTHSC 09-2246	Ethmoid sinus, Texas		
	UTHSC 09-2471	Maxillary sinus, Tennessee		
	UTHSC 10-709	Wound, Minnesota		
Curvularia sp. I	CBS 144.63 (as *C. lunata* var. *lunata*)	Leaf, India		
	UTHSC 07-2791	Cornea, Utah		
	UTHSC 07-3105	Nasal sinus, Texas		
	UTHSC 07-3184	Nasal sinus, Arkansas		
	UTHSC 07-3581	Nail, Minnesota		
	UTHSC 08-849	Eye, Louisiana	HE861837	HF565483
	UTHSC 08-1296	Nail, Texas		
	UTHSC 08-2418	Bronchial wash, Texas		
	UTHSC 08-2517	Foot, Texas		
	UTHSC 08-2905	Chest, Utah	HE861836	HF565484
	UTHSC 08-3737	Bronchial wash, Texas		
	UTHSC 09-464	Cornea, Florida		
	UTHSC 09-1692	Nasal sinus, Ohio		
	UTHSC 09-2197	Nasal sinus, Minnesota	HE861835	HF565485
	UTHSC 09-2532	Nasopharynx, Texas		
	UTHSC 09-3403	Tissue, Texas		
Curvularia sp. II	UTHSC 07-2649	Toe tissue, Texas	HE861834	HF565486
	UTHSC 08-84	Nasal sinus, Utah		
	UTHSC 08-278	Peritoneal dialysis fluid, Ohio	HE861832	HF565487
	UTHSC 08-2697	Leg, Tennessee		
	UTHSC 08-3414	Ankle, Minnesota	HE861833	HF565488
	UTHSC 09-2907	Toe nail, Oklahoma		
	UTHSC 09-2806	Bone marrow, Virginia		
	UTHSC 09-2863	Bronchial wash, Texas		
	UTHSC 10-1276	Maxillary sinus (antrochoanal polyp), California		
Curvularia sp. III	UTHSC 07-2764	Toe nail, Montana		
	UTHSC 08-1283	Nasal sinus, Nevada		
	UTHSC 09-2092	Nasal sinus, California	HE861842	HF565459

UTHSC = Fungus Testing Laboratory, University of Texas Health Science Center, San Antonio, TX; CBS = CBS-KNAW Fungal Biodiversity Centre, Utrecht, the Netherlands; [(T)] = type strain; [(NT)] = neotype strain.

[a] Sequences from Manamgoda et al. (2012).

morphologically similar genera, and they are differentiated mainly by their conidial morphology and teleomorph associations (Sivanesan, 1987). *Curvularia* and *Bipolaris* are associated to the teleomorph genus, *Cochliobolus* and *Exserohilum* to *Setosphaeria* (Ellis, 1971, 1976; Sivanesan, 1987). In a recent taxonomic re-evaluation of these genera based on a multigene analysis, Manamgoda et al. (2012) amended the concept of *Curvularia* and some clinically relevant species of *Bipolaris*, such as *Bipolaris australiensis*, *Bipolaris hawaiiensis*, and *Bipolaris spicifera*, were transferred to *Curvularia*.

In this study, we have reliably identified a large set of clinical isolates of *Curvularia* from the USA, comparing the sequences of 2 DNA regions (internal transcribed spacer [ITS] region of the rDNA and glyceraldehyde-3-phosphate dehydrogenase [GPDH] gene) with those of reference strains in order to assess the real spectrum of *Curvularia* spp. in clinical samples, and determined their susceptibility to the available antifungal drugs.

2. Materials and methods

2.1. Fungal isolates

A total of 101 clinical isolates morphologically identified as *Curvularia* were included in the study. These isolates were randomly chosen from nearly 250 *Curvularia* isolates stored in the repository of the Fungus Testing Laboratory (FTL) at the University of Texas Health Science Center at San Antonio, which had been received over a period of 5 years (2006–2010). In addition, 15 type or reference strains of *Curvularia* spp. were also included in the study (Table 1).

2.2. Morphological study

All isolates were cultured on potato carrot agar (20 g of potatoes, 20 g of carrots, 20 g of agar, 1 L of distilled water) and oatmeal agar (30 g of filtered oat flakes, 20 g of agar, 1 L of distilled water) and incubated at 25 °C for 10–21 days in the dark. The identification criteria were according to Ellis (1971, 1976) and Sivanesan (1987). Microscopic features were examined on direct wet mounts with 85% lactic acid from the different culture media using light microscopy.

2.3. Molecular study

Isolates were grown on yeast extract sucrose (2% of yeast extract, 15% of sucrose, 2% of agar, 1 L of water) for 3 days at 25 °C, and DNA was extracted using a PrepMan Ultra sample preparation reagent (Applied Biosystems, Foster City, CA, USA), following the manufacturer's protocol. The DNA was quantified using GeneQuantpro

(Amersham Pharmacia Biotech, Cambridge, United Kingdom). The 5.8S and flanking ITS regions and a fragment of the GPDH gene were amplified and sequenced with the primer pairs ITS5/ITS4 and gpd1/gpd2, respectively, following previously described protocols (Álvarez et al., 2010; Berbee et al., 1999). The ITS PCR products were purified and sequenced at Macrogen Europe (Amsterdam) with a 3739XL DNA analyzer (Applied Biosystems). The PCR and GPDH gene sequencing was carried out at the CBS-KNAW Fungal Biodiversity Centre (Utrecht, the Netherlands). This locus was sequenced using the BigDye terminator sequencing kit v. 3.1 (Applied Biosystems) and an ABI PRISM™ 3100 DNA sequencer (Applied Biosystems). SeqMan (Lasergene, Madison, WI, USA) was used to obtain consensus from the complementary sequences of each isolate. BLAST sequence identity searches with ITS sequences were carried out to compare data from the isolates studied with those deposited in the GenBank database.

2.4. Phylogenetic analysis

Nucleotide sequences were aligned with Clustal X version 1.81 (Thompson et al., 1997), followed by manual adjustments with a text editor. Phylogenetic analyses were conducted using MEGA v. 5.05 with the maximum likelihood (ML) algorithm, using Kimura 2-parameter model with pairwise deletion of gaps (Tamura et al., 2011). The robustness of branches was assessed by bootstrap analyses of 1000 replicates. Two sequences retrieved from GenBank were used as outgroup in the combined analysis, one of *Exserohilum rostratum* ATCC32197 (ITS, AF071342.1; GPDH, AF081379.1) and another of *Alternaria alternata* EGS 34–016 (ITS, AF347031.1; GPDH, AF081400.1).

2.5. Antifungal susceptibility

Antifungal susceptibility was tested using methods outlined in CLSI document M38-A2 (CLSI, 2008). With the exception of 2 isolates that did not sporulate, all the *Curvularia* isolates were included in the antifungal susceptibility study. The drugs tested included anidulafungin (AFG), amphotericin B (AMB), caspofungin (CAS), itraconazole (ITC), fluconazole (FLC), voriconazole (VRC), micafungin (MFG), posaconzole (PSC), and 5-flucytosine (5-FC).

Testing involved standardizing the inoculum by spectrophotometer to 0.4–5 × 10^4 CFU/mL (0.25–0.3 OD at 530 nm), the use of RPMI 1640 with L-glutamine but without bicarbonate, and incubation at 35 °C. The MIC endpoint was 100% inhibition for AMB, ITC, VRC, and PSC and 80% inhibition for FLU and 5-FC. The minimum effective concentration (MEC) for the echinocandins was the lowest concentration where a visible change in the growth characteristics compared to the growth control was observed. Endpoint determinations were read at 48 hours. In the event that sufficient growth was not observed at the prescribed reading time, the tests continued to be incubated until sufficient growth was observed enabling accurate endpoint determination.

3. Results

With the primers used, we were able to amplify and sequence the ITS region and a fragment of the GPDH gene, and the length of the alignment used in the combined phylogenetic analysis was 886 bp (385 bp for ITS and 501 bp for GPDH). Fig. 1 shows the ML phylogenetic tree inferred from the analysis produced from combined ITS and GPDH sequences. The isolates were placed in 14 well-supported clades, which represented different phylogenetic species of *Curvularia*. Three of them did not include any type or reference species and did not match morphologically with any known species of *Curvularia*; they were therefore considered as putative new species, which we refer to here as *Curvularia* sp. I to III.

We were able to identify 72.3% of the isolates at species level, those being: *Curvularia aeria* (23 isolates), *C. geniculata*/*C. senegalensis* (14 isolates), *C. lunata* (10 isolates), *C. verruculosa* (6 isolates), *Curvularia protuberata* (3 isolates), *Curvularia intermedia* (2 isolates), *Curvularia borreriae* (4 isolates), *Curvularia pseudorobusta* (1 isolate), *C.* cf. *inaequalis* (6 isolates), *Curvularia* cf. *sorghina* (4 isolates) and *C.* cf. *clavata* (1 isolate). *Curvularia inaequalis*, *C. sorghina*, and *C. clavata* were identified only on the basis of morphological criteria and could not be confirmed molecularly because there were no reference strains or sequences available for comparison.

The clade representing *Curvularia* sp. I contained 15 clinical isolates and 1 strain from the CBS culture collection received as *C. lunata*. The members of this clade were morphologically compatible with *C. lunata*, showing conidia usually curved, obovoid, 3-4-distoseptate, with the third cell from the base curved and often larger and darker than the others, smooth to roughly warted, intermediate and end cells pale brown to brown and with smooth walls. They measured 16–30 μm long, 7–14 μm wide in the broadest part. However, the ex-type strain of *C. lunata* nested in a different clade in the present analysis. The similarity of the sequences of this clade was 98.1–100%.

The clade of *Curvularia* sp. II comprised 9 clinical isolates with identical sequences. They were characterized by straight to curved, broadly ellipsoidal conidia, usually with the third cell much larger than the others and unequally sided, predominantly 3-distoseptate, pale brown to brown, and with a smooth to slightly rugose basal cell, and measuring 13–28 μm long by 7–15 μm wide in the broadest part.

The clade representing *Curvularia* sp. III included 3 clinical isolates. They showed conidia curved, obovoid, 3-distoseptate, with one or more septa thicker and darker than the others, with the third cell from the base frequently larger and darker than the others, end cells usually pale brown, intermediate cells brown or dark brown, smooth, 11–25 μm long, 7–12 μm wide in the broadest part. These morphological features were similar to those observed in the isolates of the clade of *C. aeria*; however, the similarity was of only 95%.

The majority of the clinical isolates included in this study were from the nasal region (36.6%), followed by skin and nails (21.8%), eyes (10.8%), bronchial washings (6.9%), and peritoneal dialysis fluid (2.9 %).

The results of the susceptibility tests are shown in Table 2. AMB, MFG, and PSC were the most active drugs. AFG showed generally good activity, although 2 isolates of *C. lunata* showed high MICs (>8 μg/mL). The 3 echinocandins displayed different results, and although AFG and MFG showed very low MICs (geometric mean [GM] total of 0.7 and 0.2 μg/mL, respectively), those of CAS were considerable higher (GM 1 μg/mL). ITC and VRC showed high MICs for some species such as *C. aeria*, *C. borreriae*, *C. protuberata*, and *C. pseudorobusta*. Fluconazole and flucytosine were not active against any of the isolates tested.

4. Discussion

This study demonstrates that a wide spectrum of *Curvularia* spp. is represented in clinical samples. In addition, the high number of clinical isolates received by the FTL over a 5-year period highlights the possible importance of *Curvularia* in the clinical setting.

Most of the isolates included in the present study were morphologically identified as *C. lunata*; however, the phylogenetic analysis demonstrates that, in many cases, that identification was incorrect. According to Sivanesan (1987) and Ellis (1971), this species comprised 2 varieties: *C. lunata* var. *aeria*, which was characterized by smooth conidia and a presence of stromata in culture; and *C. lunata* var. *lunata* characterized by smooth to roughly warted conidia and an absence of stromata. Nakada *et al.* (1994), based on DNA RFLP, considered them as 2 different species, i.e., *C. lunata* and *C. aeria*; this is confirmed in our study, in which the type strains of both species are placed in very distant clades. There is also confusion in the taxonomy of *C. geniculata* and *C. senegalensis*, which have traditionally been described as 2 different, clinically relevant species (de Hoog et al.,

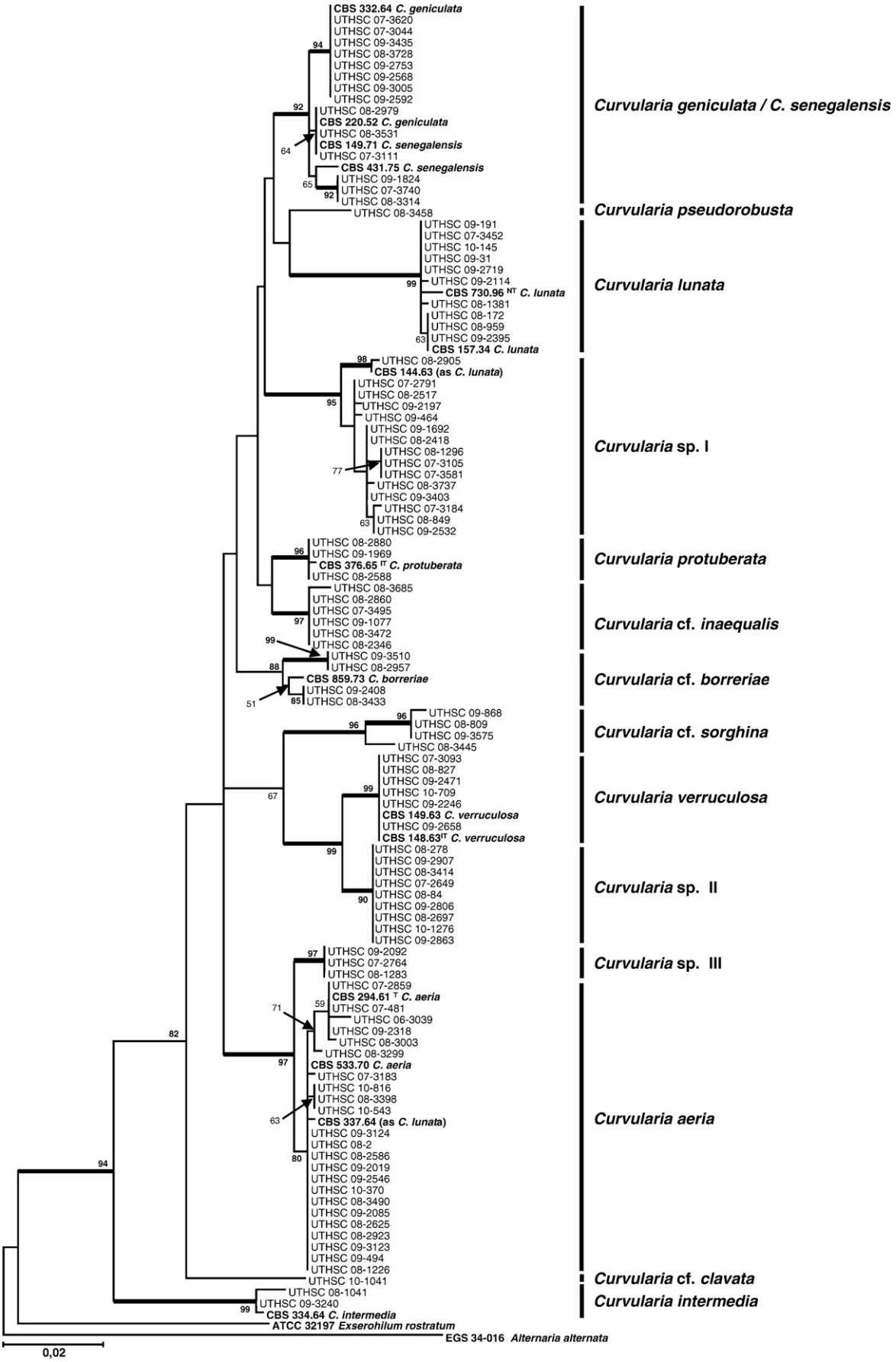

Fig. 1. ML tree inferred from combined ITS and GPDH gene sequences of the isolates listed in Table 1. Branch lengths are proportional to the distance. Bootstrap values of more than 50% are shown in the tree nodes. Branch with bootstrap support higher than 90% are indicated in bold. Ex-type ^{T} and reference strains are in bold.

Table 2
Results of in vitro antifungal susceptibility testing for *Curvularia* spp.

Species (no. of isolates)	MIC or MEC(μg/mL)														
	AFG			AMB			CAS			ITC			FLC		
	Range	GM	90%	Range	GM	90%	Range	GM	90%	Range	GM	90%	Range	GM	90%
C. aeria (23)	0.03–0.25	0.06	0.125	0.06–2	0.125	0.25	0.5–1	1	1	0.25 to >16	>16	>16	4–64	32	64
C. borreriae (3)	<0.015–0.06	0.03		0.06–0.25	0.15		0.5–1	0.79		0.5 to >16	4		8–16	10	
C. cf. clavata (1)	0.06			0.25			1			0.5			8		
C. geniculata/C. senegalensis (14)	<0.015 to >8	0.10	0.125	0.06–0.5	0.20	0.5	0.5–2	0.86	1	0.06–1	0.23	0.25	2–16	5.65	8
C. cf. inaequalis (5)	0.06–0.125	0.06		0.125–0.25	0.14		1	1		0.125–2	0.32		2–4	2.63	
C. intermedia (2)	0.03	0.03		0.5–4	1.41		0.5	0.5		0.125–0.25	0.17		2–8	4	
C. lunata (10)	<0.015 to >8	0.06	>8	0.125 to >16	0.3	0.5	0.5 to >8	1	1	0.125 to >16	0.25	0.25	2–64	3	8
C. protuberata (3)	0.03–0.06	0.03		0.25–0.5	0.31		0.5	0.5		>16	>16		64 to >64	>64	
C. pseudorobusta (1)	0.06			0.06			0.5			>16			64		
C. cf. sorghina (4)	0.03–0.5	0.10		0.125	0.125	0.125	0.5–1	0.84		<0.03–0.5	0.17		1–4	2.37	
C. verruculosa (6)	0.03–0.125	0.05		0.125–0.25	0.19	0.25	0.5–1	0.79		0.5–1	0.7		4–32	11.3	
Curvularia sp. I (15)	0.03 to >8	0.06	0.125	0.25–1	0.5	1	0.5–1	1	1	0.25–1	0.5	1	2–32	8	32
Curvularia sp. II (9)	0.03–0.125	0.05		0.06–0.25	0.19	0.25	0.5–1	0.62		0.125–2	0.42		2–16	5.03	
Curvularia sp. III (3)	0.06	0.06		0.125–0.25	0.25		1	1		0.125–1	0.125		2–16	4	
Total (99)	<0.015 to >8	0.7	0.125	0.06 to >16	0.6	0.5	0.5 to >8	1	1	<0.03 to >16	9	32	1 to >64	17	32

Species (no. of isolates)	MIC or MEC (μg/mL)											
	VRC			MFG			PSC			5-FC		
	Range	GM	90%	Range	GM	90%	Range	GM	90%	Range	GM	90%
C. aeria (23)	0.5–16	8	16	0.03–0.125	0.06	0.125	0.125–2	1	1	>64	>64	>64
C. borreriae (3)	1–4	2.51		<0.015–0.06	0.02		0.125–0.5	0.31		>64	>64	
C. cf. clavata (1)	0.5			0.06			0.25			>64		
C. geniculata/C. senegalensis (14)	0.125–4	0.67	1	<0.015–0.06	0.04	0.06	<0.03–0.5	0.10	0.25	64 to >64	>64	>64
C. cf. inaequalis (5)	0.5–2	0.75		0.03–0.125	0.06		<0.03–1	0.06		>64	>64	
C. intermedia (2)	0.125–0.5	0.25		0.06	0.06		0.06–0.125	0.08		>64	>64	
C. lunata (10)	0.25–1	0.5	1	0.015 to >8	0.03	0.06	<0.03–0.5	0.06	0.25	>64	>64	>64
C. protuberata (3)	8–16	10		<0.015–0.03	<0.015		0.5–1	0.79		>64	>64	
C. pseudorobusta (1)	>16			0.03			4			>64		
C. cf. sorghina (4)	0.25–2	0.70		0.015–0.06	0.04		0.06–0.025	0.12		>64	>64	
C. verruculosa (6)	0.5–2	1.12		0.015–0.125	0.06		0.06–1	0.22		>64	>64	
Curvularia sp. I (15)	0.5–2	1	2	<0.015–0.125	0.03	0.06	0.06–1	0.25	0.25	>64	>64	>64
Curvularia sp. II (9)	0.5–1	0.62		<0.015–0.03	0.02		<0.03–0.5	0.10		> 64	>64	
Curvularia sp. III (3)	0.5–1	0.5		0.06–0.125	0.06		<0.03–0.25	0.125		>64	>64	
Total (99)	0.125 to >16	3	8	<0.015 to >8	0.2	0.125	<0.03–4	0.4	1	64 to >64	>64	>64

GM, geometric mean; AFG, anidulafungin; AMB, amphotericin B; CAS, caspofungin; ITC, itraconazole; FLC, fluconazole; VRC, voriconazole; MFG, micafungin; PSC, posaconazole; 5-FC, flucytosine.

2000; Guarro et al., 1999). However, our study seems to confirm data published previously that indicates they could be conspecific (Hosokawa et al., 2003; Sun et al., 2003). Our study also demonstrated that several species, not previously identified and probably new, are also widely represented in clinical samples.

Although the BLAST search using ITS sequences is a useful tool for the identification of a variety of pathogenic fungi, it is not so for *Curvularia* because there are so few reference sequences deposited in GenBank. Isolate identification carried out in this study was made by comparing ex-type or reference strains from international culture collections and sequenced in our laboratory. However, even using this procedure only 72.3% of them could be confidently identified. This was due mainly to the fact that some isolates belonged to putative new species and that the taxonomy of the genus is not yet resolved. Although *Curvularia* spp. have considerable economic importance being plant pathogens, either in the production of secondary metabolites or as biological control agents, few molecular studies on the taxonomy of the genus have been carried out, and only a few species have been included (Berbee et al., 1999; Hosokawa et al., 2003; Manamgoda et al., 2011; Sun et al., 2003). In a recent phylogenetic study using a multigene analysis, Manamgoda et al. (2012) tried to clarify the taxonomy of *Curvularia*, redefining 20 species and concluding that there are still many species of *Curvularia* that need to be delimited.

As indicated above, *Curvularia* may cause a wide spectrum of human infections that affect various organs. This study agrees with several clinical studies that have reported the eyes, nasal region, and nails as the most common sites of *Curvularia* infections (Alvarez et al., 2011; Berbel et al., 2011; Ehlers et al., 2011; Gupta et al., 2007; Moody et al., 2012; Tessari et al., 2003; Varughese et al., 2011). It is worth mentioning that, in this study, most of the strains identified as *C. aeria* (73.9%) were isolated from the nasal region, whereas only 1 isolate of *C. lunata* and another of *C. geniculata/C. senegalensis* were found from that anatomical area.

In the reported clinical cases, different antifungal drugs such as AMB, natamycin, and azoles have been used, with variable results (Arora et al., 2011; Ehlers et al., 2011; Moody et al., 2012; Posteraro et al., 2010; Singh et al., 2008; Varughese et al., 2011). In allergic fungal sinusitis, treatment usually consists of surgery and administration of steroids and ITC or AMB, with good tolerance and favorable clinical outcomes (Alvarez et al., 2011; Revankar and Sutton, 2010). Allergic bronchopulmonary mycosis by these fungi is often treated with steroids and ITC (Revankar and Sutton, 2010). Cutaneous infections have been treated successfully with ITC, VRC, and ketoconazole (Moody et al., 2012; Vermeire et al., 2010). In ocular infections, the suggested therapy is natamycin and azoles, such as ITC, FLC, PSC, or VRC (Arora et al., 2011; Qiu et al., 2005; Revankar and Sutton, 2010). However, treatment failures of AMB in peritonitis (Pimentel et al., 2005; Varughese et al., 2011) and of AMB, VRC, and 5-FC in CNS and disseminated infections (Carter and Boudreaux, 2004; Singh et al., 2008; Tessari et al., 2003) have also been reported. The in vitro activity of the drugs tested here is similar to that reported in previous in vitro studies that tested a small number of *Curvularia* isolates (González, 2009; Guarro et al., 1999; Qiu et al.,

2005; Unal et al., 2011) and also to the results obtained in recent studies against *Exserohilum* and *Bipolaris*, 2 genera closely related to *Curvularia* (da Cunha et al., 2012a, 2012b). However, the general in vitro activity showed by VRC and ITC, 2 of the drugs most commonly used to treat infections by *Curvularia*, was considerably lower in the present study, with MICs 90 of 8 and 32 μg/mL, respectively.

The fact that several clinically relevant *Curvularia* spp. were unequivocally identified here mainly by comparing them with type strains and their sequences deposited in GenBank, we hope that will be of help in any future identification of clinical isolates. Similarly and for the same reason, the in vitro data provided here could be useful for guiding the therapy against human infections caused by these opportunistic fungi.

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