

Intestinal prevalence of the neurotropic black yeast *Exophiala* (*Wangiella*) *dermatitidis* in healthy and impaired individuals

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Summary

A screening of 2300 samples of faeces from humans with and without underlying disease revealed that the black yeast *Exophiala dermatitidis* is present at a frequency of 5.2‰ ($n = 12$). Eight individuals positive for the fungus had diarrhoea at the moment of its isolation, out of 11 where relevant information was available. Judging from repeated isolation over several weeks in one patient, the organism is able to persist in the human intestinal tract.

Key words: black yeast, intestines, faeces, diarrhoea, *Exophiala dermatitidis*.

Introduction

One of the black yeasts most commonly involved in human infections is *Exophiala* (*Wangiella*) *dermatitidis*. It is regularly encountered in (sub)cutaneous lesions of traumatic origin¹ as well as in systemic and disseminated disease.^{2–4} Furthermore it is particularly known as an asymptomatic colonizer of the lungs of patients with cystic fibrosis,^{5, 6} whereas infections involving the central nervous system are preponderantly found in East Asia.^{7–9}

In search of potential sources of contamination and routes of infection, Matos *et al.*¹⁰ found a focus of this species in Turkish steam baths in The Netherlands and Slovenia. This is concordant with earlier findings of Nishimura *et al.*, who consistently found the species in bathing facilities in Japan.¹¹ The species has now also been found in steam baths in Austria (A. Mayr, personal communication) and Thailand (M. Sudhadham, unpublished data). The cell density in the steam baths is remarkably high.¹⁰ As the fungus has never been reported to be carried by air or steam, it remains unclear how it enters the facilities. Therefore, one possibility

may be through asymptomatic intestinal carriage and dispersal by humans via anal carriage. The prevalence of the species in the human intestinal tract is investigated in the present paper.

Materials and methods

Patients and clinical data

Samples analysed (Table 1) were 250, 950 and 100 faecal specimens of human patients with intestinal disorders collected in Aachen (Germany), Ljubljana (Slovenia) and Gouda (the Netherlands) respectively. Additionally, 1000 samples from healthy and asymptomatic individuals in Ljubljana were investigated. The latter samples were collected in the framework of legally obligatory health testing for workers dealing with foodstuffs. Samples were processed by direct plating of approximately 200 µl of faeces on culture plates with Sabouraud's glucose agar with penicillin and streptomycin and erythritol-chloramphenicol agar,¹² and were incubated for 5–30 days at 40 °C. Black, pinhead-shaped colonies transferred to potato dextrose agar were purified and maintained until identification.

Identification and genotyping

Relevant isolates (one per sample) were identified by sequencing of the nuclear internal transcribed spacer

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Table 1 Strains of *Exophiala dermatitidis* isolated from human faeces.

Strain	Geography	M/F	Age	Date of isolation	Underlying disease	Symptoms at time of specimen collection	Occupation	ITS genotype
1. T-6734 = DH 13600	Ljubljana, Slovenia	F	?	24 October 2003	Colicæ abdominalis	Gastroenterocolitis acuta	Nurse	<i>Exophiala heteromorpha</i>
2. T-6544 = DH 12770	Ljubljana, Slovenia	F	69	14 November 2001	Melanoma malignum	Diarrhoea	?	A
3. T-4611 = DH 13251/ T-5262 = DH 13252	Ljubljana, Slovenia	F	3	21 July 2003/ 12 August 2003	Acute leukaemia, BMT	Diarrhoea	-	A
4. CBS 109148 = DH 11838	Gouda, Netherlands	?	?	?	None	Diarrhoea	?	A
5. GHP 824	Aachen, Germany	F	42	31 October 1993	Chronic diarrhoea	Diarrhoea	Medical technician	A
6. GHP 882	Dresden, Germany	M	?	9 September 1993	Haemoblastosis	?	?	A
7. GHP 883	Dresden, Germany	M	?	29 September 1993	Haemoblastosis	?	?	A
8. GHP 1038	Dresden, Germany	M	?	? September 1994	?	?	?	A
9. GHP 1166	Dresden, Germany	M	0	6 June 1996	?	?	-	B
10. GHP 1348	Aachen, Germany	F	48	20 January 1998	Leukaemia	Diarrhoea	?	A
11. T-139 = DH 12772	Ljubljana, Slovenia	F	37	16 December 2001	None	None	Shop assistant	A
12. T-13831 = DH 12773	Ljubljana, Slovenia	F	43	13 November 2001	None	None	Nurse	A
13. T-508 = DH 12771	Ljubljana, Slovenia	M	45	6 November 2001	Chronic inflammatory intestinal disease	Diarrhoea	Shop assistant	A
14. CBS 218.88 = UAMH 8662	Angers, France	?	?	?	?	?	?	A
15. CBS 292.49	Richmond, VA, USA	?	?	4 March 1937	Chronic diarrhoea	Diarrhoea	? ¹⁵	A
16. GHP 774 = IHEM 5848	Brussels, Belgium	M	?	?	None	Diarrhoea	Bank employee	A

Strains 1–10 were isolated from outpatient and inpatient with different kinds of gastrointestinal disturbance and diarrhoea. Strains 11–13 were isolated out of 1000 samples of faeces from otherwise healthy individuals working with foodstuffs. Strains 14–16 were available in reference collections. F, female; M, male; CBS, Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; DH, G.S. de Hoog working collection; GHP, G. Haase working collection; IHEM, Scientific Institute of Public Health, Mycology Section, Brussels, Belgium; UAMH, University of Alberta Microfungus Collection and Herbarium, Edmonton, Canada.

Table 2 Genotypes within *Exophiala dermatitidis* characterized by polymorphisms in rDNA internal transcribed spacer (ITS) 1.

Position	ITS1 (210)			ITS2 (220)
	162	184	196	None
<i>E. dermatitidis</i>				
Genotype A	T	–	A	
Genotype B	C	T	C	

Lengths of spacers are mentioned in brackets.

(ITS) rDNA region according to Matos *et al.*¹³ Strains were attributed to genotypes A or B¹³ (classified as genotypes I and IV according to Uijthof *et al.*)¹⁴ on the basis of three phylogenetically informative mutations in ITS1 (Table 2).

Results and discussion

Twelve samples were positive in total (2–13, Table 1), with 1–3 (–8) colonies per culture plate. One strain appeared to be the closely related species *Exophiala heteromorpha*. Nine strains (2–10) were recovered from a total of 1300 samples received in three hospitals in Germany, Slovenia and The Netherlands. Three strains (11–13) were isolated from 1000 faecal samples collected in the framework of screening of basically otherwise healthy individuals, which is legally obligatory in Slovenia for workers occupied with handling of foodstuffs. Two of these individuals (11: DH 12772, 12: DH 12773) were indeed without symptoms; the third (13: DH 12771) appeared to have chronic diarrhoea. Three additional strains originating from human faeces were available from reference culture collection. One of the reference strains (14: CBS 218.88) lacked any patient data; one (15: CBS 292.49, originally referred to as *Mycotorula schawi*) was described by Pereira-Filho¹⁵ from a patient with chronic diarrhoea, while another (16: IHEM 5848) originated from an otherwise healthy patient with diarrhoea.

Excluding the reference strains, the species was recovered with a frequency of 12 per 2300, which is 5.2‰ of stool samples analysed. Eight of 10 patients with intestinal black yeasts and where such data were available had diarrhoea or other intestinal disorders at the moment of isolation; of four, no data were available. Nine of 13 patients were suffering from underlying disease, which in four patients was of intestinal nature. In a leukaemic patient after BMT (3; Table 1) the strain was isolated consecutively during a period of 3 weeks, under continuing condition of diarrhoea. Apparently

E. dermatitidis is able to persist in the intestines for prolonged periods in predisposed patients.

The clinical significance of the intestinal carriage of *E. dermatitidis* is as yet unknown. In addition, the nature of pulmonary colonization in CF patients is insufficiently understood. The fungus cannot be isolated from air, and is not carried in detectable numbers in steam rooms of public bathing facilities, where it is commonly isolated from tiles.¹⁰ Inhalation thus may be a rare event. We hypothesize an additional, rare portal of entry in the human systemic infection. This may be similar to that suggested for wild animals (see above), namely by translocation from the intestinal tract. Passage through the intestinal tract of warm-blooded animals seems to be possible without significant decrease of live counts,¹⁰ possibly enhanced by the existence of an acidotolerant, meristematic form of growth.¹⁶ Given the recurrence over at least 3 weeks in a leukaemic patient (no. 3 in Table 1), combined with the rarity of the organism in human intestines, the fungus must be present in the digestive tract at a much higher frequency (although probably with a very small fungus load), and can be isolated in detectable numbers only when cells are liberated at high levels of intestinal activity during diarrhoea.

A hypothesis of a route of systemic infection through ingestion rather than by inhalation has been put forward by Hiruma *et al.*⁷ and has also been suggested for *E. spinifera* by De Hoog *et al.*¹⁷ Blaschke-Hellmessen *et al.*⁴, however, did not mention isolation from faeces in a case of *E. dermatitidis* sepsis in a leukaemic child. Particularly patients with cystic fibrosis are likely to swallow pulmonary mucus with colonizing *E. dermatitidis* on a regular basis, so that recurrent intestinal isolation is insufficient proof for maintenance and translocation. Isolation from faeces of CF patients may, however, require dedicated methods (G. Haase, unpublished data). This may explain why Table 1 does not list any patient with CF as underlying disease.

It is remarkable that all but one strains from faeces analysed thus far belong to ITS-genotype A.¹³ This is the same genotype found in all cerebral cases.⁷ A single disseminated case of fungemia in a leukaemic patient published by Blaschke-Hellmessen *et al.*⁴ had genotype B. The single genotype B faecal strain originated from another patient in the same city: Dresden, Germany. In the natural environment (faeces of tropical, fruit-eating warm-blooded animals) genotype B seems to be more common, while A is practically absent (unpublished observations).

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