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In Vitro Activities of Nine Antifungal Drugs against 81 *Phialophora* and *Cyphellophora* Isolates

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***Cyphellophora guyanensis* ($n = 15$), other *Cyphellophora* species ($n = 11$), *Phialophora europaea* ($n = 43$), and other *Phialophora* species ($n = 12$) were tested *in vitro* against nine antifungal drugs. The MIC_{90}s across all of the strains ($n = 81$) were, in increasing order, as follows: posaconazole, 0.063 μg/ml; itraconazole, 0.5 μg/ml; voriconazole, 1 μg/ml; micafungin, 1 μg/ml; terbinafine, 2 μg/ml; isavuconazole, 4 μg/ml; caspofungin, 4 μg/ml; fluconazole, 8 μg/ml; amphotericin B, 16 μg/ml.**

Although dermatophytes and yeasts account for the majority of superficial and cutaneous fungal infections, members of the black yeast-like fungi are reported regularly (2, 18). Humid indoor environments such as bathrooms and swimming pools (14, 15) are potential reservoirs for black yeast infections. *Cyphellophora* and *Phialophora* species are black yeast-like fungi that form a phylogenetic "europaea clade" within the order *Chaetothyriales* (9) and are recovered from human skin and nails (8, 18). *Phialophora europaea* was found in 27% of positive cultures with black yeasts (18) which are found simultaneously with dermatophytes on the skin of diabetic patients (11). The taxonomy of the genus *Cyphellophora* has recently been revised, and 11 independent species were recognized in the "europaea clade," i.e., *Cyphellophora laciniata*, *C. vermispora*, *C. pluriseptata*, *C. suttonii*, *C. fusarioides*, *C. pauciseptata*, *C. guyanensis*, *Phialophora ambigua*, *P. oxyspora*, *P. reptans*, and *P. europaea* (9, 12). *Cyphellophora laciniata*, *C. pluriseptata* (5, 10), *C. pauciseptata*, and *P. ambigua* thus far have been isolated exclusively from superficial lesions in human infections (7, 9, 12), while *C. fusarioides* originated from bronchoalveolar lavage fluid from a patient after heart bypass surgery (19). *Cyphellophora suttonii* was originally isolated from a phaeohyphomycotic lesion in a dog's ear (1) but also from ulcerating skin lesions in a patient with sarcoidosis (17). At present, there is no information available on the antifungal susceptibility profiles of *Cyphellophora* and its relatives. This study aimed to determine the *in vitro* susceptibilities of a large collection of clinical and environmental isolates of *Cyphellophora* and *Phialophora* species belonging to the black yeast "europaea clade" to eight antifungal drugs and isavuconazole (13, 20, 21), a new triazole that currently is undergoing phase III clinical trials.

A total of 81 strains were obtained from the Centraalbureau voor Schimmelcultures (CBS; Utrecht, The Netherlands) Fungal Biodiversity Centre, which included *Cyphellophora guyanensis* ($n = 15$), *C. laciniata* ($n = 3$), *C. pauciseptata* ($n = 1$), *C. pluriseptata* ($n = 3$), *Cyphellophora suttonii* ($n = 1$), *C. vermispora* ($n = 3$), *Phialophora ambigua* ($n = 1$), *P. europaea* ($n = 43$), *P. oxyspora* ($n = 3$), and *P. reptans* ($n = 8$) (see Table S1 in the supplemental material). The strains were obtained from human ($n = 55$) and animal ($n = 1$) clinical samples, environmental samples ($n = 12$), plant materials ($n = 11$), and unknown sources ($n = 2$). The set included all available ex-type strains of the species described and was supplemented with newly isolated strains. In addition to genotyping, species identity was confirmed by molecular characterization of the internal transcribed spacer region, the partial DNA-dependent RNA polymerase II largest-subunit gene, the beta-tubulin gene, and the nuclear large-subunit rRNA gene (9, 12). Antifungal susceptibility testing was performed as described in CLSI document M38-A2, with some modifications (6). Briefly, isolates were cultured on potato dextrose agar in the dark (25°C) for up to 7 days to induce sporulation. Inocula were prepared by scraping the surface of the fungal colonies with a cotton swab moistened with sterile physiological saline containing 0.05% Tween 40. Large particles were allowed to settle for 5 min, and then a suspension of spores was adjusted with a spectrophotometer (Spectronic 20D; Milton Roy, Rochester, NY) to 68 to 71% transmission (at 530 nm) and diluted 10-fold to yield a final inoculum of 1.5×10^4 to 5×10^4 CFU/ml. The test concentrations of amphotericin B (Bristol-Myers Squibb, Woerden, The Netherlands), itraconazole (Janssen Research Foundation, Beerse, Belgium), voriconazole (Pfizer Central Research, Sandwich, United Kingdom), posaconazole (Schering-Plough, Kenilworth, NJ), isavuconazole (Basilea Pharmaceutica International AG, Basel, Switzerland), and terbinafine (Novartis Pharma, Basel, Switzerland) ranged from 0.016 to 16 μg/ml; those of fluconazole (Pfizer) ranged from 0.063 to 64 μg/ml; and those of caspofungin (MSD, Haarlem, The Netherlands) and micafungin (Astellas Pharma, Ibaraki, Japan) ranged from 0.008 to 8 μg/ml. After 72 h of incubation at 25°C, MICs and minimum effective concentrations

Received 3 June 2012 **Returned for modification** 29 July 2012
Accepted 25 August 2012
Published ahead of print 4 September 2012
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Supplemental material for this article may be found at http://aac.asm.org/.

doi:10.1128/AAC.01112-12

TABLE 1 Geometric mean MICs, MIC ranges, MIC_{50}s, and MIC_{90}s obtained by susceptibility testing of antimycotic agents against *Cyphellophora* and relatives

Organism (no. of strains/genotype[a]) and drug	MIC/MEC (μg/ml)			
	Range	50%	90%	Geometric mean
Total ($n = 81$)				
Amphotericin B	0.125–16	4	16	4
Fluconazole	2–16	8	8	27.655
Itraconazole	≤0.016–1	0.125	0.5	0.097
Voriconazole	0.063–2	0.25	1	0.323
Posaconazole	≤0.016–0.125	≤0.016	0.063	0.027
Isavuconazole	0.25–4	1	4	1.928
Caspofungin	0.25–8	1	4	1.116
Micafungin	≤0.008–4	0.125	1	0.07
Terbinafine	≤0.016–4	0.5	2	0.341
Cyphellophora guyanensis ($n = 15$/group C4)				
Amphotericin B	1–16	16	16	5.879
Fluconazole	4–16	8	16	23.516
Itraconazole	0.063–1	0.25	0.5	0.25
Voriconazole	0.25–1	0.5	1	0.5
Posaconazole	≤0.016–0.125	0.063	0.125	0.05
Isavuconazole	0.25–4	2	4	3.703
Caspofungin	1–4	1	2	1.361
Micafungin	≤0.008–1	0.031	0.25	0.037
Terbinafine	0.125–1	0.25	0.5	1.167
Cyphellophora laciniata ($n = 3$/group A1)				
Amphotericin B	1–4	NC[b]	NC	1.587
Fluconazole	4–16	NC	NC	25.398
Itraconazole	0.063–0.125	NC	NC	0.079
Voriconazole	0.25–1	NC	NC	0.63
Posaconazole	≤0.016–0.031	NC	NC	0.02
Isavuconazole	1–2	NC	NC	3.175
Caspofungin	1–4	NC	NC	1.587
Micafungin	≤0.008–≤0.008	NC	NC	0.008
Terbinafine	0.125–0.125	NC	NC	0.5
Cyphellophora pauciseptata ($n = 1$/group A6)				
Amphotericin B	0.5	NC	NC	NC
Fluconazole	32	NC	NC	NC
Itraconazole	0.125	NC	NC	NC
Voriconazole	0.5	NC	NC	NC
Posaconazole	0.031	NC	NC	NC
Isavuconazole	2	NC	NC	NC
Caspofungin	0.5	NC	NC	NC
Micafungin	0.031	NC	NC	NC
Terbinafine	0.063	NC	NC	NC
Cyphellophora pluriseptata ($n = 3$/groups C1, C2)				
Amphotericin B	4–8	NC	NC	6.35
Fluconazole	4–16	NC	NC	40.317
Itraconazole	0.125–0.25	NC	NC	0.157
Voriconazole	0.25–0.5	NC	NC	0.315
Posaconazole	0.031–0.063	NC	NC	0.039
Isavuconazole	1–2	NC	NC	2.52
Caspofungin	2–8	NC	NC	4
Micafungin	≤0.008–0.016	NC	NC	0.01
Terbinafine	≤0.016–0.25	NC	NC	0.316
Cyphellophora suttonii ($n = 1$/group A4)				
Amphotericin B	2	NC	NC	NC
Fluconazole	32	NC	NC	NC
Itraconazole	0.25	NC	NC	NC
Voriconazole	0.5	NC	NC	NC

(Continued on following page)

TABLE 1 (Continued)

Organism (no. of strains/genotype[a]) and drug	MIC/MEC (μg/ml)			
	Range	50%	90%	Geometric mean
Posaconazole	0.063	NC	NC	NC
Isavuconazole	8	NC	NC	NC
Caspofungin	1	NC	NC	NC
Micafungin	0.25	NC	NC	NC
Terbinafine	2	NC	NC	NC
Cyphellophora vermispora (n = 3/group A2)				
Amphotericin B	0.5–1	NC	NC	0.707
Fluconazole	8–8	NC	NC	32
Itraconazole	0.063–0.5	NC	NC	0.177
Voriconazole	0.5–2	NC	NC	1
Posaconazole	0.031–0.063	NC	NC	0.044
Isavuconazole	2–4	NC	NC	5.657
Caspofungin	0.5–4	NC	NC	0.707
Micafungin	0.063–1	NC	NC	0.251
Terbinafine	0.063–0.063	NC	NC	0.25
Phialophora ambigua (n = 1/group C3)				
Amphotericin B	8	NC	NC	NC
Flucon0azole	32	NC	NC	NC
Itraconazole	0.5	NC	NC	NC
Voriconazole	0.25	NC	NC	NC
Posaconazole	0.125	NC	NC	NC
Isavuconazole	1	NC	NC	NC
Caspofungin	4	NC	NC	NC
Micafungin	0.25	NC	NC	NC
Terbinafine	1	NC	NC	NC
Phialophora europaea (n = 43/group E)				
Amphotericin B	0.125–16	2	16	2.341
Fluconazole	2–16	4	8	21.246
Itraconazole	≤0.016–0.5	0.063	0.25	0.071
Voriconazole	0.125–1	0.25	0.5	0.25
Posaconazole	≤0.016–0.125	≤0.016	0.031	0.021
Isavuconazole	0.25–2	1	1	1.506
Caspofungin	0.25–4	1	2	1.065
Micafungin	≤0.008–4	0.25	1	0.167
Terbinafine	0.25–2	0.5	1	0.533
Phialophora oxyspora (n = 3/group D)				
Amphotericin B	4–16	NC	NC	11.314
Fluconazole	4–8	NC	NC	32
Itraconazole	0.25–0.25	NC	NC	0.25
Voriconazole	0.5–1	NC	NC	0.707
Posaconazole	≤0.016–0.125	NC	NC	0.089
Isavuconazole	2–4	NC	NC	8
Caspofungin	1–4	NC	NC	4
Micafungin	0.5–2	NC	NC	2
Terbinafine	0.5–2	NC	NC	1.414
Phialophora reptans (n = 8/group B)				
Amphotericin B	1–16	NC	NC	4
Fluconazole	4–8	NC	NC	22.627
Itraconazole	≤0.016–0.125	NC	NC	0.044
Voriconazole	0.063–0.5	NC	NC	0.177
Posaconazole	≤0.016–0.063	NC	NC	0.019
Isavuconazole	0.25–2	NC	NC	1.414
Caspofungin	0.25–2	NC	NC	0.917
Micafungin	≤0.008–0.5	NC	NC	0.045
Terbinafine	≤0.008–0.063	NC	NC	0.037

[a] Molecular groups are those of Feng et al. (12).
[b] NC, no comparison because <10 strains per species were available for testing.

(MECs) were determined visually by comparison of the growth in the wells containing the drug with that of the drug-free control. Quality control strains *Paecilomyces variotii* ATCC 22319, *Candida parapsilosis* ATCC 22019, and *Candida krusei* ATCC 6258 were included in each assay run.

Two species with sufficient numbers of isolates ($n = \geq 10$) were included to calculate the MIC_{50} and MIC_{90} values, viz., *Phialophora europaea* and *Cyphellophora guyanensis* (Table 1; see Table S2 in the supplemental material). All of the strains of *Cyphellophora* and *Phialophora* tested had low MICs of itraconazole, voriconazole, posaconazole, and micafungin, while most of the strains had high MICs of fluconazole and amphotericin B. The highest geometric mean MICs were 27.6 μg/ml of fluconazole, followed by 4 μg/ml of amphotericin B, and much lower geometric mean MICs of the triazoles, echinocandins, and allylamine (isavuconazole, 1.9 μg/ml; caspofungin, 1.1 μg/ml; voriconazole, 0.3 μg/ml; terbinafine, 0.3 μg/ml; itraconazole, 0.1 μg/ml; micafungin, 0.07 μg/ml; posaconazole, 0.03 μg/ml). Posaconazole was the drug with the best overall activity. The eight species for which few or single isolates were available (*P. ambigua* CBS 235.93; *C. pauciseptata* CBS 284.85; *C. suttonii* CBS 449.91; *C. laciniata* CBS 174.79, CBS 190.61, and CBS 239.91; *C. pluriseptata* CBS 285.85, CBS 286.85, and CBS 109633; *C. vermispora* CBS 277.86, CBS 228.86, and CBS 122852; *P. oxyspora* CBS 416.89, CBS 698.73, and CBS 124686; and *P. reptans* CBS 113.85, CBS 152.90, CBS 458.92, CBS 101467, CBS 110814, CBS 120903, CBS 120913, and CBS 123271) yielded the lowest MICs of posaconazole, micafungin, itraconazole, and voriconazole (Table 1). In general, the MIC_{90}s of itraconazole, voriconazole, and isavuconazole were at least 2 $\log_2$ dilution steps higher than those of posaconazole. The environmental isolates of *C. guyanensis* had higher MICs of terbinafine and isavuconazole than the clinical strains of *P. europaea*. The two echinocandins showed marked differences in their MECs; in most cases, micafungin had potent activity, with a geometric mean MEC and MEC_{90} showing at least 2 $\log_2$ dilution steps higher activity than caspofungin. These data are in agreement with previously reported findings on the black yeasts *Cladophialophora* (3), *Fonsecaea* (16), and *Rhinocladiella* (4). There was a significant difference ($P < 0.001$) between the MICs of the eight antifungals for *P. europaea* (group E), *P. reptans* (group D), and *C. guyanenesis* (group B) (Table 1). *Phialophora europaea* isolates, when divided into skin and nail subgroups, showed no difference in susceptibility (see Table S2 in the supplemental material). The present paper provides the first antifungal susceptibility data on clinical species of *Cyphellophora* and its relatives in the genus *Phialophora*, which compose a phylogenetic "europaea clade" within the order *Chaetothyriales*. The results suggest that the species in this clade of black yeast-like organisms are susceptible *in vitro* to the newer azoles and to micafungin; however, these *in vitro* data still need to be complemented by clinical confirmation *in vivo*.

ACKNOWLEDGMENTS

This study was partly sponsored by Basilea Pharmaceutica International AG, Basel, Switzerland.

J.F.M. received grants form Astellas, Merck, Basilea, and Schering-Plough. He has been a consultant to Basilea and Merck and received speaker fees from Merck, Pfizer, Schering-Plough, Gilead, and Janssen Pharmaceutica. The rest of us have no potential conflicts of interest.

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