MYCOSES **45,** 373–377 (2002) ACCEPTED: SEPTEMBER 3, 2001

High prevalence of the neurotrope *Exophiala dermatitidis* and related oligotrophic black yeasts in sauna facilities

Hohe Keimdichte der neurotropen *Exophiala dermatitidis* und verwandter oligotropher schwarzer Hefen in Sauna-Einrichtungen

T. Matos[1], G. S. de Hoog[1], A. G. de Boer[2], I. de Crom[3] and G. Haase[4]

Key words. *Exophiala dermatitidis*, black yeasts, neurotropism, oligotrophy, sauna, steam bath.
Schlüsselwörter. *Exophiala dermatitidis*, Schwarze Hefen, Neurotropismus, Oligotrophie, Sauna, Dampfbad.

Summary. The black yeast *Exophiala dermatitidis,* an agent of fatal brain infections in East Asia, is common in European steam baths. The related fungi *Sarcinomyces phaeomuriformis* and *Exophiala mesophila* were isolated from locations in these complexes with lower ambient temperature and/or moisture. The latter two species had dry, rather than slimy, colonies and lower maximum growth temperatures (38 °C, 32 °C) than *E. dermatitidis* (42 °C). *Exophiala dermatitidis* produces abundant extracellular polysaccharide (EPS). The only *E. dermatitidis* strains lacking EPS were found outside the steam baths. Therefore it is likely that the extracellular polysaccharides commonly produced by *E. dermatitidis* are significant to survival under hot and moist conditions. Substrates sampled as controls, such as fruit surfaces and human faeces, yielded *Exophiala dermatitidis* at very low frequency.

Zusammenfassung. Die schwarze Hefe *Exophiala dermatitidis*, die ein aetiologisches Agens von tödlich verlaufenden Gehirninfektionen in Ostasien ist, ist häufig innerhalb von Dampfbäder in Europa zu finden. Dahingegen wurden die phylogenetisch verwandten Arten *Sarcinomyces phaeomuriformis* und *Exophiala mesophila* in Dampfbädern nur von Stellen mit Zimmertemperatur und/oder üblicher Luftfeuchte isoliert. Diese beiden Arten weisen im Gegensatz zu *E. dermatitidis*, welcher aufgrund einer üppigen extrazellulären Polysaccharidproduktion (EPS) typischerweise einen schleimig erscheinenden Kolonietyp produziert, einen trockenen Kolonietyp auf, wobei ihre Wachstumsgrenze bei 38 °C bzw. 32 °C liegt im Gegensatz zu *E. dermatitidis* mit 42 °C. Der einzige *E. dermatitidis* Stamm ohne EPS-Bildung wurde außerhalb des Dampfbades isoliert. Daraus ergibt sich die Schlussfolgerung, dass die EPS-Produktion bei *E. dermatitidis* wesentlich für das Überleben in einer heißen und feuchten Umgebung ist. Untersuchte Kontrollsubstrate, wie beispielsweise Oberflächen von Früchten und menschliche Stuhlproben, wiesen nur eine sehr geringe Dichte von *E. dermatitidis*-Isolaten auf.

[1]Centraalbureau voor Schimmelcultures, Utrecht, [2]Division of Pharmacology, Leiden-Amsterdam Center for Drug Research, University of Leiden, Leiden, [3]Groene Hart Hospital, Gouda, The Netherlands, and [4]Institute for Medical Microbiology, University Hospital RWTH Aachen, Aachen, Germany.

Correspondence: Professor Dr G. S. de Hoog, Centraalbureau voor Schimmelcultures, PO Box 85167, NL-3508 AD Utrecht, The Netherlands.
Tel: + 31-30-2122663 Fax: + 31-30-2512097
E-mail: de.hoog@cbs.knaw.nl

Introduction

Exophiala dermatitidis (Kano) de Hoog is a black yeast with a world-wide distribution [1]. Epidemiological data [2–5] and results from experimental inoculation [6, 7] have demonstrated that this species has a marked predilection for the human central nervous system. Cerebral infections due to *E. dermatitidis* are chronic, but death of infected patients has only been reported from Asia. In other parts of the world, *E. dermatitidis* infections occur in skin following trauma [8], keratitis [9], onychomycosis, otitis externa [10], subclinically in the lungs of patients with cystic fibrosis [11], and very rarely as a

disseminated infection that does not involve the brain [12, 13]. Of note, cystic fibrosis is limited to Caucasians (Caucasian 1 : 2500, versus 1 : 350 000 in Japan) and hence is mainly found outside Asia. Strain typing using random amplified polymorphic DNA (RAPD) [14] proved that Asian and non-Asian strains may belong to a single population and thus differences in observed virulence are unlikely due to strain differences. So far no plausible explanation has been advanced to explain the exclusive occurrence of cerebral infections caused by *E. dermatitidis* in East Asia. Horré & de Hoog [1] suggested that host factors may be involved.

One possible explanation could be a different degree of exposure to this fungus. For example, the frequent occurrence of *E. dermatitidis* in Japanese bathing facilities [15, 16] might enhance its entry as a systemic invader. To address this question, we sampled similar facilities in Europe and compared the presence of *E. dermatitidis* and related black yeasts with that in various environments from which currently held strains originated.

Materials and methods

In order to obtain an overview of possible habitats for *E. dermatitidis*, we collected data on all 151 strains presently identified with certainty with the species, namely from: (a) IFM collection [17]; (b) Centraalbureau voor Schimmelcultures [18]; (c) Uijthof *et al.* [19]; (d) G. Haase; and (e) G.S. de Hoog. Strains of (a) were identified by physiological key features, whereas strains of (b) (c) (d) and (e) were confirmed by ITS1 sequencing.

Samples from 18 private bathrooms and five public saunas and steam baths in the Netherlands and in Slovenia, and 12 natural hot springs (temperatures between 28 and 60 °C) in Slovenia, were taken from sinks, walls, floors, bathtubs, taps, windows and direct vicinities of wells, using sterile cotton swabs. Ten-litre water samples from hot springs were divided and filtered over 0.8 µm pore membrane filters (Millipore, Bedford, MA, USA). Filters were subsequently placed on erythritol-chloramphenicol agar (ECA) in culture plates. Suspensions from plants and soil were made by rinsing or submersion in sterile water with 0.1% Tween 80 and subsequent shaking at 50 r.p.m. for 30 min Swabs were vortexed with 2 ml sterile water and 0.1% Tween 80 for 15 s; the suspensions were treated as described above. For all other samples ECA and cherry decoction agar (ChA) [20] were used. The air of steam baths was sampled by incubating open 8 cm ECA plates for 10 min. Soil and faeces were shaken for 30 min in sterile water with 0.1% Tween 80 and processed in similar way. Aliquots of 0.5 ml of 1 : 10 series-diluted suspensions were inoculated per culture plate.

Samples of fruit and orchard soil were collected during August and September in The Netherlands in sterile plastic containers and processed within 2 days. The fruits screened were commercially sold apples and plums, as well as wild-grown raspberries, blueberries and rowan berries (*Sorbus aucuparia*). Thirty samples of bat faeces from an artificial cave in the Rotterdam Zoo were screened, as well as 12 samples from a bat-inhabited cave near Valkenburg (The Netherlands). A total of 250 samples of faeces of human patients with intestinal disorders were processed by direct plating of approximately 200 µl on culture plates with Sabouraud glucose agar (SGA) and ECA [21]. For all other samples ECA and ChA [20] were used. The efficiency of the media used was established by re-isolating known concentrations of fresh cells of *Exophiala dermatitidis* (CBS 525.76) mixed in sterile water together with autoclaved substrate; the recovery rates were 86% for ECA and 92% for ChA. In the case of samples from plant surfaces, growth of the phyllosphere fungus *Aureobasidium pullulans* at 27 °C on malt yeast extract agar (MYEA; Difco, Detroit, MI, USA) was used as a positive control for the efficacy of the isolation procedure for obtaining living fungal cells from sticky plant leaves. All plates were incubated for at least 3 weeks at 36 and 40 °C and checked for growth every 2 days. Brownish yeast-like colonies were examined microscopically and subcultured for further identification. Up to four isolates were selected from a location.

Selected isolates were identified by macro- and micromorphology, and by their ability to grow at 40 °C. Species identification was confirmed by analysis of rDNA ITS1-2 sequence analysis [22] and comparison with a set of reference sequences [23].

Results and discussion

Of 35 *Aureobasidium pullulans*-containing samples from phyllosphere, honeydew-covered objects, and fruits (i.e. 75% of a total of approximately 12 000 cm^2 sampled), one was positive for *Exophiala dermatitidis* with a single colony (T-15). This sample was a rowan berry from *S. aucuparia* in a garden. Fifty-one *A. pullulans*-positive (85%) samples from commercially sold fruit were negative for other black yeasts. Of the 250 samples from human faeces, three were positive for *E. dermatitidis* (G-21, GHP 824, GHP 1348). All isolates appeared after about 10 days of incubation. Samples taken from soil, bat caves and hot springs were all negative for this species. *Sarcinomyces phaeomuriformis* Matsumoto *et al.* was isolated from one of the hot springs tested.

Of 18 bathrooms sampled (Table 1), black yeasts could be recovered from sinks [5–50 colony-forming units (CFU)/plate] and taps (1–20 CFU/plate) of four. *Sarcinomyces phaeomuriformis* (B-8, B-19) was isolated twice and *Exophiala mesophila* Listemann & Freiesleben (B-20) once. The only strain of this species known thus far originated from a silicone seal in a hospital shower cabin in Hamburg, Germany [24]. Furthermore, an as yet, undescribed, *Exophiala* species (B-6) was encountered. The latter species has the same ITS1 sequence as isolate CBS 314.90, which originated from tiles of a swimming pool in Berlin, Germany. One out of 12 natural hot springs sampled, with a temperature of 28 °C, was positive for *S. phaeomuriformis* (about 50 CFU/plate after 7 days incubation).

The steam baths (Turkish baths) of all five sauna complexes tested were positive for *E. dermatitidis* (Table 1). From each swab-sampled synthetic or tile surface of about 5 cm^2, over 10^3 colonies grew within 3 days. Two sauna halls (H-9, L-14) and a water bowl within a Finnish sauna (H-7) were also positive for *E. dermatitidis*, but colonies appeared only after 7 days of incubation, with much lower counts. Neither *Sarcinomyces phaeomuriformis* nor *Exophiala mesophila* was isolated from any steam bath.

Most isolates of *E. dermatitidis* grew at 42 °C, with the exception of three strains that were dry rather than slimy. *Sarcinomyces phaeomuriformis* strains were invariably dry and had a maximum temperature for growth of 38 °C. *Exophiala mesophila* did not grow above 32 °C.

The diversity of environments sampled, necessarily using different methods, interfered with the collection of comparable quantitative data. Data based on numbers of CFU per plate and germination velocity are comparable within the same isolation category. These data suggested that soil, faeces, plant surfaces and fruits, although *E. dermatitidis* has been reported from such substrates, do not commonly harbour large quantities of the fungus. *Exophiala dermatitidis* has previously been isolated from humidifiers [15], Japanese house baths [16] and public bathing facilities [16]. All these environments have moderate to high temperatures, high moisture and low nutrient levels. The species was consistently and abundantly present in the Turkish steam baths of European sauna complexes, where temperatures of over 60 °C are reached on a daily basis, but was much less common in adjacent localities, which are about 25 °C. In the hot Finnish sauna (> 80 °C) the species also was rare, perhaps due to the relative dryness of this environment. Isolates from steam baths grew in 2–4 days, showing that the cells apparently occurred in an active metabolic state. In all other environments growth was observed only after 8–12 days.

Nishimura *et al.* [16] found *E. jeanselmei* in the sinks of Japanese houses. They also noted a relatively low maximum growth temperature of 37 °C for this species. Thus *S. phaeomuriformis, E. jeanselmei* and *E. mesophila* inhabit niches with a lower ambient temperature, whereas *E. dermatitidis*, which displayed a maximum growth temperature of

Table 1. List of isolated strains with origins, germination time and maximum temperature of growth

Strain	Name	Origin	Germination time (days/abundance)		Max. temperature of growth
T-15	*Exophiala dermatitidis*	fruit	10	+	42 °C
GHP-824	*Exophiala dermatitidis*	human faeces		+	42 °C
GHP-1348	*Exophiala dermatitidis*	human faeces		+	42 °C
G-21	*Exophiala dermatitidis*	human faeces	9	+	42 °C
H-9	*Exophiala dermatitidis*	hall of sauna	8	+	42 °C
H-7	*Exophiala dermatitidis*	water in Finnish sauna	7	+	42 °C
L-12	*Exophiala dermatitidis* (dry)	Finnish sauna	4	+	40 °C
L-14	*Exophiala dermatitidis* (dry)	hall of sauna	8	+	40 °C
L-16	*Exophiala dermatitidis* (dry)	shower cabin-sauna	7	+	40 °C
H-1	*Exophiala dermatitidis*	steam bath	3	+ + + +	42 °C
L-10	*Exophiala dermatitidis*	steam bath	4	+ + + +	42 °C
A-17	*Exophiala dermatitidis*	steam bath	4	+ + + +	42 °C
C-18	*Exophiala dermatitidis*	steam bath	3	+ + + +	42 °C
S-22	*Exophiala dermatitidis*	steam bath	4	+ + + +	42 °C
B-8	*Sarcinomyces phaeomuriformis*	bathroom, sink	12	+	36 °C
B-19	*Sarcinomyces phaeomuriformis*	bathroom, tap	12	+	36 °C
S-23	*Sarcinomyces phaeomuriformis*	hot spring	7	+ +	36 °C
B-6	*Exophiala* sp.	bathroom, tap	10	+	36 °C
B-20	*Exophiala mesophila*	bathroom, sink	8	+	32 °C

Sauna facilities: A = Amsterdam-1; C = Amsterdam-2; H = Hilversum; L = Laren; S = Slovenia.
Abundance per culture plate: +, ≤ 5 colonies; + +, 5–50 colonies; + + +, 50–10^2 colonies; + + + +, 10^2– > 10^3 colonies.

42 °C, is particularly abundant in baths at temperatures above 60 °C with dense aerosols. The EPS covering budding cells may be an essential factor for survival in this environment. The three *E. dermatitidis* strains lacking EPS had a temperature maximum of 40 °C. Interestingly, these strains did not originate from steam baths but from adjacent localities in the same bathing facilities.

The abundance of *E. dermatitidis* in steam baths suggests that large numbers of north-western Europeans, where visits to public saunas are a common practice, are regularly exposed to the fungus. Uijthof *et al.* [14] demonstrated that some of the cerebral isolates from Asia, as well as subclinical European strains from cystic fibrosis patients, belong to a single RAPD population with world-wide distribution. Cerebritis caused by black yeasts outside Asia is associated with *Cladophialophora bantiana* [1]. Thus, the black yeast infection of the brain seen exclusively in Asians probably can not be attributed to large differences in exposure levels. The possibility of the involvement of host factors, as suggested by Horré & de Hoog [1], seems an attractive hypothesis for explaining the observed difference.

Steam baths seem to provide an optimal environment for growth of *Exophiala dermatitidis*, but nevertheless its natural ecological niche remains to be discovered. The temperature of the hot springs sampled ranged between 20 and 60 °C; thus the species would be expected to be present. Its absence is perhaps explained by the absence of dense aerosols.

In conclusion, the observed high prevalence of *E. dermatitidis* in European steam baths indicates that this fungus is also abundant in European man-made bathing facilities. Therefore, differences in exposure between Europe and Asia are a less likely explanation for the observed difference in infection patterns. The natural ecological niche of *E. dermatitidis* needs further elucidation but is probably characterized by high temperature and humidity and a low concentration of organic compounds. The three *Exophiala* and one *Sarcinomyces* species mentioned above are all anamorphs of the ascomycete family Herpotrichiellaceae, as demonstrated on the basis of inferred rDNA phylogeny [25]. They each have preference for slightly different niches within heated, moist, low-nutrient environments. Oligotrophy seems to be an evolutionary tendency within the group.

Acknowledgements

A. H. G. Gerrits van den Ende and is thanked for guidance with the molecular part of the study, and M. de Boer, Blijdorp Zoo, Rotterdam, for enabling sampling of a bat cave. We are indebted to A. Koppen and R. Vermeulen for preparing large amounts of culture media.

References

1 Horré, R. & De Hoog, G. S. (1999) Primary cerebral infections by melanized fungi: a review. *Stud. Mycol.* **43**, 176–193.
2 Chang, H. L., Kim, D.-S., Park, D. J., Kim, H. J., Lee, C. H. & Shin, H. J. (2000) Acute cerebral phaeohyphomycosis due to *Wangiella dermatitidis* accompanied by cerebrospinal fluid eosinophilia. *J. Clin. Microbiol.* **38**, 1965–1966.
3 Hiruma, M., Kawada, A., Ohata, H., *et al.* (1993) Systemic phaeohyphomycoses caused by *Exophiala dermatitidis*. *Mycoses* **36**, 1–7.
4 Matsumoto, T., Padhye, A. A., Ajello, L., Standard, P. & McGinnis, M. R. (1984) Critical review of human isolates of *Wangiella dermatitidis*. *Mycologia* **76**, 232–249.
5 Matsumoto, T., Matsuda, T., McGinnis, M. R. & Ajello, L. (1993) Clinical and mycological spectra of *Wangiella dermatitidis* infections. *Mycoses* **36**, 145–155.
6 Dixon, D. M., Migliozzi, J., Cooper, C. R., Solis, O., Breslin, B. & Szaniszlo, P. J. (1992) Melanized and non-melanized multicellular form mutants of *Wangiella dermatitidis* in mice: mortality and histopathology studies. *Mycoses* **35**, 17–21.
7 Nishimura, K. & Miyaji, M. (1983) Defense mechanisms of mice against *Exophiala dermatitidis* infection. *Mycopathologia* **81**, 9–21.
8 Kim, D. S., Yoon, Y. M. & Kim, S. W. (1999) Phaeohyphomycosis due to *Exophiala dermatitidis* successfully treated with itraconazole. *Korean J. Med. Mycol.* **4**, 79–83.
9 Benoudia, F., Assouline, M., Pouliquen, Y., Bouvet, A. & Guého, E. (1999) *Exophiala (Wangiella) dermatitidis* keratitis after keratoplasty. *Med. Mycol.* **37**, 53–56.
10 Kerkmann, M.-L., Piontek, K., Mitze, H. & Haase, G. (1999) Isolation of *Exophiala (Wangiella) dermatitidis* in a case of otitis externa. *Clin. Infect. Dis.* **29**, 929–930.
11 Haase, G., Skopnik, H., Groten, T., Kusenbach, G. & Posselt, H.-G. (1991) Long-term fungal cultures from sputum of patients with cystic fibrosis. *Mycoses* **34**, 373–376.
12 Kabel, P. J., Illy, K. E., Holl, R. A., Buiting, A. G. M. & Wintermans, R. G. F. (1994) Nosocomial intravascular infection with *Exophiala dermatitidis*. *Lancet* **344**, 1167–1168.
13 Blaschke-Hellmessen, R., Lauterbach, I., Paul, K. D., Tintelnot, K. & Weißbach, G. (1994) Nachweis von *Exophiala dermatitidis* (Kano) de Hoog 1977 bei Septikämien eines Kindes mit akuter lymphatischer Leukämie und bei Patienten mit Mukoviszidose. *Mycoses* **37** (Suppl. 1), 89–96.
14 Uijthof, J. M. J., De Hoog, G. S., De Cock, A. W. A. M., Takeo, K. & Nishimura, K. (1994) Pathogenicity of strains of the black yeast *Exophiala (Wangiella) dermatitidis:* an evaluation based on polymerase chain reaction. *Mycoses* **37**, 235–131.
15 Nishimura, K. & Miyaji, M. (1987) Studies on a saprophyte of *Exophiala dermatitidis* isolated from a humidifier. *Mycopathologia* **77**, 173–181.
16 Nishimura, K., Miyaji, M., Taguchi, H. & Tanaka, R. (1987) Fungi in bathwater and sludge of bathroom drainpipes. I. Frequent isolation of *Exophiala* species. *Mycopathologia* **97**, 17–23.
17 Nishimura, K., Miyaji, M., Takeo, K. *et al.*, eds. (1998) *IFM List of Pathogenic Fungi and Actinomycetes with Photomicrographs*. Chiba: Chiba University, pp. 305.

18 CBS (1996) *List of Cultures*, 34th edn and Internet edn. Baarn: Centraalbureau voor Schimmelcultures.
19 Uijthof, J. M. J., Van Belkum, A., De Hoog, G. S. & Haase, G. (1998) *Exophiala dermatitidis* and *Sarcinomyces phaeomuriformis*: ITS1-sequencing and nutritional physiology. *Med. Mycol.* **36**, 143–151.
20 Gams, W., Hoekstra, E. S. & Aptroot, A., eds. (1998) *CBS Course of Mycology*. Baarn: Centraalbureau voor Schimmelcultures, pp. 165.
21 De Hoog, G. S. & Haase, G. (1993) Nutritional physiology and selective isolation of *Exophiala dermatitidis*. *Antonie Van Leeuwenhoek* **64**, 17–26.
22 Gerrits van den Ende, A. H. G. & De Hoog, G. S. (1999) Variability and molecular diagnostics of the neurotropic species *Cladophialophora bantiana*. *Stud. Mycol.* **43**, 151–162.
23 Matos, T., De Hoog, G. S. & Haase, G. (2001) Comparison of Asian and non-Asian populations of the neurotropic black yeast *Exophiala dermatitidis*. *Antonie van Leeuwenhoek*. in press.
24 Listemann, H. & Freiesleben, H. (1996) *Exophiala mesophila* spec. nov. *Mycoses* **39**, 1–3.
25 Haase, G., Sonntag, L., Melzer-Krick, B. & De Hoog, G. S. (1999) Phylogenetic interference by SSU-gene analysis of members of the *Herpotrichiellaceae* with special reference to human pathogenic species. *Stud. Mycol.* **43**, 80–97.