mycoses
Diagnosis, Therapy and Prophylaxis of Fungal Diseases

Original article

Virulence markers of opportunistic black yeast in *Exophiala*

Hafize Sav,[1] Fatma Ozakkas,[1] Rabiye Altınbas,[1] Nuri Kiraz,[1] Ayşegül Tümgör,[2] Ramazan Gümral,[3] Aylin Döğen,[4] Macit Ilkit[5] and G. Sybren de Hoog[6,7,8,9]

[1]*Department of Microbiology, Cerrahpasa Medical Faculty, Istanbul University, Istanbul, Turkey,* [2]*Department of Microbiology, Adana Numune Training and Research Hospital, Adana, Turkey,* [3]*Department of Microbiology, Gülhane Military Medical Academy, Ankara, Turkey,* [4]*Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Mersin, Mersin, Turkey,* [5]*Division of Mycology, Department of Microbiology, Faculty of Medicine, University of Çukurova, Adana, Turkey,* [6]*CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands,* [7]*Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, Amsterdam, The Netherlands,* [8]*Basic Pathology Department, Federal University of Paraná State, Curitiba, Paraná, Brazil and* [9]*King Abdulaziz University, Jeddah, Saudi Arabia*

Summary

The black yeast genus *Exophiala* is known to cause a wide variety of diseases in severely ill individuals but can also affect immunocompetent individuals. Virulence markers and other physiological parameters were tested in eight clinical and 218 environmental strains, with a specific focus on human-dominated habitats for the latter. Urease and catalase were consistently present in all samples; four strains expressed proteinase and three strains expressed DNase, whereas none of the strains showed phospholipase, haemolysis, or co-haemolysis activities. Biofilm formation was identified in 30 (13.8%) of the environmental isolates, particularly in strains from dishwashers, and was noted in only two (25%) of the clinical strains. These results indicate that virulence factors are inconsistently present in the investigated *Exophiala* species, suggesting opportunism rather than pathogenicity.

Key words: Biofilm, carbohydrate, opportunistic, pathogen, proteinase, virulence.

Introduction

Pathogens are ecologically defined as organisms that take advantage of a mammalian host at any stage of their life cycle to enhance their fitness, whereas opportunists are organisms that are able to survive in the host only due to coincidental similarities of the host tissue with its own natural niche. A pathogen is therefore hypothesised to be well-adapted to the host through correlated trait evolution. In contrast, for an opportunist, the host tissue needs to be only minimally compatible; therefore, the organism can survive under non-optimal conditions through resource tracking over a sloppy fitness landscape.[1]

Correspondence: Macit Ilkit, Division of Mycology, Department of Microbiology, Faculty of Medicine, University of Çukurova, Adana 01330, Turkey. Tel.: +90 532 286 0099. Fax: +90 322 457 3072.
E-mail: milkit@cu.edu.tr

Submitted for publication 3 December 2015
Revised 10 January 2016
Accepted for publication 12 January 2016

Despite the gamut of infections in immunocompetent patients caused by black yeast and related taxa,[2] little is known about their virulence determinants.[3–7] Members of the genus *Exophiala* are able to disseminate in an apparently healthy host and cause fatal disease,[2] but do not seem to possess factors that enhance entry to the host; indeed, *Exophiala* infections are often implantation diseases generated by trauma. Among the generally known virulence factors are those associated with morphogenesis, including phenotypic switching; production of phospholipases, proteinases, and esterases; siderophores; and adherence and biofilm formation.[8,9]

Outside of a human host, members of the genus *Exophiala* are frequently encountered in human-made environments, including creosote-treated wood such as that found in railway sleepers,[10–15] as well as in hot and humid, nutrient-poor environments such as Turkish and Finnish saunas,[16] bathrooms,[17] dishwashers[3,18,19] and washing machines.[20] Some *Exophiala* species also occur on rocks,[21] and in drinking and ground water.[22] The ability to colonise a high

doi:10.1111/myc.12478

diversity of extreme environments has been referred to as 'polyextremotolerance'.[23] In general, *Exophiala* species seem to evade habitats that are occupied by competitors and are thus pushed into nutrient-poor, toxic, or otherwise extreme environments.

The virulence of *Exophiala* may differ significantly between closely related species.[24] Some species are strictly saprobic (e.g. *E. crusticola*), some are rare opportunists (e.g. *E. heteromorpha* and *E. xenobiotica*), whereas others are found regularly on the human host (e.g. *E. dermatitidis* and *E. phaeomuriformis*).[13–15,18,19] Clinical studies revealed that *E. dermatitidis* may be more virulent than *E. phaeomuriformis*, regardless of the anatomical site of infection.[25] Recently, Seyedmousavi *et al.* [24] reviewed the known pathomechanisms and host defence mechanisms in *Exophiala* infection.

In this study, we evaluated a number of characteristics that are generally associated with infection, i.e. the potential of biofilm formation and the secretion of hydrolytic enzymes (acid-proteinase, phospholipase, DNase, urease, catalase and haemolytic and co-haemolytic activities). In addition, we examined the assimilation patterns of the same *Exophiala* species using a commercial API ID32C kit, to describe the basic metabolic traits of the fungi.

Materials and methods

Strains analysed

A total of 218 environmental *Exophiala* isolates [114 from dishwashers (52.3%) and 104 from oak railway sleepers (47.7%)] were tested, including 42 reference environmental strains from the CBS-KNAW Fungal Biodiversity Center, Utrecht, The Netherlands, as follows: 137 *E. dermatitidis* (genotypes A, B, and C [95 : 27 : 15]), 54 *E. phaeomuriformis*, 14 *E. crusticola*, and 13 *E. heteromorpha* (Table 1). The strains were previously identified using internal transcribed spacer (ITS)-sequencing (Refgen Biotechnology, Ankara, Turkey), genotyped, and their physiological characteristics were determined.[13–15,18,19] All strains are stored in the Division of Mycology at Çukurova University, Adana, Turkey. In addition, clinical isolates of seven *E. dermatitidis* strains and one *E. castellanii* strain were examined, which were obtained from the CBS collection, Utrecht, The Netherlands (Table 2).

Table 1 Distribution of environmental *Exophiala* isolates according to sample type.

	Sample type		
Isolate	Dishwasher	Railway sleeper	Total
E. crusticola	–	14	14
E. dermatitidis genotype A	44	51	95
E. dermatitidis genotype B	23	4	27
E. dermatitidis genotype C	11	4	15
E. heteromorpha	–	13	13
E. phaeomuriformis	36	18	54
Total	114	104	218

Acid-proteinase activity

Proteinase activity was analysed by measuring the zone of clearance on bovine serum albumin agar medium, as previously described.[26] A suspension of each *Exophiala* test strain was prepared in a broth of yeast extract, peptone and glucose; the final density was adjusted as McFarland Standard No. 0.5. Turbidity was measured using McFarland densitometry. A 10-µl aliquot of this suspension was placed on a sterile paper disc placed on the surface of the bovine serum albumin agar medium (pH 5.0), and the plates were incubated at 30 °C for 6 days. Plates were observed daily for an increasing opacity around the discs caused by growing fungi, and transparent zones around the colonies were measured.

Phospholipase activity

Phospholipase activity was analysed using an egg-yolk agar method described by Price *et al.* [27] and modified by Samaranayake *et al.* [28]. Test isolates were grown on Sabouraud glucose agar (Merck, Darmstadt, Germany) with the density adjusted to 1–2 × 10^6, and 1-µl aliquots were transferred to the egg-yolk agar. The plates were incubated at 37 °C for 48–72 h.

Table 2 Characteristics of clinical *Exophiala dermatitidis* isolates used in this study.

CBS No	Genotype	Substrate of isolation	Country
115663	B	Endotracheal aspirate	Doha, Qatar
424.67	A	–	Germany
581.76[1]	A	Disseminated chromomycosis	–
686.92	A	Blood culture	Hannover, Germany
120429	A	Cystic fibrosis	Finland
120473	A	Brain infection	USA
109154	A	Fatal brain infection	South Korea
149.90	A3	Cystic fibrosis	Aachen, Germany

[1]*Exophiala castellanii.*

Phospholipase activity was determined by dividing the colony diameter by the diameter of the precipitation zone (Pz) around the colony formed on the plate. Phospholipase activity was graded as follows: Pz = 1, absent; Pz = 0.64–0.99, moderately positive; Pz ≤ 0.63, strongly positive.[29]

Haemolysis activity

Haemolytic activity was examined using Columbia agar supplemented with 5% bovine (CBA) and Columbia agar supplemented with 5% bovine (COA) erythrocytes (Besimik, Istanbul, Turkey). Plates were incubated at 27 °C for 7 days. To increase haemolytic activity, strains were additionally incubated at 36 °C for 1–5 days. Haemolytic activity was measured macroscopically, and test results were recorded each day.[30,31]

CAMP factor

The Christie, Atkins, and Munch-Peterson (CAMP) test was performed using CBA and COA media (Besimik). *Exophiala* isolates were inoculated on the medium at a single point using a needle. The tester strains: (i) a β-haemolytic strain (*Staphylococcus aureus* ATCC 25923); (ii) an α-haemolytic strain (*Streptococcus pneumoniae* ATCC 6303) and (iii) a non-haemolytic strain (*Enterococcus faecalis* ATCC 29212) were intersected in straight lines across the plate, at a distance of 10 mm from the border of the colony, using the edge of a loop, and then were incubated at 27 °C for 1–7 days. CAMP-like reactions were examined daily for 7 days. The presence of a distinct arrowhead of haemolysis at the intersection between the tester strain and the test *Exophiala* streaks was considered as positivity.[31,32]

Biofilm formation

Biofilm formation was tested with a modification of the crystal violet staining method described by Toledo-Arana *et al.* [33]. Briefly, one colony of each isolate was placed into a tube containing 2 ml of brain-heart infusion broth (BHIB) with glucose (0.25%). Tubes were incubated at 37 °C for 24 h, and suspensions were diluted at a ratio of 1 : 20 in freshly prepared BHIB with glucose. A 200 µl aliquot of this dilution was added to each well of a microplate. After incubation for 24 h at 37 °C, the microplate was rinsed three times with phosphate-buffered saline (PBS) and then inverted to blot, and 200 µl of 1% crystal violet was added to each well. After incubation for 15 min at room temperature, the microplate was again rinsed three times with PBS. Next, 200 µl of an ethanol: acetone mixture (80 : 20 w/v) was added to each well. The plates were read at 450 nm using a plate reader (Biotek ELx808, USA). The percent transmittance (%T) value of each test sample was subtracted from the %T value of the reagent blank to obtain a measure of the relative amount of light blocked by the sample (% Tbloc). Biofilm production by each isolate was assessed as negative (%Tbloc, <5), +(%Tbloc, 5–20), ++(%Tbloc, 20–50) or +++(%Tbloc, >50).

DNase

To detect extracellular DNase production, plates of DNase Test Agar (HiMedia, Mumbai, India) were prepared.[34] All samples were plated on DNase medium and incubated at 30 °C for 7 days. The plates were then flooded with 1N hydrochloric acid and the clear zones formed around the colonies were measured.

Urease activity

Urease activity was investigated as described by Christensen [35]. Strains were inoculated in urease test medium (Sigma, Milan, Italy) and incubated for 3 days at 25 °C. Discoloration was monitored and when the medium became pinkish, the strain was recorded as urease-positive.

Catalase activity

Samples of 20-µl were added to cuvettes containing 0.1% hydrogen peroxide in 1 ml of 50 mmol l^{-1} KPO_4, pH 7.0. The rate of hydrogen peroxide decomposition at 37 °C was recorded as the decrease in absorption at 240 nm, and activity was calculated based on the initial rate (up to 20 s). One unit was defined as the decomposition of 1 µmol of hydrogen peroxide per minute.[36]

Nutritional physiology

The API ID 32C Yeast Identification System (bioMérieux, Marcy L'Etoile, France) was applied, consisting of 32 cupules with 29 different carbon source substrates, one susceptibility test (cycloheximide), and a negative control. A test for esculin activity is also included in the system and was employed in this study. The system was modified for use with melanised fungi, which included an extended culture incubation time of 3 days.[37] API strips were used according to

the manufacturer's instructions, with manual inoculation. The culture was grown on malt extract agar (Merck, Darmstadt, Germany) and black colonies were covered and placed in API suspension medium. Final turbidity was adjusted to the equivalent of McFarland Standard No. 2, and the suspension was distributed to each cupule. Strips were examined visually, and growth was determined to be positive or absent based upon the presence or absence of turbidity in the wells. Numerical profiles were constituted from the reaction patterns and were used to obtain identifications with the identification software.

Quality control strains

As controls, proteinase activity was determined for quality control (QC) strain *Candida albicans* CBS 2730 as ++, and phospholipase activity was determined for QC in *C. albicans* SC 5314 as +. Biofilm activity was measured for the QC strain ATCC *C. albicans* 92228 as + (%Tbloc, 5–20). DNase and catalase activities were determined for QC strain *Staphylococcus aureus* ATCC 25923 as +, and urease activity was determined for QC strain *Proteus mirabilis* ATCC 29906 as +.

Statistical analysis

Distributions of data were tested with the Shapiro–Wilk test. Two-group comparisons were evaluated with the Mann–Whitney U test, and multiple-group comparisons were evaluated with Kruskal–Wallis analysis of variance with *post hoc* Tukey HSD assessment for pairwise comparisons. In comparisons of categorical variables, the Pearson chi-squared test was used. Data analysis was conducted with R STUDIO software, and the significance level was accepted as $P < 0.05$.

Results

Virulence factors

Proteinase activity was observed as (+) in four *E. dermatitidis* isolates (genotypes A and C [3 : 1]) from dishwashers ($n = 2$) and oak-wood sleepers ($n = 2$). DNase activity was detected in only three of 218 environmental isolates, which were restricted to *E. dermatitidis* genotype A and dishwasher isolates. However, none of the clinical *Exophiala* isolates showed DNase and proteinase activities. All strains studied showed urease and catalase activity, but none showed phospholipase, haemolytic and co-haemolytic activities. Proteinase, phospholipase and biofilm formation characteristics of the 218 environmental and eight clinical *Exophiala* isolates are summarised in Table 3.

Biofilm forming ability was determined in 30 (13.8%) environmental *Exophiala* isolates, including 25 dishwasher isolates and five oak-sleeper isolates, which represented a statistically significant difference ($P < 0.05$). Specifically, the numbers of biofilm-positive isolates were 21 for *E. dermatitidis* (70%), eight for *E. phaeomuriformis* (26.7%) and one for *E. heteromorpha* (3.3%). Notably, biofilm activity was commonly observed in genotypes C and A of *E. dermatitidis* and *E. phaeomuriformis* isolates respectively. The highest biofilm activity was observed in *E. dermatitidis* genotype C, and this difference was statistically significant ($P < 0.05$). Biofilm activity was not detected in any of the strictly environmental *E. crusticola* isolates, and only in one of the *E. heteromorpha* isolates. Two clinical strains, namely *E. dermatitidis* CBS 109154 (genotype A) and CBS 115663 (genotype B), were determined to have biofilm forming abilities; the

Table 3 Virulence factors of environmental and clinical *Exophiala* isolates.

Species (*n*)	Proteinase	Phospholipase	DNase	Urease	Catalase	Biofilm
Environmental isolates						
E. crusticola (14)	0/14	0/14	0/14	14/14	14/14	0/14
E. dermatitidis genotype A (95)	3/95	0/95	3/95	95/95	95/95	8/95
E. dermatitidis genotype B (27)	0/27	0/27	0/27	27/27	27/27	1/27
E. dermatitidis genotype C (15)	1/15	0/15	0/15	15/15	15/15	12/15
E. heteromorpha (13)	0/13	0/13	0/13	13/13	13/13	1/13
E. phaeomuriformis (54)	0/54	0/54	0/54	54/54	54/54	8/54
Clinical isolates						
E. castellanii (1)	0/1	0/1	0/1	1/1	1/1	0/1
E. dermatitidis (7)	0/7	0/7	0/7	7/7	7/7	2/7
Total	4/226	0/226	3/226	226/226	226/226	32/226

strains were recovered from a fatal brain infection and endotracheal aspirate respectively.

Assimilation profiles

The carbohydrate assimilation profiles of *Exophiala* species determined with the commercial API ID32C kit are shown in Table 4. All environmental *Exophiala* isolates could assimilate D-xylose and glucose, except one and two isolates respectively. Most of the environmental strains (>50%) assimilated arabinose, trehalose, 2-keto-D-gluconate, sorbitol and glycerol. In addition, 14 *E. crusticola* isolates could not assimilate lactose and α-methyl-D-glucopyranoside, but the other carbohydrates tested were found to be well assimilated. Notably, all *E. crusticola* isolates showed esculin activity, whereas none of the *E. phaeomuriformis* isolates showed this activity. All clinical *Exophiala* isolates assimilated trehalose. All clinical isolates, except for CBS 581.76 (genotype A) assimilated galactose, α-methyl-D-glucopyranoside, sorbitol and glucose.

Discussion

Fungi that are adapted to reside in mammalian tissues are hypothesised to possess the ability to secrete the appropriate hydrolytic enzymes needed to feed on host cell compounds. The main categories of such compounds are proteinases that hydrolyse peptide bonds, phospholipases to digest phospholipids and haemolysins.[31,38,39] *Exophiala* black yeast enter the human host from the environment and the infected hosts are not contagious,[40] suggesting that environmental strains should have a set of virulence factors similar to that of clinical strains. In this study, a selection of virulence factors and nutritional profiles of 218 environmental and eight clinical strains were compared. All strains showed urease and catalase activity, but none showed phospholipase activity. Limited DNase and proteinase activity was observed in environmental *E. dermatitidis* isolates. There was no significant differentiation or specific distribution patterns of virulence markers between strains from different sources, except biofilm formation that was particularly developed in the dishwasher isolates.

Proteolytic activity has also traditionally been used to distinguish dematiaceous fungi.[5] Members of the black yeast group (e.g. genera *Fonsecaea* and *Cladophialophora*) were found to be negative, whereas the ubiquitous contaminant *Cladosporium* showed positive proteolytic activity. This strongly suggests that this factor is not related to pathogenesis. Several

Table 4 Assimilation profiles of carbohydrates and esculin by *Exophiala* isolates with positivity and percentage values.

	Number of positivity/percentage																
Species (*n*)	GAL	SAC	NAG	ARA	CEL	RAF	MAL	TRE	2KG	MDG	LAC	INO	SOR	XYL	GLY	MLZ	GLU	ESQ
Environmental isolates																		
E. crusticola (14)	3/21	11/79	2/14	9/64	11/79	1/7	5/36	11/79	13/93	0/0	0/0	3/21	7/50	14/100	14/100	10/71	14/100	13/93
E. dermatitidis genotype A (95)	54/57	78/82	13/14	90/95	33/35	6/6	69/73	77/81	92/97	4/4	3/3	8/8	75/79	95/100	95/100	27/28	95/100	7/7
E. dermatitidis genotype B (27)	13/48	20/74	3/11	26/96	12/44	3/11	15/56	17/63	27/100	2/7	1/4	1/4	21/78	27/100	27/100	13/48	13/48	1/4
E. dermatitidis genotype C (15)	12/80	7/47	1/7	13/87	6/40	0/0	10/67	14/93	14/93	1/7	1/7	1/7	13/87	15/100	15/100	3/20	12/80	1/7
E. heteromorpha (13)	8/62	3/23	10/77	12/92	11/85	1/8	6/46	11/85	12/92	2/15	0/0	1/8	12/92	12/92	13/100	2/15	13/100	6/46
E. phaeomuriformis (54)	14/26	19/35	6/11	51/94	33/61	6/11	17/31	49/91	50/93	4/7	1/2	4/7	53/98	54/100	52/96	14/26	14/26	0/0
Clinical isolates																		
E. castellanii (1)	0/0	0/0	0/0	0/0	1/100	1/100	0/0	1/100	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	1/100	0/0
E. dermatitidis (7)	7/100	1/14	3/43	0/0	2/29	5/71	5/71	7/100	0/0	7/100	0/0	0/0	7/100	1/14	2/29	1/14	7/100	0/0

GAL, galactose; SAC, sucrose; NAG, N-acetylglucosamine; ARA, arabinose; CEL, cellobiose; RAF, raffinose; MAL, maltose; TRE, trehalose; 2KG, 2-keto-D-gluconate; MDG, α-methyl-D-glucopyranoside; LAC, lactose; INO, inositol; SOR, sorbitol; XYL, xylose; GLY, glycerol; MLZ, melezitose; GLU, glucose; and ESQ, esculine.

researchers have reported that *E. jeanselmei* and *E. dermatitidis* did not show proteolytic activity.[7] In this study, proteinase activity was observed in 1.8% of the environmental *Exophiala* isolates and in none of the clinical isolates. Consistent with our findings, phospholipase activity was not detected in a strain of *E. jeanselmei* from case of human chromoblastomycosis.[6] However, Gugnani and Okeke [41] evaluated 39 environmental dematiaceous opportunists and found positive phospholipase activity in two *E. jeanselmei* isolates.

The presence of urease, catalase and extracellular DNase has been associated with pathogenesis; e.g. in group A *Streptococcus* and in *Cryptococcus*.[42–44] Urease enables the utilisation of urea as a nitrogen source and has been reported as an extracellular enzyme produced by dematiaceous fungi.[41] Vicente *et al.* [11] reported that *E. xenobiotica* showed urease activity. In this study, urease and catalase activity was observed in all environmental and clinical *Exophiala* isolates tested. DNases may enhance evasion of the innate immune response.[43] DNase activity proved to be absent from *E. jeanselmei*.[6] In our study, the enzyme was rarely found among the isolates tested, being limited to the most common opportunist, genotype A of *E. dermatitidis*. This may suggest that the presence of this factor enhances infection by an otherwise environmental species.

Haemolysin mediates the severity of infectious diseases, and loss of this activity often results in avirulence, particularly in bacteria.[45] Haemolytic activity has been described in *Aspergillus*,[46] *Candida*[47] and dermatophytes.[30,31] Dermatophyte strains recovered from both symptomatic and asymptomatic lesions have been shown to have haemolytic and co-haemolytic activities using COA and CBA.[31] However, these activities were absent from the black yeast evaluated.

Biofilm formation was observed in 13.8% and 25% of the environmental and clinical *Exophiala* isolates respectively. Among the environmental strains, dishwasher isolates showed significantly more biofilm forming ability compared with the oak-sleeper isolates ($P < 0.05$), suggesting that this ability is linked to habitat choice. The rare genotype C of *E. dermatitidis* showed greater biofilm forming ability (57.1%) compared to genotypes A (38.1%) and B (4.8%) of this species; C strains originated predominantly from dishwashers compared to oak-wood sleepers with a ratio of 3 : 1. Heinrichs *et al.* [4] noted that *E. lecanii-corni*, and occasionally *E. dermatitidis* and *E. phaeomuriformis*, were present in municipal water networks with biofilm formation. Sudhadham *et al.* [48] noted that genotype A of *E. dermatitidis* is more frequently encountered as an aetiological agent of systemic disease in immunocompetent humans than genotype B, which is mainly environmental; it was repeatedly recovered from the faeces of frugivorous animals.[48] In this study, biofilm forming ability was also identified in two of eight clinical strains (two isolates each of genotype A and B); this factor may contribute to infection, but given the environmental habitat choice of these black yeast, a role in their natural niche seems more likely. The limitation of this study is that only eight clinical isolates have been investigated. Future studies with a higher number of clinical isolates are required before reaching any solid conclusion about the difference in virulence markers between environmental and clinical *Exophiala* strains.

Exophiala species are able to decompose aromatic hydrocarbons,[49,50] assimilate different detergents,[51] and can survive under harsh and extreme conditions, including high temperature, high pH, and up to 17% NaCl.[3,13–15,18,19] Clinically significant species tend to be thermotolerant.[3,13–15,18,19] These abilities suggest that *Exophiala* species by nature are clinical species, occupying relatively extreme (micro)niches to evade competition by rapidly growing microbes. The infectious ability, even though occurring relatively frequently in immunocompetent individuals, is therefore judged to be opportunistic. The frequently infectious species did not differ markedly in their virulence profile from the strict saprobe, *E. crusticola*. In the literature, there are no indications of host-to-host transmission in *Exophiala*, and enhancement of fitness by use of an animal vector as yet seems unlikely. However, the possibility of adaptation to a mammalian host with successful and repeated infection, as observed in agents of chromoblastomycosis, cannot be excluded.

Funding

This study was supported in part by the Division of Mycology, Cerrahpaşa Faculty of Medicine, Istanbul University, Istanbul.

Acknowledgments

The study strains were provided by the Division of Mycology, Faculty of Medicine, Çukurova University, Adana, Turkey, and the Fungal Biodiversity Centre (CBS), Utrecht, The Netherlands. The authors also thank the Collection, Preservation, and Digitalisation Group at the CBS, Utrecht, The Netherlands, for their kind help in processing the clinical *Exophiala* isolates.

Conflict of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the article.

References

1 Araujo SB, Braga MP, Brooks DR *et al.* Understanding host-switching by ecological fitting. *PLoS ONE* 2015; **10**: e0139225.
2 Li DM, de Hoog GS. Cerebral phaeohyphomycosis—a cure at what lengths? *Lancet Infect Dis* 2009; **9**: 376–83.
3 Zalar P, Novak M, de Hoog GS, Gunde-Cimerman N. Dishwashers—a man-made ecological niche accommodating human opportunistic fungal pathogens. *Fungal Biol* 2011; **115**: 997–1007.
4 Heinrichs G, Hübner I, Schmidt CK, de Hoog GS, Haase G. Analysis of black fungal biofilms occurring at domestic water taps. II: potential routes of entry. *Mycopathologia* 2013; **175**: 399–412.
5 Espinel-Ingroff A, Goldson PR, McGinnis MR, Kerkering TM. Evaluation of proteolytic activity to differentiate some dematiaceous fungi. *J Clin Microbiol* 1988; **26**: 301–7.
6 Souza TF, Scroferneker ML, Costa JM, Carissimi M, Corbellini VA. Secretion of five extracellular enzymes by strains of chromoblastomycosis agents. *Rev Inst Med Trop Sao Paulo* 2008; **50**: 269–72.
7 Mok WY. Nature and identification of *Exophiala werneckii*. *J Clin Microbiol* 1982; **16**: 976–8.
8 Hogan LH, Klein BS, Levitz SM. Virulence factors of medically important fungi. *Clin Microbiol Rev* 1996; **9**: 469–88.
9 Karkowska-Kuleta J, Rapala-Kozik M, Kozik A. Fungi pathogenic to humans: molecular bases of virulence of *Candida albicans*, *Cryptococcus neoformans* and *Aspergillus fumigatus*. *Acta Biochim Pol* 2009; **56**: 211–24.
10 Sudhadham M, Pirakitsin S, Sivichai S *et al.* The neurotropic black yeast *Exophiala dermatitidis* has a possible origin in the tropical rain forest. *Stud Mycol* 2008; **61**: 145–55.
11 Vicente VA, Attili-Angelis D, Pie MR *et al.* Environmental isolation of black yeast-like fungi involved in human infection. *Stud Mycol* 2008; **61**: 137–44.
12 Seyedmousavi S, Badali H, Chlebicki A, Zhao J, Prenafeta-Boldú FX, de Hoog GS. *Exophiala sideris*, a novel black yeast isolated from environments polluted with toxic alkyl benzenes and arsenic. *Fungal Biol* 2011; **115**: 1030–7.
13 Dogen A, Kaplan E, Ilkit M, de Hoog GS. Massive contamination of *Exophiala dermatitidis* and *E. phaeomuriformis* in railway stations in subtropical Turkey. *Mycopathologia* 2013; **175**: 381–6.
14 Dogen A, Ilkit M, de Hoog GS. Black yeast habitat choices and species spectrum on high altitude creosote-treated railway ties. *Fungal Biol* 2013; **117**: 692–6.
15 Gumral R, Tumgor A, Saraclı MA, Yıldıran ST, Ilkit M, de Hoog GS. Black yeast diversity on creosoted railway sleepers changes with ambient climatic conditions. *Microb Ecol* 2014; **68**: 699–707.
16 Matos T, de Hoog GS, de Boer AG, de Crom I, Haase G. High prevalence of the neurotrope *Exophiala dermatitidis* and related oligotrophic black yeasts in sauna facilities. *Mycoses* 2002; **45**: 373–7.
17 Lian X, de Hoog GS. Indoor wet cells harbour melanized agents of cutaneous infection. *Med Mycol* 2010; **48**: 622–8.
18 Dogen A, Kaplan E, Oksuz Z, Serin MS, Ilkit M, de Hoog GS. Dishwashers are a major source of human opportunistic yeast-like fungi in indoor environments in Mersin, Turkey. *Med Mycol* 2013; **51**: 493–8.
19 Gumral R, Ozhak-Baysan B, Tumgor A *et al.* Dishwashers provide a selective extreme environment for human-opportunistic yeast-like fungi. *Fungal Divers* 2016; **76**: 1–9.
20 Babič MN, Zalar P, Ženko B, Schroers HJ, Džeroski S, Gunde-Cimerman N. *Candida* and *Fusarium* species known as opportunistic human pathogens from customer-accessible parts of residential washing machines. *Fungal Biol* 2015; **119**: 95–113.
21 Sterflinger K. Temperature and NaCl-tolerance of rock-inhabiting meristematic fungi. *Antonie Van Leeuwenhoek* 1998; **74**: 271–81.
22 Babic MN, Zalar P, Gunde-Cimerman N.Presence of emerging fungal pathogens in drinking water and ground water. IBBS Conference, Water Microbiology Current and Emerging Issues in Healthcare, 7th and 8th September 2015, Great Britain, 2015.
23 Gostincar C, Grube M, Gunde-Cimerman N. Evolution of fungal pathogens in domestic environments? *Fungal Biol* 2011; **115**: 1008–18.
24 Seyedmousavi S, Netea MG, Mouton JW, Melchers WJ, Verweij PE, de Hoog GS. Black yeasts and their filamentous relatives: principles of pathogenesis and host defense. *Clin Microbiol Rev* 2014; **27**: 527–42.
25 Zeng JS, Sutton DA, Fothergill AW, Rinaldi MG, Harrak MJ, de Hoog GS. Spectrum of clinically relevant *Exophiala* species in the United States. *J Clin Microbiol* 2007; **45**: 3713–20.
26 Chakrabarti A, Nayak N, Talwar P. *In vitro* proteinase production by *Candida* species. *Mycopathologia* 1991; **114**: 163–8.
27 Price MF, Wilkinson ID, Gentry LO. Plate method for detection of phospholipase activity in *Candida albicans*. *Sabouraudia* 1982; **20**: 7–14.
28 Samaranayake LP, Raeside JM, MacFarlane TW. Factors affecting the phospholipase activity of *Candida* species *in vitro*. *Sabouraudia* 1984; **22**: 201–7.
29 Mohandas V, Ballal M. Proteinase and phospholipase activity as virulence factors in *Candida* species isolated from blood. *Rev Iberoam Micol* 2008; **25**: 208–10.
30 Schaufuss P, Steller U. Haemolytic activities of *Trichophyton* species. *Med Mycol* 2003; **41**: 511–6.
31 Döğen A, Gümral R, Ilkit M. Haemolytic and co-haemolytic (CAMP-like) activity in dermatophytes. *Mycoses* 2015; **58**: 40–7.
32 Schaufuss P, Brasch J, Steller U. Dermatophytes can trigger cooperative (CAMP-like) haemolytic reactions. *Br J Dermatol* 2005; **153**: 584–90.
33 Toledo-Arana A, Valle J, Solano C *et al.* The enterococcal surface protein, Esp, is involved in *Enterococcus faecalis* biofilm formation. *Appl Environ Microbiol* 2001; **67**: 4538–45.
34 Sánchez-Porro C, Martin S, Mellado E, Ventosa A. Diversity of moderately halophilic bacteria producing extracellular hydrolytic enzymes. *J Appl Microbiol* 2003; **94**: 295–300.
35 Christensen WB. Urea decomposition as a means of differentiating *Proteus* and paracolon cultures from each other and from *Salmonella* and *Shigella* types. *J Bact* 1946; **52**: 461–6.
36 Baureder M, Barane E, Hederstedt L. *In vitro* assembly of catalase. *J Biol Chem* 2014; **289**: 28411–20.
37 Espinel-Ingroff A, McGinnis MR, Pincus DH, Goldson PR, Kerkering TM. Evaluation of the API 20C yeast identification system for the differentiation of some dematiaceous fungi. *J Clin Microbiol* 1989; **27**: 2565–9.
38 Ghannoum MA. Potential role of phospholipases in virulence and fungal pathogenesis. *Clin Microbiol Rev* 2000; **13**: 122–43.
39 Naglik JR, Challacombe SJ, Hube B. *Candida albicans* secreted aspartyl proteinases in virulence and pathogenesis. *Microbiol Mol Biol Rev* 2003; **67**: 400–28.
40 Hofmann H, Choi SM, Wilsman-Theis D, Horré R, de Hoog GS, Bieber T. Invasive chromoblastomycosis and sinusitis due to *Phialophora verrucosa* in a child from northern Africa. *Mycoses* 2005; **48**: 456–61.
41 Gugnani HC, Okeke CN. Physiological characteristics of environmental isolates of pathogenic dematiaceous fungi. *Mycoses* 1989; **32**: 78–83.
42 Sánchez M, Colom F. Extracellular DNase activity of *Cryptococcus neoformans* and *Cryptococcus gattii*. *Rev Iberoam Micol* 2010; **27**: 10–3.
43 Sumby P, Barbian KD, Gardner DJ *et al.* Extracellular deoxyribonuclease made by group A *Streptococcus* assists pathogenesis by enhancing evasion of the innate immune response. *Proc Natl Acad Sci USA* 2005; **102**: 1679–84.

44 Feder V, Kmetzsch L, Staats CC *et al. Cryptococcus gattii* urease as a virulence factor and the relevance of enzymatic activity in cryptococcosis pathogenesis. *FEBS J* 2015; **282**: 1406–18.
45 Vázquez-Boland JA, Kuhn M, Berche P *et al. Listeria* pathogenesis and molecular virulence determinants. *Clin Microbiol Rev* 2001; **14**: 584–640.
46 Wartenberg D, Lapp K, Jacobsen ID *et al.* Secretome analysis of *Aspergillus fumigatus* reveals Asp-hemolysin as a major secreted protein. *Int J Med Microbiol* 2011; **301**: 602–11.
47 Guzel AB, Kucukgoz-Gulec U, Aydın M *et al. Candida* vaginitis during contraceptive use: the influence of methods, antifungal susceptibility and virulence patterns. *J Obstet Gynaecol* 2013; **33**: 850–6.
48 Sudhadham M, Gerrits van den Ende AH, Sihanonth P *et al.* Elucidation of distribution patterns and possible infection routes of the neurotropic black yeast *Exophiala dermatitidis* using AFLP. *Fungal Biol* 2011; **115**: 1051–65.
49 Isola D, Selbmann L, de Hoog GS *et al.* Isolation and screening of black fungi as degraders of volatile aromatic hydrocarbons. *Mycopathologia* 2013; **175**: 369–79.
50 Prenafeta-Boldú FX, Summerbell R, de Hoog GS. Fungi growing on aromatic hydrocarbons: biotechnology's unexpected encounter with biohazard? *FEMS Microbiol Rev* 2006; **30**: 109–30.
51 Hamada N, Abe N. Physiological characteristics of 13 common fungal species in bathrooms. *Mycoscience* 2009; **50**: 421–9.