Antonie van Leeuwenhoek
DOI 10.1007/s10482-006-9080-z

ORIGINAL PAPER

Exophiala xenobiotica sp. nov., an opportunistic black yeast inhabiting environments rich in hydrocarbons

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Received: 10 January 2006 / Accepted: 19 April 2006

Abstract A new black yeast species, *Exophiala xenobiotica*, is described, a segregant of the *Exophiala jeanselmei* complex. It is morphologically very similar to *E. jeanselmei*, though with less melanized conidiogenous cells, but deviates unambiguously on the basis of molecular phylogeny. The species is a relatively common agent of cutaneous infections in humans, whereas *E. jeanselmei* is associated with subcutaneous infections. Environmental strains of *E. xenobiotica* are frequently found in habitats rich in monoaromatic hydrocarbons and alkanes.

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Keywords Black yeast · Exophiala jeanselmei · Taxonomy · Ecology · Cutaneous infection · Hydrocarbon

Introduction

Among the black yeast species most frequently reported in the clinical laboratory is *Exophiala jeanselmei* (Langer.) McGinnis and Padhye. However, relatively soon after its recognition as a black yeast (McGinnis and Padhye 1977), it was realized that this taxon is actually quite heterogeneous. De Hoog (1977) introduced three varieties on the basis of morphological and cultural features: var. *heteromorpha* (Nannf.) De Hoog and var. *lecanii-corni* (Benedek & Specht) De Hoog were described in addition to the typical variety. Matsuda et al. (1989) provided the first molecular confirmation of this diversity using molecular data by dot-blot nDNA reassociation. With recent data of all genes sequenced thus far, such as the rDNA small subunit (SSU) gene (Haase et al. 1999), the rDNA internal transcribed spacer (ITS) region (Vitale and De Hoog 2002), the mitochondrial cytochrome *B* gene (Wang et al. 2001), and RFLP analysis of mitochondrial DNA (Kawasaki et al. 1999), all variants of *E. jeanselmei* were proven to represent separate species, now known as *E. heteromorpha* (Langer.) De Hoog and Haase (De Hoog et al.

2003), *E. lecanii-corni* (Benedek & Specht) Haase and De Hoog (Haase et al. 1999) and *E. jeanselmei s. str*. The latter entity, *E. jeanselmei* itself, appears to be relatively rare (De Hoog et al. 2003). It is the only black yeast proven to be repeatedly involved in human mycetoma: two of the four strains identified with certainty using ITS sequencing (De Hoog et al. 2003) were agents of mycetoma (Langeron 1928; Murray et al. 1963). Of 23 strains of the closely related species *E. oligosperma* De Hoog and Tintelnot (De Hoog et al. 2003) only a single strain was reported to cause this disorder (Neumeister et al. 1995). It thus seems probable that the different opportunistic *Exophiala* species on humans and previously identified as *E. jeanselmei* differ in predilection, clinical behavior and ecology.

In general, *Exophiala* species also differ in their environmental autecology. Several authors (Middelhoven et al. 1989; Middelhoven 1993; Cox et al. 1997; Prenafeta-Boldú et al. 2006) noticed that black yeasts and their filamentous relatives of the ascomycete order *Chaetothyriales* are potent degraders of monoaromatic xenobiotics, and eventually tend to accumulate in industrial biofilters. A species regularly encountered in clinical samples as well as in phenolic environments was an as yet undescribed taxon recognized through ITS sequencing as "cluster 8" by De Hoog et al. (De Hoog et al. 2003). Additional studies involving a large number of environmental and clinical *Exophiala* strains (Zeng et al. 2006) proved that this is a consistent entity, comprising clinical strains in addition to a striking number of isolates from sites polluted by toxic xenobiotics. The present article formally introduces this taxon as a new species of *Exophiala*.

Materials and methods

Fungal strains, morphology and physiology

A total of 55 strains studied are listed in Table 1. For morphological observation, slide cultures were made of strains grown on potato dextrose agar (PDA) and mounted in lactophenol cotton blue. Thermotolerance was tested by incubation of freshly inoculated culture plates at 30, 33, 36 and 40°C. The in vitro production of pseudothecia was observed with 11 strains (Table 1) following the method of Untereiner (1994). The strains tested were incubated on PDA in artificial light at room temperature for 1 week. Three 4-mm plugs taken from actively growing edges of each culture were transferred to a 25 ml Erlenmeyer flask containing 10 ml MP (maltpepton) broth and incubated on a rotary shaker at 100 rpm for 3 days at room temperature. Segments of peduncle (*Urtica dioica*, 20 mm, sterile, moistened) were added to each Erlenmeyer flask and incubated for an additional 3–21 days on a rotary shaker. Colonized peduncle segments were transferred to plastic petri dishes with sterile filter paper moistened with sterile water. The dishes were sealed with parafilm, illuminated with black blue light (20 W, 360–370 nm) at 21–25°C and checked weekly for the production of pseudothecia. One set of peduncles was seeded with all pooled strains according to the same method. Antimycotic susceptibility test of 4 current antifungal agents (amphotericin B, itraconazole, voriconazole and posaconazole) was performed with 37 strains according to NCCLS guidelines (M38-A) (National National Committee for Clinical Laboratory Standards 2002).

Isolation

Selective isolation was performed using a sample (Hilversum b101b, kindly delivered by BioSoil BV, Hendrik Ido Ambacht, The Netherlands) of soil polluted by volatile aromatic compounds (naphtalene 4.8 mg/kg, xylene 2.2 mg/kg, ethylbenzene 0.15 mg/kg, toluene < 0.05 mg/kg, benzene < 0.05 mg/kg) and mineral oil (C_{10}–C_{12} 9.8%, C_{12}–C_{22} 87.7%, C_{22}–C_{30} 2.3%, C_{30}–C_{40} 0.3%, total concentration of C_{10}–C_{40} 4,600 mg/kg). Fungi were isolated using a mineral oil flotation method modified after Iwatsu et al. (1981). Enrichment was done in a closed system on Sabouraud‘s dextrose agar plates under naphtalene and phenol atmospheres after incubation for 4–5 weeks at 30°C. Of the few fungi isolated (*Fusarium*, *Scedosporium, Exophiala* and *Paecilomyces*) the black yeast was identified by ITS sequencing.

Table 1 Strain examined

CBS no.	Other number	GenBank accession	Origin	Geography	Provisional name
118157	dH 13236 [Arias]	DQ182571, DQ182579, DQ182587	Oil-contaminated soil	Venezuela	*Exophiala* sp.
110555	AK 9813 = T4, A. Kuhn [Prenafeta 2001]		Gasoline-polluted soil	Germany	*Exophiala* sp.
	NH3-6, K. Sterflinger [Sterflinger 2001]	AJ301704	Limestone, urban façade	Austria	*Exophiala* sp.
115831	dH 12868, U. Hölker		Lignite browncoal	Germany	*Exophiala* sp.
204.50	H. Lüthi		Spoilt apple juice	Switzerland	*E. castellanii*
102606*	dH 11749, P.J. Szaniszlo		Moist bathroom floor	USA	*Rhinocladiella* sp.
102177	dH 11293, G. Phillips [Phillips 1998]		Hospital water after decontamination	UK	*Phialophora* sp.
580.76*	NRRL YB-4163 [Watson 1976]		Paper pulp mill	USA	*Rhinocladiella elatior*
	dH 11807, D. Adelmann		Railway tie	Netherlands	*Exophiala* sp.
522.76	IMI 173394, L.H.G. Morton		Decaying timber joinery	UK	*E. castellanii*
117653*	UTHSC 94–1718, D.A. Sutton	DQ182578, DQ182586, DQ182594	Peritoneal dialysis fluid	USA	*E. jeanselmei*
117658	UTHSC 95–1261, D.A. Sutton		Dialysis fluid	USA	*E. jeanselmei* var. *lecanii-corni*
117674	UTHSC 92–1983, D.A. Sutton	DQ182573, DQ182581, DQ182589	Blood	USA	*E. jeanselmei*
117235	UTHSC 02–483, D.A. Sutton		Sputum	USA	*Exophiala* sp.
648.76A	UAMH 570, J.W. Carmichael		Sputum	Canada	*E. jeanselmei*
117641	UTHSC 99–791, D.A. Sutton	DQ182575, DQ182583, DQ182591	Knee, cyst	USA	*E. jeanselmei*var. *lecanii-corni*
117669	UTHSC 01–895, D.A. Sutton		Elbow, cyst	USA	*Exophiala*sp.
117648	UTHSC 92–891, D.A. Sutton		Eye, sclera	USA	*E. jeanselmei*var. *lecanii-corni*
102455	HC-5 / LM 02, M.A. Resende		Eye infection	Brazil	*E.jeanselmei*
117671	UTHSC 93–2633, D.A. Sutton		Eye, vitreous fluid	USA	*Exophiala* sp.
117661*	UTHSC 96–2382, D.A. Sutton		Eye, vitreous fluid	USA	*Exophiala* sp.
117668	UTHSC R-3427, D.A. Sutton		Nasal mass	USA	*Exophiala* sp.
117643	UTHSC 97–260, D.A. Sutton		Hand lesion	USA	*E. jeanselmei*var. *lecanii-corni*
117676	UTHSC 95–2059, D.A. Sutton	DQ182576, DQ182584, DQ182592	Finger lesion	USA	*Exophiala* sp.
117646	UTHSC 99–1958, D.A. Sutton		Finger lesion	USA	*E. jeanselmei*
117660	UTHSC 97–1139, D.A. Sutton		Finger, fluid	USA	*E. jeanselmei*var. *lecanii-corni*
117647	UTHSC 00–2163, D.A. Sutton		Wrist, wound	USA	*E. jeanselmei*
117663	UTHSC 90–311, D.A. Sutton		Forearm, lesion	USA	*Exophiala* sp..
117651	UTHSC 94–592, D.A. Sutton		Forearm, lesion	USA	*Exophiala* sp.
117650*	UTHSC 94–586, D.A. Sutton		Arm, abscess	USA	*Exophiala* sp.
117667	UTHSC 04–1239, D.A. Sutton		Arm, biopsy	USA	*Exophiala* sp.
117670*	UTHSC 98–2285, D.A. Sutton		Toe	USA	*E. jeanselmei*
117675	UTHSC 98–2286, D.A. Sutton		Toe	USA	*E. jeanselmei*
117642	UTHSC 97–242, D.A. Sutton	DQ182577, DQ182585, DQ182593	Foot, wound	USA	*E. jeanselmei* var.*lecanii-corni*
117753*	UTHSC 00–1074, D.A. Sutton		Leg, wound	USA	*E. jeanselmei* var. *lecanii-corni*
117644	UTHSC 98–2412, D.A. Sutton		Foot, abscess	USA	*E. jeanselmei* var.*lecanii-corni*
117656	UTHSC 93–16, D.A. Sutton		Foot, ulcer	USA	*E. jeanselmei* var. *lecanii-corni*
718.76	UAMH 2034, J.W. Carmichael		Foot lesion	Canada	*E. jeanselmei*
117655*	UTHSC 87–282, D.A. Sutton		Buttock lesion	USA	*E. jeanselmei*
117654	UTHSC 94–2670, D.A. Sutton		Knee, wound	USA	*E. jeanselmei*

Table 1 continued

CBS no.	Other number	GenBank accession	Origin	Geography	Provisional name
117657	UTHSC 92–1962, D.A. Sutton		Knee, lesion	USA	*E. jeanselmei* var. *lecanii-corni*
117662	UTHSC 96–938, D.A. Sutton		Leg tissue	USA	*Exophiala* sp.
117672	UTHSC 02–1843, D.A. Sutton		Scalp lesion	USA	*Exophiala* sp.
117673*	UTHSC 96–232, D.A. Sutton	DQ182574, DQ182582, DQ182590	Scalp lesion	USA	*E. jeanselmei* var. *lecanii-corni*
234.90	R.W. Vreede		Skin, scales	Netherlands	*E. jeanselmei* var. *lecanii-corni*
101271	dH 11314, E.J. Kuijper		Toe, osteomyelitis	Netherlands	*Exophiala* sp.
117666	UTHSC 03–782, D.A. Sutton		Genital	USA	*Exophiala* sp.
117665	UTHSC 02–70, D.A. Sutton	DQ182572, DQ182580, DQ182588	Skin tissue	USA	*Exophiala* sp.
117649	UTHSC 96–197, D.A. Sutton		Skin, wound	USA	*Exophiala* sp.
117652	UTHSC R-904, D.A. Sutton		Human	USA	*E. jeanselmei* var. *lecanii-corni*
117645	UTHSC 00–955, D.A. Sutton		Human	USA	*E. jeanselmei*
117659	UTHSC 93–2109, D.A. Sutton		Human	USA	*E. jeanselmei* var. *lecanii-corni*
117664*	UTHSC 02–47, D.A. Sutton		Animal	USA	*Exophiala* sp.
	UTHSC 02–48, D.A. Sutton		Animal	USA	*Exophiala* sp.
358.29	M. Engelhardt		Unknown source	Germany	*E. castellanii*

*: strains whose *in vitro* production of pseudothecia were observed

DNA extraction

About 1 cm^2 of fungal material was transferred to a 2 ml Eppendorf tube containing a 2:1 (w/w) mixture of silica gel and Celite (silica gel H, Merck 7736/Kieselguhr Celite 545, Machery) and 300 µl TES buffer. The fungal material was ground with a micropestle for 1–2 min. Volume was adjusted by adding 200 µl TES buffer. After vigorous shaking and adding 10 µl 10 mg/ml proteinase K to the tube, the mixture was incubated at 65°C for 10 min. The salt concentration was raised by adding 140 µl 5 M NaCl solution. The mixture was mixed with 1/10 volume (~65µl) cetyltrimethylammonium bromide (CTAB) buffer 10% followed by incubation for another 30 min at 65°C. One volume (~700µl) chloroform–isoamylalcohol (v/v = 24/l) was added and mixed carefully by hand. After incubation 30 min at 0°C (on icewater) and centrifugation at 14,000 rpm at 4°C for 10 min, the toplayer was transferred to a clean Eppendorf tube. The sample mixed with 225 µl 5 M NH_4 -acetate was incubated at 0°C for at least 30 min (on icewater) and spun again. The supernatant was transferred to a clean sterile Eppendorf tube and mixed with 0.55 volume (~510µl) icecold isopropanol. After being spun 7 min, 14,000 rpm, 4°C (or room temperature), supernatant was decanted. The pellet was washed with icecold ethanol 70% 2 times and dried using a vacuum dryer. The powder was re-suspended in 48.5µl TE-buffer with 1.5 µl RNAse, incubated at 37°C for 15–30 min and stored at –20°C until used.

DNA amplification and sequencing

PCR was performed in 50 µl volumes of a reaction mixture containing 10 mM Tris HCL, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2 \cdot 6H_2O$, 0.01% gelatin, 200 mM of each deoxynucleotide triphosphate, 25 pmol of each primer, 10–100 ng rDNA and 0.5 U Taq DNA polymerase (Sigma). Three primer sets ITS1 (5'-TCC GTA GGT GAA CCT GCG G-3') and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3'), EF1-728F (5'-CAT CGA GAA GTT CGA GAA GG-3') and EF1-986R (5'-TAC TTG AAG GAA CCC TTA CC-3'), Bt-2a (5'-GGT AAC CAA ATC GGT GCT

GCT TTC) and Bt-2b (5'-ACC CTC AGT GTA GTG ACC CTT GGC-3') were used to amplify and sequence ITS region of rDNA, partial elongation factor 1–α (EF 1–α) and β-tubulin (β-TUB) genes, respectively. If no amplicon of ITS was obtained, the primers ITS1 and ITS4 were changed to V9G (5'-TTA CGT CCC TGC CCT TTG TA-3') and LS266 (5'-GCAT TCC CAA ACA ACT CGA CTC-3'). SSU (18S) amplicons were generated with primers Oli4 and NS24 and sequenced with primers Oli4, Oli5, BF83, Oli9, Oli1, Oli3, BF963, BF1419, BF951, BF1438, NS3, NS6 and NS24 (De Hoog et al. 2000). Amplification of ITS and SSU was performed as follows: 95°C for 4 min, followed by 35 cycles consisting of 94°C for 45 sec, 52°C for 30 sec and 72°C for 2 min. Annealing temperature was changed to 55 and 58°C, respectively, when amplifying EF 1–α and β-TUB genes. Amplicons were cleaned with GFX columns (Amersham Pharmacia). Sequence-PCR was performed as follows: 95°C for 1 min, followed by 30 cycles consisting of 95°C for 10 sec, 50°C for 5 sec and 60°C for 2 min. DNA was purified with Sephadex G-50 Superfine and sequenced using an DYE-ET terminator.

Molecular identification

Sequences obtained were adjusted using SeqMan Π of Lasergene software (DNASTAR, Inc.). ITS, EF 1–α and β-TUB sequences were aligned iteratively using Ward's averaging in the BioNumerics package v. 4.0 (Applied Maths, Kortrijk, Belgium). Nearest neighbors were found by local Blast searches. The distance trees were based on a re-aligned file using the DCSE program (De Rijk and De Wachter 1993) and calculated with the neighbor-joining method of the Treecon package (Van de Peer and De Wachter 1994) with Kimura-2 correction. Bootstrap values >90 of 100 resampled dataset are shown. If the similarity between an ITS sequence and its nearest neighbor exeeds 99%, they are members of a single branch of the phylogenetic tree, and no re-shuffling is observed when other genes are sequenced, the strains were regarded as belonging to a single species. SSU sequences of 3 strains of *E. xenobiotica* were aligned using the ARB programme (Ludwig et al. 2004) and a parsimony tree was constructed with related species using PAUP v. 4.0b10 (Swofford 2000). A heuristic search was performed for each dataset with 100 random taxon additions and tree bisection and reconstruction (TBR) as the branch swapping algorithm. Branches of zero-length were collapsed and all multiple, equally parsimonious trees were saved. Measures calculated for parsimony included tree length, consistency index and retention index index (TL, CI and RI, respectively). The robustness of the resulting phylogenetic tree was evaluated by 100 bootsstrap replications and every replication used a maxium of 500 trees. The phylogenetic tree was printed with TreeView v. 1.6.6 (Page 1996).

Results

Five hundred most parsimonious trees (TL = 565 steps; CI = 0.625; RI = 0.760) obtained from a heuristic search with 100 random taxon additions of the 18S sequence alignment of 67 strains of *Chaetothyriales* (black yeasts and relatives). The total amount of characters was 1,548, of which 1,248 character were constant, 167 parsimony-uninformative and 133 parsimony-informative. One of the most parsimonious trees is shown in Fig. 1. Six well delimited clades are observed [Fig. 1; clade 1–5 descriptions are those of Haase et al. (1999); clade 6 is recognized in this study]. *Exophiala xenobiotica* belongs to clade 3 and is paraphyletic to *Exophila spinifera*, *E. jeanselmei* and *Exophila oligosperma*. Using the 18S sequence of *Saccharomyces cerevisiae* as a reference, 4 introns were found in *E. xenobiotica* CBS 117655, at positions 563, 1168, 1431 and 1436, and with lengths of 359, 388, 294 and 85 bp, respectively. Only 1 intron of 387 bp was found in CBS 118157 at position 1168 with reference to *S. cerevisiae*. No intron was found in the SSU of CBS 117642.

The interrelationships of 55 of the strains identified to be related to "cluster 8" of De Hoog et al. (2003) on the basis of rDNA ITS sequence data are shown in Fig. 2. The cluster contains *Exophiala jeanselmei, E. spinifera, E. oligosperma, E. nishimurae* and *E. bergeri*. Informative variation in the clade was found in 21 of 205 positions

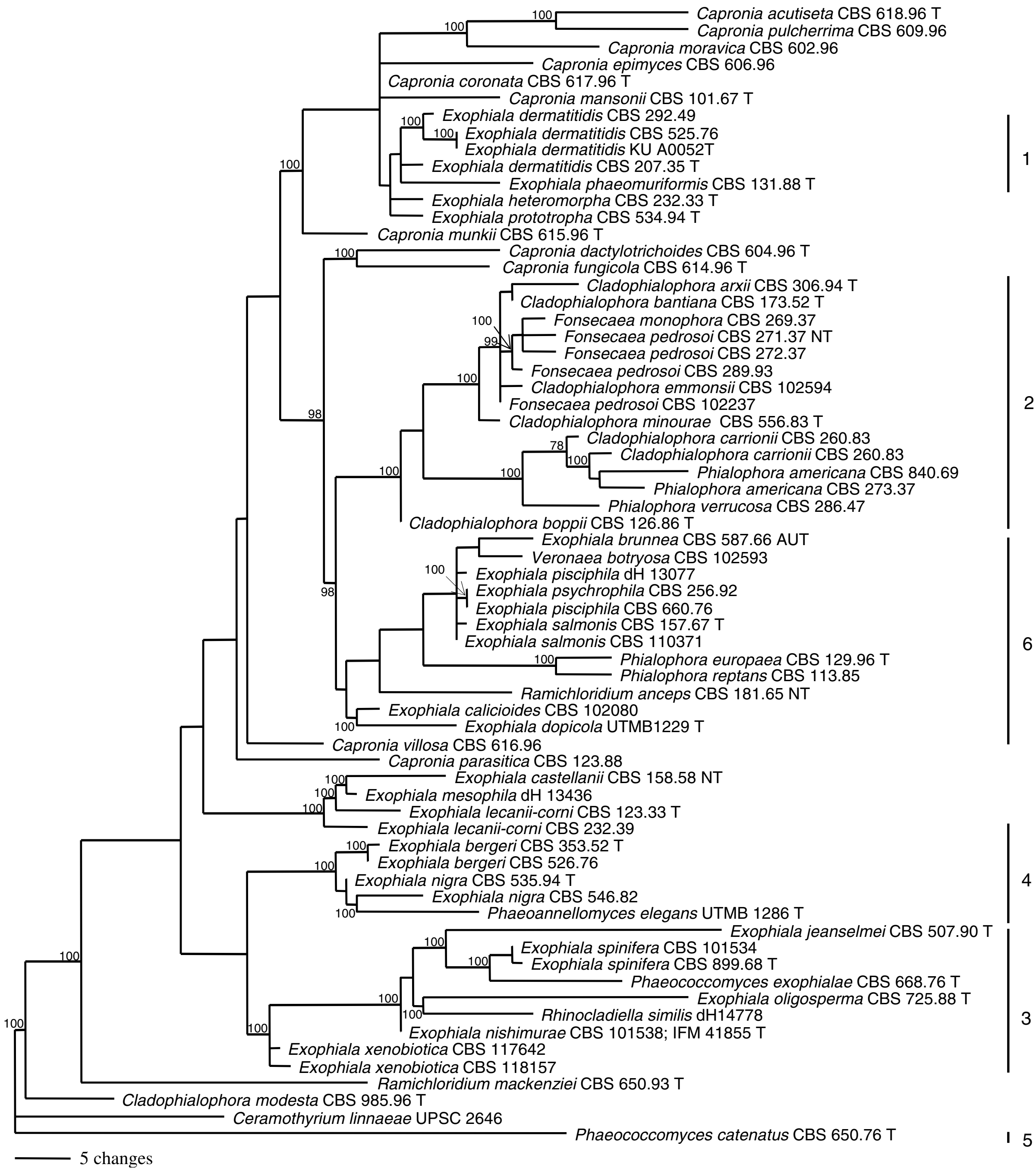

Fig. 1 One of 500 most parsimonious trees obtained from a heuristic search with 100 random taxon additions of the 18S sequence alignment. The scale bar shows 5 changes; bootstrap support values (>90%) from 100 replicates are shown at the nodes. Thickened lines indicate the restrict consensus branches. The tree was rooted to *Phaeococcomyces catenatus* CBS 650.76 T. Clade descriptions are those of Haase et al. (1999)

(10.2%) in ITS1 and in 22 of 185 positions (11.9%) in ITS2; the 159 positions of the 5.8S gene were identical in all strains.

Thirty-two of the strains were also sequenced for partial β-TUB gene and 33 strains for partial EF 1–α gene (data not shown). Highest sequence

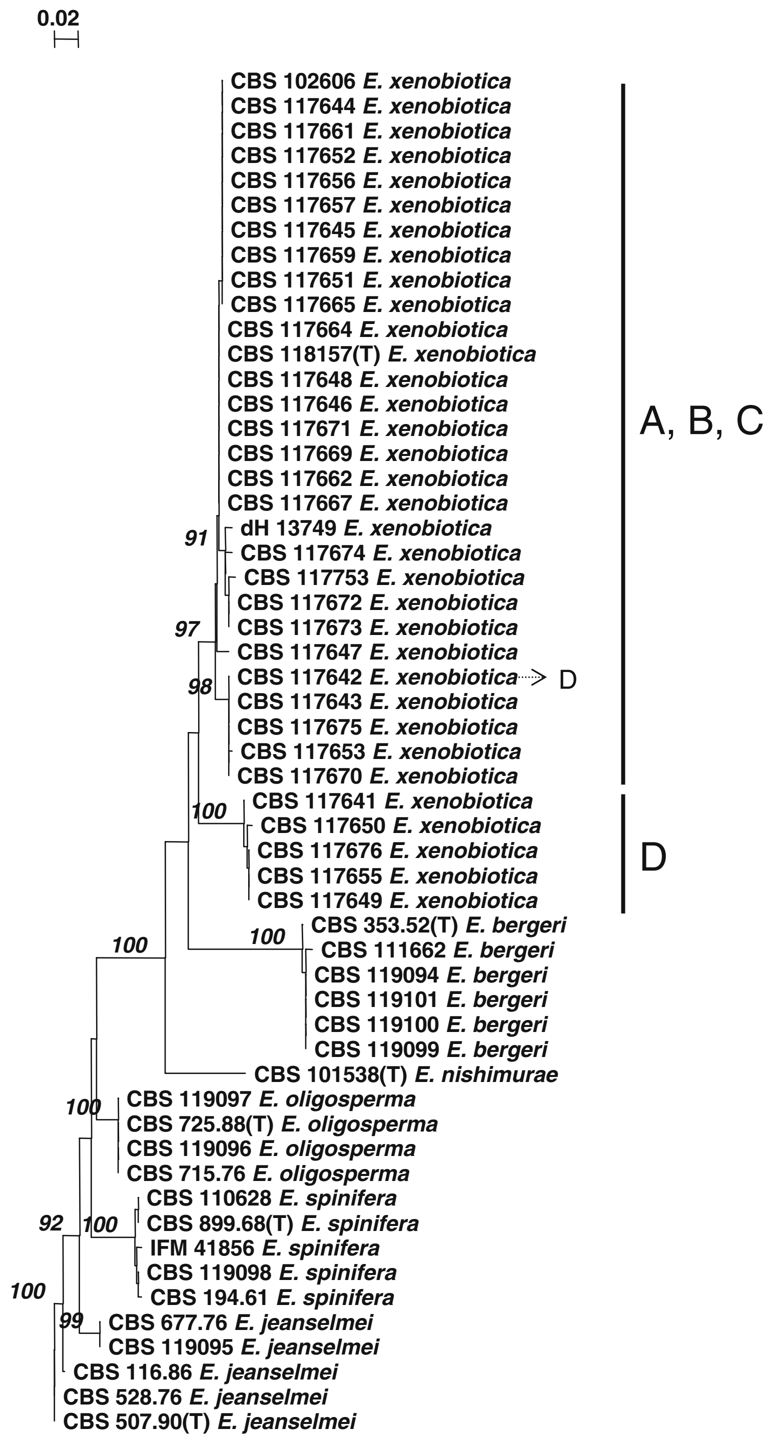

Fig. 2 Distance tree of ITS rDNA of 55 strains belonging to the *E. spinifera* clade, constructed with the Neighbor joining algorithm in the Treecon package with Kimura (2) correction and 100 bootstrap replications (values >90 are shown with the branches). *E. jeanselmei*, CBS 507.90 is selected as outgroup. Groups (A–C) and (D) are based on EF I-α data.

diversity was found in EF 1–α, where four groups (A–D) were detected at high bootstrap values. Using β-TUB sequencing, groups C and D were also recognized comprising the same strains, but groups A and B were not statistically supported. With ITS, group D was recognized, but A–C were not (Fig. 2). Although group D showed the largest distance to the remainder groups with all genes sequenced, one of the strains, CBS 117642 was found in group A–C with ITS (Fig. 2) but in D with EF 1–α and β-TUB. A possible error was excluded by re-sequencing of the same DNA for all genes. The groups A–D are therefore, regarded to belong to a single species. The new species is described below.

The black yeast selectively isolated from soil polluted by aromatic compounds and mineral oil in Hilversum, CBS 119307, was identified as *Exophiala xenobiotica* by ITS sequencing.

Exophiala xenobiotica De Hoog, Zeng, Harrak and D. A. Sutton, **sp. nov**. Mycobank 491509. – Fig. 3.

Coloniae (CBS 118157) in substrates PDA et MEA, 27°C post 14 dies, dictis restrictae, primum planae, olivaceo-atrae, mucidae, in medio velutinae et olivaceo-griseae, margine plana, deinde (post 15 dies) umbonatae, coactae, olivaceo-griseae, in medio velutinae et brunneo-griseae. Reversum olivaceo-atrum. Pigmentum diffundens absens. Cellulae gemmantes copiosae, dilute olivaceae,

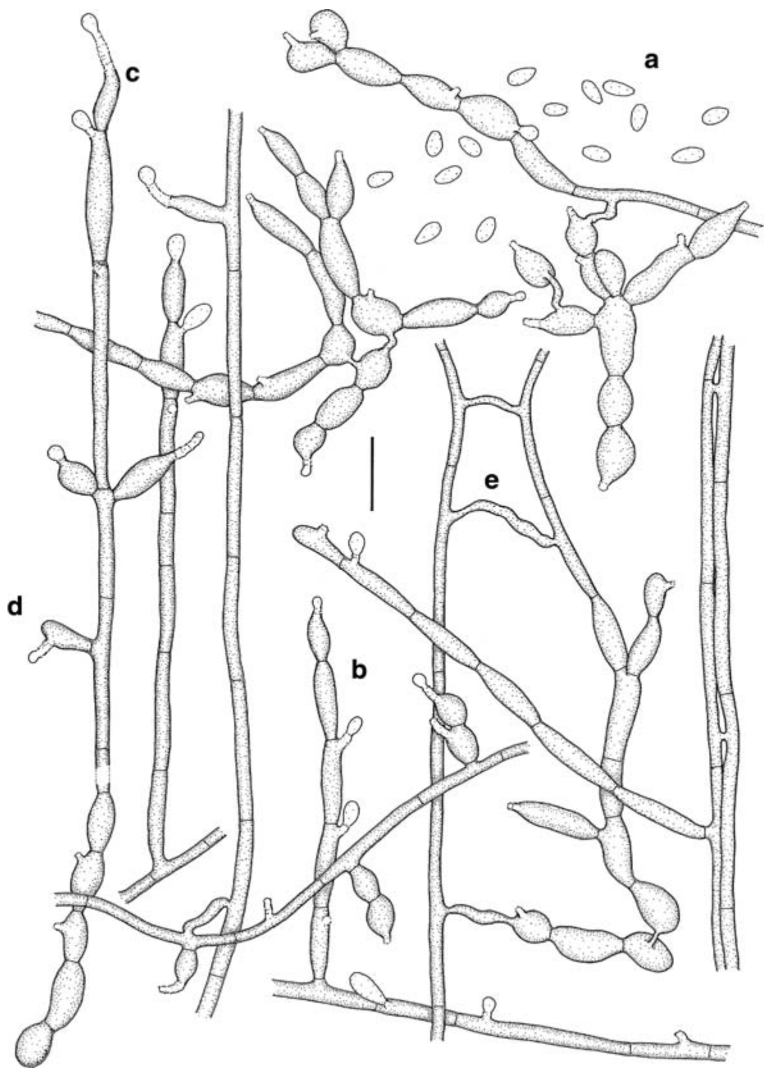

Fig. 3 *E. xenobiotica*CBS 118157. (a. conidia; b. conidiophores; c,d. conidiogenous cells; e. anastomoses. Bar =10 μm.

ellipsoideae, 5–6×2.5–3.0 μm, capsula egentes, saepe inflatae et in cellulas germinantes, 7–10×3–5 μm, transformatae quae saepe zonam irregulariter annellatam brevem formant. Hyphae pallide olivaceae vel brunneae, 1.3–2.0 μm latae, septis 7–28 μm distantibus irregulariter divisae. Anastomoses frequentes. Conidiophora 1–7-cellularia, angulis acutis vel rectis ex hyphis restantibus oriunda, hyphis concoloria, raro ramosa. Cellulae conidiogenae limoniformes vel fusiformes, zonam irregulariter annellatam extensam formantes. Conidia pauca cohaerentia, subhyalina, obovoidea, 3.3–5.4×1.6–2.0 μm. Chlamydosporae globosae, subhyalinae, ad 13 μm diam nonnumquam visae. Teleomorphosis ignota. Temperatura crescentiae optima 30°C, maxima 33–36°C.

Typus: CBS H-18220 (holotypus), cultura extypus CBS 118157, vivus et exsiccatus in CBS, Utrecht, praeservatur.

The following description is of CBS 118157 incubated at 27°C for 14 d.

Colonies on PDA and MEA restricted, circular, initially (on day 3) flat, olivaceous black, slimy with velvety, olivaceous grey center and flat margin, later (on day 14) becoming umbonate, felty, olivaceous grey, with velvety, brownish grey center. Reverse olivaceous black on PDA, olivaceous black with brownish black center on MEA. No diffusible pigment produced on any medium. Budding cells initially abundant, pale olivaceous, ellipsoidal, 5–6×2.5–3.0 μm, without capsule in India ink, often inflating and developing into broadly ellipsoidal, brown germinating cells of about 7–10×3–5 μm that often bear a short, irregular annellated zone. Hyphae pale olivaceous to brown, 1.3–2.0 μm wide, irregularly septate every 7–28 μm. Anastomoses abundant. Conidiophores 1–7-celled, arising at acute or right angles from creeping hyphae, with the same color as the hyphae, seldom branched. Conidiogenous cells lemon-shaped or fusiform with a flaring irregular annellated zones. Conidia adhering in small groups, subhyaline, obovoidal, 3.3–4.0×1.6–2.0 μm. Spherical, subhyaline chlamydospores up to 13 μm diameter may be present. Teleomorph not observed in any of the strains tested after 2 months incubation.

Cardinal temperatures: optimum 30°C, maximum growth temperature 33–36°C.

Type: deposited in Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands, as CBS H-18220 (holotype), ex-type culture CBS 118157, isolated from oil sludge, San Tome, Anzoategui State, Venezuela (Arias and Stotzky 1997). Living strain also deposited in the collection of the Institute for Hygiene and Microbiology, Brussels, Belgium, as IHEM 21721.

The MIC50 and MIC90 are 0.25 and 0.5 mg/l for amphotericin B, 0.03 and 0.125 mg/l for itraconazole, 0.125 and 0.5 mg/l for voriconazole and ≤ 0.015 and 0.03 mg/l for posaconazole.

Discussion

Like *E. jeanselmei* and *E. oligosperma*, the new species has fusiform conidiogenous cells inserted laterally on hyphae, with a single, terminal annellated zone which often is somewhat irregularly flared. The species is indistinguishable from *E. oligosperma* and from immature colonies of *E. jeanselmei* in morphology and physiology. Mature *E. jeanselmei* conidiogenous cells arise at right angles from creeping hyphae and are somewhat darker than the remaining thallus (De Hoog et al. 2000).

The phylogenetic position of the species within the order *Chaetothyriales* is shown in Fig. 1. The main teleomorph genus in this order is *Capronia*, but nearly all species sequenced thus far are relatively distant at long branches. Haase et al. (1999) supposed an ancestral position for *Capronia*, the human opportunistic anamorph species being derived. A number of clearly discernible clades is apparent. The *E. spinifera* complex (clades 3 and 4) is paraphyletic to the majority of the black yeast-like fungi (clades 1, 2 and 6). The genus *Cladophialophora* is polyphyletic, the agents of systemic disease (*C. arxii*, *C. bantiana*, *C. devriesii*) being found with *Fonsecaea*, and the agents of cutanous disorders (*C. carrionii*, *C. boppii*) (clade 2). *Exophiala dermatitidis* and its allies are found in clade 1. A group of psychrophilic species clusters in clade 6.

The *E. spinifera* complex consists of two sister groups, clades 3 and 4. *E. xenobiotica* is located in clade 3, with *E. jeanselmei, Exophila nishimurae* and *E. oligosperma*, in addition to *Rhinocladiella similis* which has an *Exophiala* synanamorph.

CBS 204.50 was originally attributed to *Exophila castellanii*, but was re-identified as *E. xenobiotica*, which was comfirmed by rDNA ITS sequence data (Table 1). Iwatsu (1984) selected this strain as neotype of *E. castellanii* Iwatsu et al., as a replacement name for *Exophila mansonii* Castell., but the present authors prefer to maintain CBS 158.58, deposited by A. Castellani in the CBS collection as representing *E. mansonii*, for this purpose. The position of *E. xenobiotica* close to as notorious a pathogen as *E. spinifera* suggests an infectious potential on humans. Indeed *E. xenobiotica* was exceptionally isolated from animals, all hosts being warm-blooded. However, clinical syndromes were predominantly mild cutaneous.

The number of SSU introns is remarkably variable. The three strains sequenced have entirely different numbers of introns, varying between 0–4. One intron at position 1168 was shared by 2 strains. Haase et al. (1999) noticed the occurrence of up to 3 introns in black yeasts, while Matos et al. (2002) found that infraspecies variation (0–2) is observed in *E. dermatitidis*.

The ITS, β-TUB and EF 1–α genes sequenced showed different degrees of resolution. The ITS sequences are sufficiently alignable over distant species to produce a robust tree of the entire *E. spinifera* SSU clade 3 including *E. xenobiotica* [ITS clade 8 in (De Hoog et al. 2003)]. The highest resolution with groups A–D was found with EF 1–α, while with ITS only a bipartition (A–C) versus (D) could be observed. Strains of group D seem to be clearly separate from the remaining strains of "cluster 8" of De Hoog (2003) with three genes sequenced, but strain CBS 117642 which was found in group A–C with ITS but in D with partial EF 1–α and β-TUB genes. So strains in 4 groups were identified as same species.

In general, the species is fairly common in mild cutaneous infections (Zeng et al. 2006): 41 of 55 strains examined and deposited in culture collections without identification bias originated from humans or animals. Unfortunately, detailed case reports are lacking for all of the strains, and we are not aware of any confirmed case report by a strain now known to be referable as *E. xenobiotica*. Consequently there is limited insight into the virulence of the species. This virulence is probably rather low. Most infections seem to have been of traumatic nature, judging from the frequent occurrence of eye, wound and (sub)cutaneous lesions and cysts. A single draining sinus of an ulcer was mentioned. The deeper infection of the positive blood culture (CBS 117674, Table 1) is likely to have been associated with immunodepression or major debilitating disease.

Several of the environmental strains listed in Table 1 were derived from moist environments in a hospital setting, including dialysis fluid, and once from a bathroom floor. Phillips et al. (1998) isolated CBS 102177 from a biofilm in a waterpipe supplying automated endoscope washer disinfectors. The strain resisted UV-light and survived subsequent decontamination with peracetic acid and hydrogen peroxide disinfectants. *E. xenobiotica* survives acidic conditions, which is demonstrated by CBS 115831 isolated from browncoal at pH 1, together with *Hortaea acidophila* (Hölker et al. 2003). Watson et al. (1976) analyzed a unique extracellular compound produced by CBS 580.76, 2-acetamido-2-deoxy-D-glucuronic acid, which is otherwise known from *Staphylococcus aureus*. This compound, when isolated as its potassium salt, dissolves readily in water and produces very viscous solutions, and may play a significant role in the protection of the fungus against oxygenic action. Two strains were derived from the lungs of patients with cystic fibrosis, where *S. aureus* is one of the prevalent colonizers (Elborn 1999).

The prevalence of black yeasts with clinical potential in drinking water networks may be underestimated (Göttlich et al. 2002). Fungal load is generally low, but accumulation may take place in cells with decreased flow. Their resistance to disinfection gives the fungi a competitive advantage. For example, Porteous et al. (2003) noted biofilms dominated by *Exophiala mesophila* in chemical cleaners to reduce bacterial load in dental unit waterlines. Several reports of pseudoepidemics resulting from inoculation of fluids contaminated with black yeasts have been reported (Nucci et al. 2002; Woollons et al. 1996), eventually with fatal outcome (Engemann et al. 2002).

In the remaining environmental strains we witness a striking association with toxic, aromatic xenobiotics (Table 1). Strain CBS 118157 was isolated from oil-contaminated soil, and CBS

110555 from soil polluted by gasoline. The timber strain CBS 522.76 was derived from wood treated with phenolic preservatives. Strain dH 11807 came from a creosote-treated railway tie. Also browncoal (CBS 115831) is rich in phenolic compounds. Sterflinger and Prillinger (2001) encountered the species on the blackened surface of an urban building, where it was supposed to thrive at the expense of polycyclic aromatic hydrocarbons, degradation products of industrial activity and heavy traffic. Prenafeta-Boldú et al. (2001, 2006) noted a distinct association with monoaromates in black yeasts and filamentous relatives of the order *Chaetothyriales*, confirming earlier reports (Cox et al. 1997; Middelhoven 1993).

In conclusion, *E. xenobiotica* is remarkable in combining an obvious clinical potential —although at low virulence—with an environmental preference to grow in soils or waters containing xenobiotics. With this dual ecology the chaetothyrialean black yeasts and their relatives are unique in the fungal Kingdom (Prenafeta-Boldú et al. 2006). The frequent statement in the literature that human-infecting black yeasts are common degraders of dead plant material is obviously incorrect. In contrast, they inhabit quite specific environmental niches, many of which are rich in aromatic pollutants. How this predilection enhances their ability to infect humans is still a mystery.

Judging from the breakpoints of antifungal susceptibility (Sutton et al. 1998, 1999a), most strains tested were susceptible to the antifungal agents in vitro. Particularly, posaconazole is highly effective.

Acknowledgements K. Sterflinger (Vienna, Austria), U. Hölker (Bonn, Germany), L. Sigler (Edmonton, Canada), G. Haase (Aachen, Germany) and the curator of the IFM culture collection (Chiba, Japan) are acknowledged for sending strains. A. H. G. Gerrits van den Ende and K. F. Luijsterburg are thanked for technical assistance, and R.C. Summerbell for comments on the text.

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