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Nutritional physiology and selective isolation of *Exophiala dermatitidis*

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Abstract

The nutritional physiology of ten strains of *Exophiala dermatitidis* was investigated. The growth reactions to lactose, citrate, nitrate, nitrite, lysine, creatine and creatinine differ from those found in closely related black yeasts. In addition, it is the only *Exophiala* species which is able to grow at 40° C. A selective medium containing *meso*-erythritol as sole carbon source was evaluated. This medium is particularly useful for the isolaton of black yeasts from lungs of patients with cystic fibrosis as well as from the environment.

Introduction

Exophiala dermatitidis (Kano) de Hoog is an opportunistic black yeast known from cases of chromoblastomycosis (Kano 1937), phaeohyphomycosis (Matsumoto et al. 1984), phaeohyphomycotic cysts (Scott et al. 1986), onychomycosis (Matsumoto et al. 1992) or keratomycosis (Pospísil et al. 1990), all developing after traumatic inoculation. More recently, a pulmoneous route of infection was established by the regular occurrence of the fungus in patients with cystic fibrosis (Haase et al. 1991), occasionally leading to pneumonia (Barenfanger et al. 1989; Kusenbach et al. 1992). Systemic infections may be localized, as in endocarditis (Ventin et al. 1987) or disseminated (Hohl et al. 1983). For dissemination, promotive factors are required such as immunodeficiency or corticosteroid therapy (Crosby et al. 1989). Such patients often have multiple fungal infections (Hohl et al. 1983). The neurotropic pathology of the fungus, confirmed experimentally in mice (Dixon et al. 1989), is responsibe for a high fatality rate (Matsumoto et al. 1984).

The object of the present study is twofold. The occurrence of two potential routes, subcutaneous *vs.* pulmoneous, raises the possibility that two taxonomic entities might be concerned. A set of morphological, cultural and physiological parameters is applied to verify this. Secondly, multiple infections may conceal the occurrence of the slow-growing *Exophiala dermatitidis.* In CF-patients, the contamination of bronchial secretions with *Candida albicans* obscures the successful recovery of *Exophiala dermatitidis,* because growth of the former fungus is more rapid. A selective medium for the recovery of black yeasts from respiratory tract samples of these patients, as well as from the environment, is therefore necessary.

Based on the fact that some *Exophiala* species originate from seawater and that all are able to grow on Sabouraud's glucose agar with seawater (containing approximately 3.5% salt; Haase et al. 1992) we searched for a selective medium based on compounds present in this environment. The carbohydrate *meso*-erythritol is regularly found in the biotope as a compound of algae. It is assimilated very well by *E. dermatitidis,* but not by most *Candida* species. The addition of *meso*-erythritol to a synthetic medium is evaluated below as a possible selective substrate.

Materials and methods

Strains studied are listed in Table 1. Stock cultures were maintained on 2% malt/1% yeast extract agar (MYA) slants. Growth and fermentative ability were tested in duplicate in liquid medium in test tubes at 25° C, 50 rpm (van der Walt & Yarrow 1984). Diazonium Blue B staining was performed according to Hagler & Ahearn (1981). Urease activity was tested in Christensen's liquid medium. Acid production was tested on 2% MYA with calcium carbonate; on this medium, as well as on Sabouraud's glucose agar (SGA) production of extracellular, brown pigments was optimal. Cycloheximide tolerance was tested both in liquid medium at 0.01, 0.05 and 0.1% and on commercial Mycosel agar (0.04%; Bacto, Dickinson, USA). Production of alkaline components and siderophores was tested on CAS agar (Chrome Azurol S; De Hoog & Gerrits-van den Ende 1992); halos of clearance and final pH were measured after incubation for 30 d at room temperature. Gelatin (12%) liquefaction was tested in tubes at 21° C and read after 10 min incubation at 4° C, and also on agar plates with 0.4% gelatin, using $HgCl_2$ in 7.6% HCl as reagent.

The selective medium (ECA = Erythritol-Chloramphenicol-Agar) was prepared with Yeast-Nitrogen-Base medium (Difco, Detroit, USA) with 1% *meso*-erythritol (1,2,3,4,-butantetrol; Roth, Karlsruhe, Germany), 0.05% chloramphenicol (Merck, Darmstadt, Germany) and 2.5% agar (Unipath, Wesel, Germany). Incubation was at 30° C. Type and other strains of *Exophiala* species, especially *E. dermatitidis* (most of the isolates originated from sputum of CF-patients), supplemented by some other medically relevant fungi, were tested.

The growth-kinetics of *E. dermatitidis,* CBS 148.90 were estimated in the broth formula of the same EC medium and compared to a medium containing glucose instead of *meso*-erythritol. In order to remove the excreted brown pigment produced by the fungus, cells were thoroughly washed before optical densities (at 520 nm) were determined. The performance of EC medium was further evaluated in a clinical trial with 200 sputum samples from 47 CF-patients, in comparison to SGA with chloramphenicol (Sanofi Pasteur Diagnostica, Freiburg, Germany). Plates were observed over a period of 30 days.

Results

On SGA plates, *Exophiala dermatitidis* forms waxy, smooth, blackish-brown colonies; some rust-brown pigment is exuded into the medium. Initial growth is yeastlike, but the colonies soon become hyphal and tough. Sclerotial bodies are rarely seen. Funnel-shaped, sessile phialidic collarettes are locally produced. Phialoconidia are (sub)spherical, about 2 µm in diam. Collarettes soon deteriorate. Annelloconidia are broadly ellipsoidal and 2.5–4.0 µm in length. In liquid medium, *E. dermatitidis* usually grows as a milky, dark grey yeast, with few or no filaments. Shake cultures in Erlenmeyer flasks may show a rust brown or purplish tinge. Growth is ab-

Table 1. List of strains studied.

CBS 207.35	(= CDC B-1775), T of *Hormodendrum dermatitidis,* ex human skin lesion, Japan, M. Ota (Kano 1937).
CBS 292.49,	T of *Mycotorula schawii,* ex faeces of human patient with chronic diarrhoea (Pereiro-Filho 1948).
CBS 525.76,	P. Szaniszlo No. 8656 (Geis & Szaniszlo 1984).
CBS 577.76	(= CDC B-1782), ex human cervical lymph node, Taiwan (Jen 1966).
CBS 578.76,	ex case of cerebral infection, Taiwan (Tsai et al. 1966).
CBS 971.87,	Iraq, F.Y. Al-Ani.
CBS 218.88,	ex human faeces, France.
CBS 748.88,	ex sputum, CF, Norway, P. Sandven.
CBS 148.90,	154.90, 156.90, 213.90, ex sputum, CF, Germany, G. Haase.
CBS 149.90,	ex sputum, CF, Germany, B. Hövener.
CBS 150.90,	ex sputum, pneumonia, Netherlands, W. Pauw.
CBS 153.90,	ex sputum, CF, Germany, G. Haase.
CBS 106.92,	culture contaminant, Italy, A. Vaughan Martini.

sent or with D-glucosamine, lactose, inulin, L-arabinitol, D-galacturonate, citrate, methanol and nitrite (Table 2). Variable reactions were noted with D-ribose, D-arabinose, arbutin, melibiose, soluble starch, galactitol, DL-lactate, nitrate, creatine and creatinine.

Ten percent NaCl and 0.01% cycloheximide are not tolerated; all strains grow, at least weakly, with 10% $MgCl_2$. Fermentation and gelatin liquefaction are absent. In two strains some clarification of chalk agar is obtained. On CAS agar a marked siderophore production is observed. The original pH 4.5 of this medium is raised to 8.4 due to the production of extracellular, alkaline products.

The following strains are exceptional in their growth responses: CBS 292.49 (from faeces: pigment absent, D-ribose +), CBS 577.76 (from lymph node: nitrate +, creatinine +), CBS 971.87 (source unknown: DL-lactate +), CBS 218.88 (from faeces: D-arabinose –, soluble starch –), CBS 748.88 (from sputum: melibiose –), CBS 150.90 (from sputum: arbutin –, creatine +, creatinine +). The remaining strains have been isolated from skin lesions, sputum and unspecified sources (Table 1).

The species is able to grow at 43° C; some strains, particularly those originating from CF patients, grow at 46° C. Most strains still show some growth at 4° C.

All 90 strains of *Exophiala dermatitidis* tested grew very well on ECA medium, as did most other *Exophiala* species (Table 3). *E. dermatitidis* grew better in broth containing glucose ($t_{1/2max}$ glucose = 42.6 h; $t_{1/2max}$ *meso*-erythritol = 85.3 h; Fig. 1). Using ECA, three times as many isolates of *E. dermatitidis* were recovered from the samples of CF-patients than with SGA. This was primarily due to the suppression of growth of *C. albicans* on ECA (Fig. 2). *Candida lipolytica* was recovered twice in low quantities on ECA. No other fungal species was obtained on ECA plates from clinical specimens.

Discussion

The black yeast genus *Exophiala* Carmichael (including *Wangiella* McGinnis) comprises a number of closely related species several of which are potential agents of opportunistic mycoses. *Exophiala dermatitidis* is recognized by waxy colonies, attaining about 5 mm diam after 10 days at room temperature. Often a rust-brown diffusable pigment is exuded into the agar, particularly when Sabouraud's glucose or chalk agars are applied. Morphologically, the flat, non-elongating annellated zones ('phialides-without-collarettes'; McGinnis 1977), often in groups of 2 to 3, are characteristic. Similar structures are known in *E. mansonii* (Castell.) de Hoog (de Hoog 1977; = *E. castellanii* Iwatsu et al. 1984), but cultures of this species show less expansion growth, are not waxy and brown pigments are absent. Both species produce broadly ellipsoidal conidia. The species *E. jeanselmei* (Langeron) McGinnis & Padhye, though extremely variable in its morphology, can be distinguished by somewhat faster growing colonies, tapering annellated zones and a preponderance of narrow conidia.

The minute cultural and morphological characteristics render species identification in *Exophiala* rather difficult. *E. dermatitidis* is well-delimited serologically (Espinel-Ingroff et al. 1984; Matsumoto et al. 1984). Nutritional physiology provides a useful set of additional diagnostic criteria (Miyaji & Nishimura 1985; Espinel-Ingroff et al. 1989; De Hoog & Gerrits-van den Ende 1992; Tintelnot et al. 1992).

The milky, evenly yeastlike growth of *E. dermatitidis* in suspension enables an easier evaluation of test results than *Hortaea werneckii.* The latter shows a strain- and condition-dependent dimorphism, leading to hydrophobic clumps of mycelium in some of the growth compounds tested (De Hoog & Gerrits-van den Ende 1992). To evaluate reproducibility, eight strains from cases of cystic fibrosis, partly originating from a single patient, were tested and found to be nearly identical (results not shown). Slight differences were found only in assimilation of D-ribose and lactose, which were sometimes scored as weak rather than negative. In contrast to findings of Espinel-Ingroff et al. (1989) using API 20C identification strips, we found *E. dermatitidis* to be consistently positive with galactose, xylitol, myo-inositol, melezitose and raffinose. As pointed out by De Hoog & Gerrits-van den Ende (1992), miniaturized methods are less suitable for testing black yeast physiology. Some strain-depend-

Table 2. Growth reactions and other tests of ten strains of *Exophiala dermatitidis*.

	207.35	292.49	525.76	577.76	578.76	971.87	218.88	748.88	149.90	150.90	Species signature
D-Glucose	+	+	+	+	+	+	+	+	+	+	+
D-Galactose	+	+	+	+	+	+	+	+	+	+	+
L-Sorbose	w	+	w	w	w	w	+	w	w	+	w/+
D-Glucosamine	–	–	w	w	–	–	–	–	–	–	–/w
D-Ribose	w	+	–	w	–	–	–	w	–	–	v
D-Xylose	+	+	+	+	+	+	+	+	+	+	+
L-Arabinose	+	+	+	+	+	+	+	+	+	+	+
D-Arabinose	+	+	w	w	w	+	–	+	w	+	v
L-Rhamnose	+	+	w	+	+	+	+	w	+	+	+/w
Sucrose	+	+	+	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+	+	+	+
αα-Trehalose	+	+	+	+	+	+	+	+	+	+	+
methyl-α-Glucoside	w	+	+	w	w	+	+	+	w	w	+/w
Cellobiose	+	+	+	+	+	+	+	+	+	+	+
Salicin	+	+	+	+	+	+	+	+	+	+	+
Arbutin	+	+	+	+	+	+	+	+	+	–	+(–)
Melibiose	+	+	w	+	+	+	+	–	+	+	+(–)
Lactose	w	w	–	w	w	w	–	w	–	–	w/–
Raffinose	+	+	+	+	+	+	+	+	+	+	+
Melezitose	+	+	+	+	+	+	+	+	+	+	+
Inulin	w	–	–	w	–	–	–	w	–	–	–/w
Soluble starch	w	w	w	w	w	+	–	+	w	w	v
Glycerol	+	+	+	+	+	+	+	+	+	+	+
meso-Erythritol	+	+	+	+	+	+	+	+	+	+	+
Ribitol	+	+	+	+	+	w	+	+	w	+	+/w
Xylitol	+	+	+	+	+	+	+	+	+	+	+
L-Arabinitol	–	w	w	–	–	–	w	–	w	–	–/w
D-Glucitol	+	+	+	+	+	+	+	+	+	+	+
D-Mannitol	w	+	+	+	+	+	+	+	+	+	+(w)
Galactitol	w	+	+	–	w	+	+	+	–	+	v
myo-Inositol	+	+	+	+	w	+	+	+	+	+	+(w)
Glucono-δ-lactone	+	+	+	+	+	+	+	+	+	w	+(w)
2-keto-D-Gluconate	+	+	+	+	+	+	+	+	+	+	+
D-Gluconate	+	+	+	+	+	w	+	+	+	+	+(w)
D-Glucuronate	+	+	+	+	+	+	+	+	+	+	+
D-Galacturonate	w	w	w	w	w	–	w	–	w	w	w/–
DL-Lactate	–	–	–	–	–	+	–	–	–	w	v
Succinate	w	+	+	+	w	+	+	+	+	+	+/w
Citrate	–	–	–	–	–	–	–	–	–	–	–
Methanol	–	–	–	–	–	–	–	–	–	–	–
Ethanol	+	+	+	+	+	+	+	+	+	+	+
Nitrate	–	–	–	+	–	–	–	–	–	–	–(+)
Nitrite	–	–	–	–	–	–	–	–	–	–	–
Ethylamine	+	+	+	+	+	+	+	+	+	+	+
L-Lysine	+	+	+	+	+	+	+	+	+	+	+
Cadaverine	+	+	+	+	+	+	+	+	+	+	+
Creatine	–	–	–	–	–	–	–	–	–	+	–(+)
Creatinine	–	–	–	+	–	–	–	–	–	+	v
2% NaCl	+	+	+	+	+	+	+	+	+	+	+
5% NaCl	+	w	+	w	w	+	+	+	w	+	+/w
10% NaCl	–	–	–	–	–	–	–	–	–	–	–

Table 2. Continued

	207.35	292.49	525.76	577.76	578.76	971.87	218.88	748.88	149.90	150.90	Species signature
2% $MgCl_2$	+	+	+	+	+	+	+	+	+	+	+
5% $MgCl_2$	+	+	+	+	+	+	+	+	+	+	+
10% $MgCl_2$	+	w	+	w	+	+	w	+	+	+	+/w
0.01% Cycloheximide	–	–	–	–	–	–	–	–	–	–	–
Mycosel	–	–	–	–	–	–	w	–	–	–	–/(w)
Urease	+	+	+	+	+	+	+	+	+	+	+
4° C	–	–	+	–	+	+	w	+	+	+	v
10° C	+	+	+	+	+	+	+	+	+	+	+
40° C	+	+	+	+	+	+	+	+	+	+	+
43° C	+	+	+	+	+	+	+	+	+	+	+
46° C	–	–	w	–	–	w	w	–	w	–	v
50° C	–	–	–	–	–	–	–	–	–	–	–
Gelatin liquef.	–	+	–	–	–	–	–	+	–	+	v
Fermentation	–	–	–	–	–	–	–	–	–	–	–
Acid production	–	+	+	–	–	–		–	–	–	v
DBB	–	–	w	–	–	–	w	–	–	–	–/w
CAS	3	3	3	v	3	2	2	w	w	4	
final pH	8.4	5.7	6.4	v	8.0	5.6	5.4	v	v	8.1	
Pigment	+	–	w	w	+	+	v	+	+	+	v

DBB = Diazonium Blue B; CAS = Chrome Azurol Blue; w = weak growth reaction. (+/w or –/w several reactions scored as weak; between brackets single isolate deviating; v = variable several +/w or –/w).

ent variation has regularly been found in the amount of biomass produced, leading to many 'weak' growth reactions (Table 2) which can be interpreted either as positive or as negative. Definite (+/–) strain-dependent variation in growth reactions have been noted with D-ribose, D-arabinose, arbutin, melibiose, galactitol, DL-lactate, nitrate, creatine and creatinine.

Exophiala dermatitidis is physiologically characterized by absence of growth or weak assimilation of lactose, citrate and nitrite, mostly no assimilation of nitrate, creatine and creatinine, by assimilation of lysine, and no tolerance of 10% NaCl (Table 2). It is the only *Exophiala* species which is able to grow at 40° C (Padhye et al. 1978; Espinel-Ingroff et al. 1988). *Exophiala jeanselmei* and *E. mansonii* do not grow at 37° C, but assimilate nitrate and nitrite (Tintelnot et al. 1992). *Hortaea werneckii* differs by absence of growth with lysine but it grows with lactose, nitrate, nitrite and 10% NaCl (De Hoog & Gerrits-van den Ende 1992).

The variation in growth responses (Table 2) does not coincide with sources of isolation and is therefore regarded as intra-specific variability. The species was found to be homogeneous on the basis of nDNA/DNA reassociation experiments (Masuda et al. 1989) and mtDNA restriction patterns (Kawasaki et al. 1990). The tolerance of high temperatures and the inability to take up inorganic nitrogen suggests a natural association with animals. Urine is probably not a main substrate, since only a few strains are able to assimilate creatine and creatinine. Outside humans, the species has mainly been found in waters with slightly raised electrolyte contents, e.g., in sewers, swimming pools, humidifiers or purification trains (Nishimura & Miyaji 1982). Dixon et al. (1980) isolated the species from wood after injection of extracts in mice. Reiss & Mok (1979) isolated black yeasts identified as *Wangiella dermatitidis* from internal organs of bats in Brazil. The factors determining the natural ecological niche of *E. dermatitidis* have not been established.

Exophiala dermatitidis grows easily in submerged culture, producing an abundance of yeast cells. Cells soon become thick-walled (Oujezdsky et al. 1973) and accumulate fatty acids in vacuoles, which are subsequently utilized in an increasing endogenous respiration (Calderone 1976). The yeast-

Table 3. Growth of black yeasts and other clinically significant fungi on ECA selective medium.

Black yeasts	Source of strains	Growth
Capronia mansonii	MUCL 10097	+
Exophiala alcalophila	CBS 520.82T	–
Exophiala angulospora	CBS 482.92T	+
Exophiala dermatitidis	CBS 207.35T	+
Exophiala dermatitidis	85 clinical strains	+
Exophiala dermatitidis	ATCC 44504	+
Exophiala dermatitidis	Ku A 0052	+
Exophiala dermatitidis	ATCC 34100	+
Exophiala dermatitidis	Mc 3 (multicellular 34100)	+
Exophiala dopicola	UTMB 1229T	+
Exophiala jeanselmei var. heteromorpha	CBS 232.33T	–
Exophiala jeanselmei var. jeanselmei	CBS 537.73	+
Exophiala jeanselmei var. lecanii-corni	CBS 680.76	+
Exophiala jeanselmei var. lecanii-corni	CBS 530.76	+
Exophiala jeanselmei var. lecanii-corni	CBS 123.33T	+
Exophiala mansonii	CBS 158.58NT	+
Exophiala mansonii	UTMB 3284	+
Exophiala moniliae	CBS 520.76T	+
Exophiala pisciphila	CBS 537.73T	+
Exophiala psychrophila	CBS 191.87T	+
Exophiala salmonis	CBS 157.67T	+
Exophiala spinifera	CBS 899.68T	+
Exophiala sp.*	CBS 725.88	+
Fonsecaea pedrosoi	3 clinical strains	+
Hortaea werneckii	CBS 107.67NT	+
Nadsoniella nigra var. heuselica	CBS 546.82	–
Phaeococcomyces elegans	UTMB 1286T	–
Phaeococcomyces exophialae	CBS 668.76T	+
Phaeoannelomyces nigricans	CBS 652.76T	–
Rhinocladiella atrovirens	CBS 264.49	+
Rhinocladiella atrovirens	CBS 265.49	+
Sarcinomyces phaeomuriformis	CBS 131.88T	+
Aspergillus clavatus	CBS 114.48	+
Aspergillus flavus	DSM 62065	+
Aspergillus fumigatus	CBS 110.46, 192.65 + 4 clinical strains	–
Aspergillus oryzae	ATCC 114.94	+
Aspergillus terreus	DSM 826	+
Candida albicans	ATCC 10231 + 15 clinical strains	–
Candida glabrata	DSM 70125 + 10 clinical strains	–
Candida guilliermondii	DSM 70051 + 7 clinical strains	–
Candida krusei	DSM 70075 + 8 clinical strains	–
Candida lusitaniae	4 clinical strains	–
Candida parapsilosis	DSM 70125 + 7 clinical strains	–
Candida pseudotropicalis	CBS 2235 + 5 clinical strains	–
Candida tropicalis	CBS 2424 + 10 clinical strains	–
Clavispora lusitaniae	DSM 70102	–
Cryptococcus neoformans var. gattii	BAL2, B32.64, AB34.3	+
Cryptococcus neoformans var. neoformans	3 clinical strains	+
Rhodotorula rubra	clinical strain	+
Saccharomyces cerevisiae	DSM 1333	–
Scedosporium apiospermum	LMA 90.630 + 2 strains	–
Sporothrix schenckii	4 clinical strains	+
Yarrowia lipolytica	DSM 70562 + 2 clinical strains	+

* Possibly representing an undescribed species (Tintelnot et al. 1991).

Abbreviations used: DSM – Deutsche Sammlung von Mikroorganismen u. Zellkulturen; ATCC – American Type Culture Collection; UTMB – University of Texas Microbiology Branch; LMA – Laboratoire Mycologie, Angers; KU – Kyushu University; MUCL – Mycotheque de L'Université Catholique de Louvain; CBS – Centraalbureau voor Schimmelcultures.

T = Type strain; NT = neotype.

like growth of *E. dermatitidis* probably facilitates haematogenous dissemination in the animal host. Jen & Kuo (1978) demonstrated the fungistatic effects of a gamma-globulin preparation from human serum. Numerous authors have reported neurotropic involvement with a high mortality in systemic infections (e.g. Matsumoto et al. 1984). This may be explained by siderophore production (Table 2), demonstrating the fungus' notable requirement for free iron; halos are significantly larger than those found in the superficial fungus *Hortaea werneckii* (De Hoog & Gerrits-van den Ende 1992).

Granular growth forms have been described in several black yeasts (Naka et al. 1989), e.g., as a response to stress temperatures. In *E. dermatitidis* this change of growth form, occasionally provoked by growth at high temperature (Roberts & Szaniszlo 1978), is reversible to yeast (Jacobs et al. 1985). In experimentally inoculated mice, yeast cells are present in tissue in addition to muriform cells (Dixon et al. 1992). Strains isolated from cerebral granulomes are frequently granular (Jotisankasa et al. 1970; Shimazono et al. 1963), but could be reverted to yeast. Our cerebral strain CBS 578.76 is not granular.

In 1986 Matsumoto et al. described *Sarcinomyces phaeomuriformis* for two isolates from skin lesions, originally referred to as granular forms of

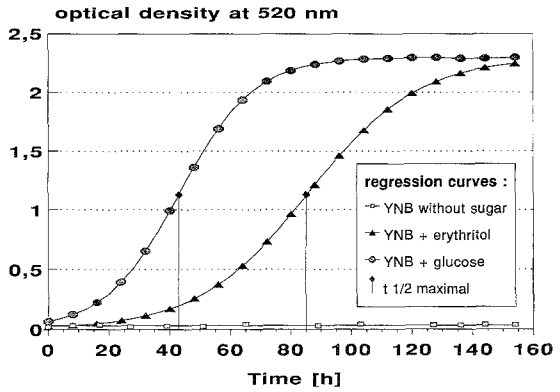

Fig. 1. Growth kinetics of *Exophiala dermatitidis,* CBS 148.90 in Yeast Nitrogen Base broth, with or without the addition of glucose or *meso*-erythritol.

Exophiala dermatitidis. Later, Matsumoto & Ajello (1991) found the two species to be indistinguishable with the exoantigen test. However, we found the type strain of *S. phaeomuriformis,* CBS 131.88 to differ physiologically from all strains of *E. dermatitidis* by growing with L-arabinitol and 0.01% cycloheximide but not with glucono-δ-lactone and 5% NaCl. On the basis of these differences we keep the species apart, although *S. phaeomuriformis* admittently must be close to *E. dermatitidis,* shown also by its inability to assimilate nitrate and nitrite, which is quite uncommon in black yeasts.

Nishimura et al. (1985) found the maximum

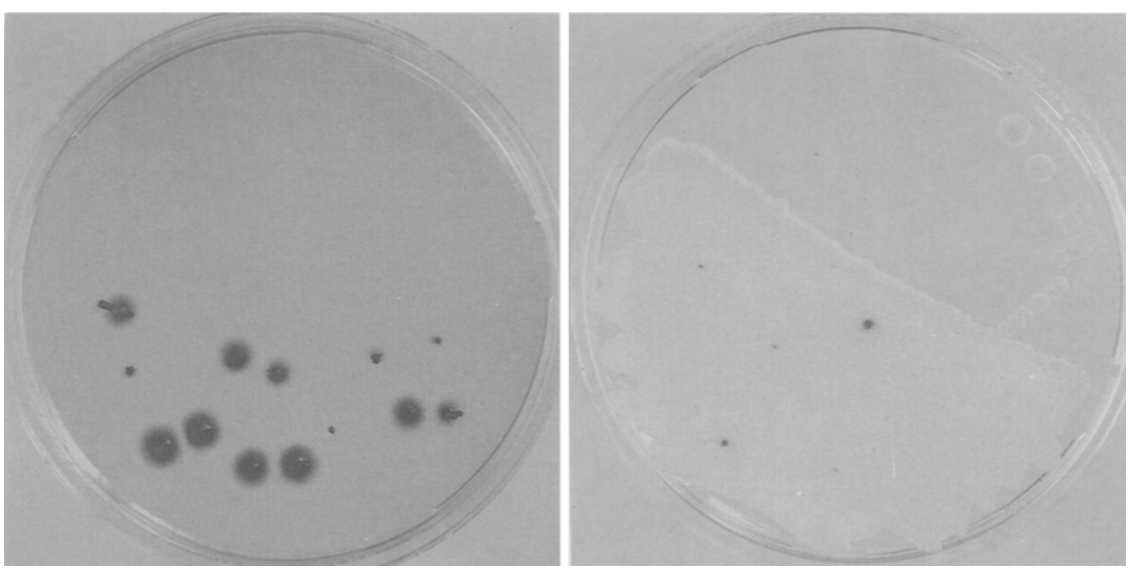

Fig. 2. Mixture of *Exophiala dermatitidis,* CBS 207.35 and *Candida albicans,* DSM 1333 on ECA (left) and SGA (right).

growth temperature of their subcutaneous isolates to be 44° C rather than 40° C as reported by Padhye et al. (1978). This is confirmed by our data (Table 2).

Broncho-alveolar lavages of patients with cystic fibrosis usually exhibit a large colony count of *Candida albicans,* which conceal the slower growing *E. dermatitidis* (Hughes & Kim 1973; Blaschke-Hellmessen et al. 1991; Haase et al. 1991; Doern & Brogden-Torres 1992). Media for the selective isolation of *E. dermatitidis* can be developed on the basis of differences in nutritional patterns of the two species. *Exophiala dermatitidis* consistently grows with cellobiose, salicin, raffinose, *meso*-erythritol and D-glucuronate, while *C. albicans* is unable to assimilate these compounds (Barnett et al. 1990). Haase et al. (1992) successfully applied a medium with *meso*-erythritol. This compound occurs in marine algae; ocean water is one of the natural ecological niches of related black yeasts. Erythritol accumulation was found to play a key role in osmoregulation of another black yeast, *Aureobasidium pullulans* (Tokuoka et al. 1992).

The origin of the clinical *E. dermatitidis* isolates tested with ECA was mainly pulmoneous, but strains from other localities gave the same result. The addition of 0.05% cycloheximide to this medium reduced the growth rate of *E. dermatitidis* (visible colonies could be detected only after 20 d incubation). The species is not tolerant to cycloheximide, although some growth may be observed on Mycosel agar (Table 2).

The performance of ECA medium in the clinical trial was very good. The reduced growth-rate in EC broth in comparison to SG medium, as revealed in growth-kinetic experiments, did not affect the usefulness of ECA. On ECA, the colony counts of *E. dermatitidis* were three times higher than on SGA, the medium currently used in diagnostic laboratories for the isolation of fungi from clinical samples. *Candida albicans* and *Aspergillus fumigatus,* species frequently occurring in lungs of CF patients, were suppressed on ECA (Table 3). No further yeasts (except *C. lipolytica*) could be recovered from the clinical samples. Judging from literature the medically significant yeast *Cryptococcus neoformans* should be able to grow on this medium. Preliminary experiments with six isolates of *C. neoformans* (3 var. *neoformans,* 3 var. *gattii*) were positive (Table 3). *Scedosporium apiospermum,* which also occurs subclinically in the lungs of leukemic and CF-patients, is not isolated because it does not assimilate erythritol (de Hoog et al. 1993).

The selective ECA medium seems to be promising for a reliable and easy recovery of *Exophiala dermatitidis* from clinical specimens. It may help to elucidate the pathogenic role of this fungus in CF-patients, which might currently be underestimated. The medium may also be helpful for the isolation of the fungus from environmental specimens which are heavily contaminated by moulds. An opportunity is thus provided to screen the environment more systematically in search of the natural habitat of this species.

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