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Ecology and physiology of the emerging opportunistic fungi *Pseudallescheria boydii* and *Scedosporium prolificans*

Ökologie und Physiologie der opportunistischen Pilze *Pseudallescheria boydii* und *Scedosporium prolificans*

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Key words. *Pseudallescheria boydii, Scedosporium prolificans*, Microascaceae, taxonomy, diagnostics, pathogenetic determinants, physiology, anaerobic growth, opportunistic fungi.

Schlüsselwörter. *Pseudallescheria boydii, Scedosporium prolificans*, Microascaceae, Taxonomie, Diagnostik, Pathogenitätsfaktoren, Physiologie, anaerobes Wachstum, opportunistische Pilze.

Summary. Nutritionally physiological patterns of *Pseudallescheria boydii* (Microascaceae) and the related species *Scedosporium prolificans* were established. Differences between the two species were found in assimilation of sucrose, ribitol, xylitol and L-arabinitol. In contrast, no physiological distinction could be made between the three intraspecific variants of *P. boydii* which have been recognized on the basis of nDNA/DNA homology data and of morphological and clinical differences. Some potential virulence factors were studied in the fungi mentioned above and in some related anamorphs. All species were capable of anaerobic growth, but differed in their temperature relations.

Zusammenfassung. Assimilations-Muster von *Pseudallescheria boydii* (Microascaceae) und der verwandten Art *Scedosporium prolificans* wurden untersucht. Dabei wurden Art-Unterschiede in der Assimilation von Saccharose, Ribitol, Xylitol und L-Arabinitol festgestellt. Im Gegensatz dazu wurden keine physiologischen Unterschiede zwischen den drei Varianten innerhalb der Art *P. boydii* nachgewiesen. Damit lassen sich die bisher verwendeten Merkmalsunterschiede, bzw. nDNA/DNA Homologie-Werte sowie morphologische und klinische Characteristika, nicht bestätigen. Einige mögliche Virulenz-Faktoren bei den oben beschriebenen sowie auch bei einigen verwandten Arten, wurden untersucht. Alle Arten erwiesen sich im gleichen Maß zum anaeroben Wachstum befähigt, unterscheiden sich aber in ihrem Temperaturverhalten.

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Introduction

The Ascomycete *Pseudallescheria boydii* (Negroni & Fischer) McGinnis *et al.* (fam. Microascaceae) is found rather commonly in soil and polluted water. For a long time it escaped attention as a potential human pathogen because it was known by a number of different names. This nomenclatural confusion was caused by its polymorphism, different strains being potentially capable of producing *Scedosporium*- and *Graphium*-like anamorphs in addition to *Pseudallescheria* cleistothecia.

Several types of pathogenicity have been associated with *P. boydii*. The species is particularly known to cause human white-grain mycetoma [1] and, more recently, it has emerged as an agent of systemic and disseminated mycoses [2, 3]. It is found subclinically in the lungs of patients with leukaemia [4] or cystic fibrosis [5]. In addition, it can cause of otitis externa with discharge [6, 7]. It rarely causes keratitis [8].

In their review of pseudallescheriasis, Warnock

& Richardson [9] distinguished between the morbidity of localized and disseminated deep mycoses by *P. Boydii*. Disseminated mycoses, in which neurotropic involvement is frequent, are mostly fatal despite antimycotic therapy. The portal of entry also varies, being via the sinuses in localized mycoses and possibly via the lungs in deep mycoses. Guého & de Hoog [10] suggested a possible molecular basis for the purported differences in pathogenesis. Using nDNA/DNA reassociation techniques, they established three infraspecific entities with different sources of isolation, namely lungs, sinuses and soil. As a consequence, human infection by *P. boydii* might be more complicated than originally supposed. The present paper focuses on the ecology and nutritional physiology of the *P. boydii* variants and of related species in order to characterize further the clinically relevant taxa of Microascaceae. Among these, *Scedosporium prolificans* (Hennebert & Desai) Guého & de Hoog is particularly emerging as an opportunist in humans [11–13] and is therefore considered in more detail.

Materials and methods

Strains and culture conditions

The strains used, which are listed in Fig. 1, are maintained in the culture collections of the Centraalbureau voor Schimmelcultures, Baarn (CBS), The Netherlands, and of the Unité de Mycologie, Institut Pasteur, Paris (IP). Stock cultures were inoculated with conidial suspensions and grown on oatmeal agar (OA) or 4% malt extract agar (MEA) slants at 30 °C. Strains 9.1 and 1.2 were isolated under 80% N_2/20% CO_2 and cultivated on 1% malt extract (ME)/0.4% yeast extract/0.4% glucose flushed with N_2, final pH 7.0. For karyology, strains were grown on permeable PT cellophane membranes, eventually covered by a second membrane. Colony diameters were measured twice weekly on MEA petri dishes incubated in the dark at 24, 30, 36 and 42 °C until the stationary growth phase.

Anaerobic growth

Strains were grown in Hungate tubes (16 × 125 mm) fitted with butyl septum stoppers (Bellco Glass, Vineland, NJ, USA), containing 10-ml aliquots of 1% malt extract/0.4% yeast extract/0.4% glucose/2% agar medium, flushed with O_2-free CO_2 and subsequently autoclaved. Filter-sterilized vitamin and haemin solutions were added to a basal growth medium [14]. Fungi were grown under O_2-free N_2 (80%)/CO_2 (20%) gas phase (pH 7.0) and subcultured every 3–4 days.

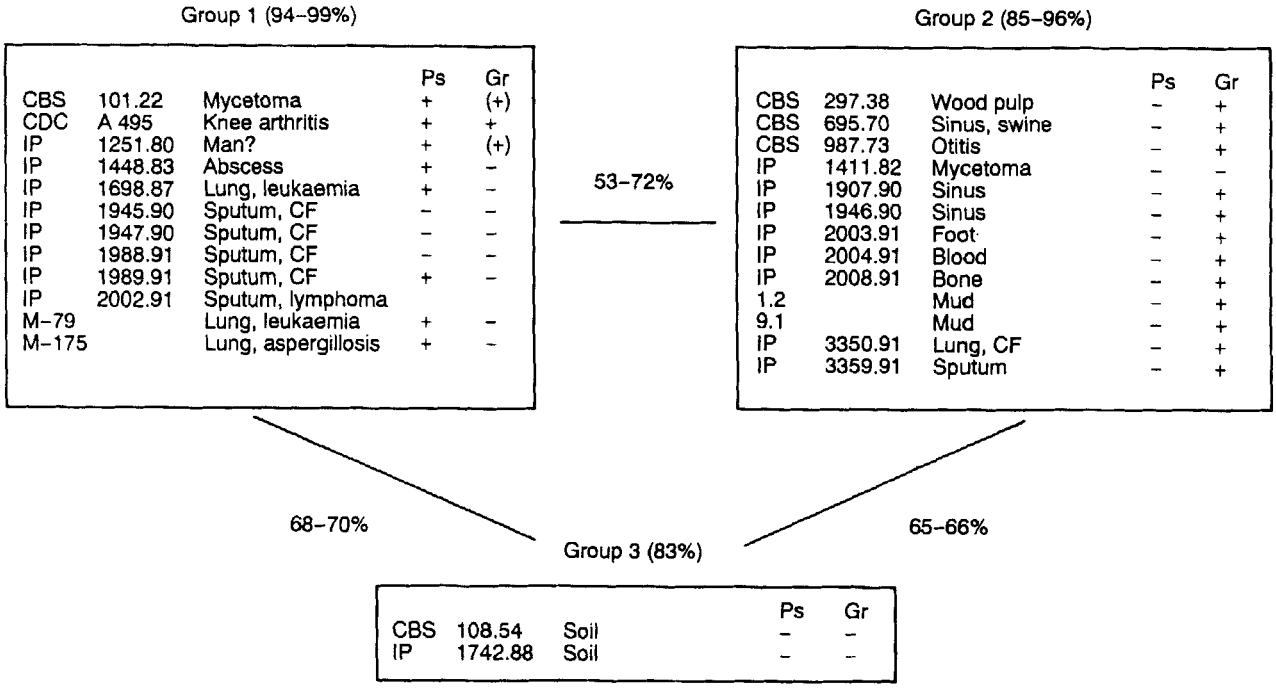

Figure 1. Genomic relationships among isolates of *Pseudoallescheria boydii* and prevalent form of sporulation in addition to *Scedosporium* anamorph. Ps, *Pseudallescheria*; Gr, *Graphium* synnemata; CDC, Centers for Disease Control (Atlanta, GA, USA); CBS, Centraalbureau voor Schimmelcultures, Baarn, The Netherlands; IP, Institute Pasteur (France); CF, cystic fibrosis.

Analysis of metabolic products

Alcohols and short-chain fatty acids, both volatile and non-volatile, were analysed by gas chromatography in cultures grown for 2 weeks at 30 °C in 0.5×, 1×, 2× and 4× concentrations of the standard medium. Hydrogen concentrations were measured as described by Gerritse *et al.* [15]. Protein contents were measured in co-cultures by the method of Lowry *et al.* [16].

Physiology

Comparative nutritional tests were performed in liquid medium at 24 °C by methods standardized by Van der Walt & Yarrow [17]. Gas production in non-agitated liquid 2% glucose/nitrogen base medium was tested with Durham inserts. Tolerances of NaCl and $MgCl_2$ (2%, 5% and 10%) were tested using 5% glucose with nitrogen base as growth medium. Cycloheximide tolerance (0.01, 0.05 and 0.1%) was tested in liquid medium according to Van der Walt & Yarrow [17], as well as on commercial Mycosel agar (BBL; 0.04%), in which case inhibition was measured relative to growth on Sabouraud's glucose agar (SGA) and scored as 'weak' when growth was 50–70%. Diazonium blue B (DBB) reactions were performed according to Hagler & Ahearn [18], with *Cryptococcus neoformans* as control. The presence of urease was tested in slants on Christensen's urea agar and acid production on chalk agar. Gelatin liquefaction was tested in slants at 21 °C and read after 10 min incubation at 4 °C. Proteolysis was also tested in Petri dishes with tryptone/yeast extract/gelatin medium (Brocades), using a $HgCl_2$/HCl solution as indicator. Production of siderophores was tested on CAS agar modified after Schwyn & Neilands [19] [SGA with Chrome Azurol S (Sigma) 60.5 mg, cetyltrimethylammoniumbromide (CTAB) 72.9 mg and 1 mM $FeCl_3$; final pH 4.5]. Widths of pale halos on a purple background were measured. Production of alkaline compounds was measured on the same medium with a Philips PW 9410 digital pH-meter with surface electrodes. Production of phospholipase was tested according to Price *et al.* [20], using egg yolk (Difco) as substrate; *Candida albicans* was used as control.

nDNA/DNA reassociation

Nuclear DNA was isolated from wet cells obtained from 1 l of liquid medium after 3 days of growth. Purification was performed according to Guého & de Hoog [10] using a Gilford Response II spectrophotometer with thermoprogrammer.

Staining

Cell wall glucans and nuclei were fluorescence stained after fixation in 75% ethanol followed by 0.1% Calcofluor white (Sigma), 10% dimethylsulphoxide (DMSO) and a freshly prepared mixture (1:1, v/v) of (a) 2.5 mg of ethidium bromide, 1.2 g of Tris buffer and 0.6 g of NaCl in 100 ml of H_2O (pH 7.4) and (b) 5 mg of mithramycin and 125 mg of $MgCl_2$. Chitin staining was performed using 1% primuline solution. Melanin was stained in a freshly prepared Schmorl's solution [$K_3Fe(CN)_6$ 1%, 2 ml, $FeCl_3$ 1%, 15 ml, aqua dest 3 ml], then with 0.1% nuclear fast red (Merck, Darmstadt, Germany) in 5% $Al_2(SO_4)_3$ and observed in xylol. Amyloid reactions were observed in Melzer's reagent.

Single-spore isolations

Suspensions of conidial cultures were made in 1% Tween 60. Ascomata (cleistothecia) were grown between two cellophane membranes, cut out under a stereomicroscope, vortexed several times in distilled water and squashed under a microscope slide, a series of concentrations of spore suspension in a.d. being incubated on OA at 30 °C.

Results

On the basis of DNA/DNA reassociation experiments, three variants could be distinguished within *Pseudallescheria boydii*. Strains of group 1, which have an intra-group DNA homology of 94–99%, reassociate at 53–72% with members of group 2, which have intra-group DNA homology values of 85–96%. Most strains of group 1 were isolated from the lungs of human patients; none came from the oropharynx. In contrast, four strains of group 2 were isolated from human or animal oropharynx and only two from human lungs. Both groups contain members occurring in nature: a strain isolated from a case of traumatic implantation was found in group 1 and strains from wood pulp and mud were found in group 2. A third, small group, reassociating at 65–70% with members of groups 1 and 2, contained two isolates from soil.

Fresh isolates of members of group 1 usually sporulate abundantly with ascomata. A *Graphium* synanamorph is usually absent, or some small, scattered synnemata are produced. None of the members of group 2 was found to produce cleistothecia. In these cultures, large *Graphium* synnemata were usually present in abundance (Table 1). Analysed members of group 3 lacked a *Graphium* synanamorph.

The form of propagation in *P. boydii* was analysed in strain IP 1251-80 (group 1), which produces cleistothecia and small synnemata in addition to a *Scedosporium* anamorph. Under microaerobic conditions, cleistothecium production is stimulated. Optimal production is obtained at 30 °C using ascospore or conidium suspensions in nutritionally poor media. Production is stimulated when expansion growth is arrested, e.g. at the edge of the Petri dish. Ascomata are formed deep in submersion, later becoming emergent and covered with few setae which actively turn away when approached by an inoculation needle.

Under cellophane the *Scedosporium* anamorph of *P. boydii* consists of single, lateral conidia on hyphae, while in superficial colonies differentiated conidiogenous cells producing conidia repetitively in slimy heads are observed. Small *Graphium* synnemata are found especially on drying parts of the colony, particularly near the edge of slant cultures.

Hyphal cells, conidia and ascospores were invariably found to be uninucleate. Ascospore nuclei stained with difficulty, while conidial nuclei showed intense staining with ethidium bromide/mithramycin. Hyphal cell walls stained well with Calcofluor, but conidia remained unstained. Extracellular clumps containing melanin-like compounds were demonstrated alongside hyphae with Schmorl's solution. No amyloid reactions were noted.

Single-spore isolations were made from ascospores and from *Scedosporium* and *Graphium* conidia of *P. boydii*, IP 1251-80. No significant differences were observed between conidium and ascospore subcultures, all being able to produce cleistothecia. Some isolates formed sectors of higher growth rate, lacking cleistothecia, or formed some scattered cleistothecia only. In the latter case subcultures of these sectors could be restored to their original cleistothecial state when ascospores were transferred.

Strains grown in non-agitated test tubes with Durham inserts showed insignificant gas production. In anaerobic culture, small amounts of carbon dioxide, ethanol, acetate, lactate and succinate were detected (Table 1). Hydrogen and butyrate were often found to be produced in trace amounts; propionate and formate remained absent. The quantities of fermentation products increased linearly with the concentration of the growth medium. No significant differences were observed between strains and species from nature and strains from humans.

All *P. boydii* and *Scedosporium prolificans* isolates grew optimally between 30 and 37 °C. Some other synnematous microascaceous species not known to occur on humans showed no growth at 37 °C.

Most strains of *P. boydii* were unable or weakly able to assimilate L-sorbose, D-arabinose, methyl-α-glucoside, raffinose, melezitose, inulin, glycerol, methanol or higher alcohols and sugar acids (Table 2). Nitrates were assimilated, with the exception of creatine and creatinine. The species differed from *Scedosporium prolificans* by assimilation of sucrose, ribitol, xylitol and L-arabinitol and no or little growth with glycerol. *S. prolificans* could not grow in the presence of cycloheximide, while the sensitivity of *P. boydii* was variable. Little growth of *P. boydii* was observed in 5% NaCl or 10% $MgCl_2$. Urease was consistently produced; DBB reactions were insignificant. No phospholipases or starch-like compounds were detected. In most strains small amounts of siderophores were excreted. Alkaline compounds were generally detected, staining chrome azure SCAS agar deep blue; in the two strains of *P. boydii* group 3 the entire Petri dish showed a neutral or slightly alakaline pH (Table 2).

Discussion

Pseudallescheria boydii is closely related to *Scedosporium prolificans* (= *Sc. inflatum* Malloch & Salkin), judging from morphology of anamorphs and from similarities in nutritional physiology. *P. boydii* is physiologically distinguished from the latter by being able to assimilate sucrose, ribitol, xylitol and L-arabinitol, but it is unable or weakly able to utilize glycerol. Salkin *et al.* [11] and Dixon & Polak-Wyss [21] found the two species to differ in cycloheximide tolerance when tested on Mycosel agar. This was confirmed in our study, at least weak growth being obtained in *P. boydii* and none in *Sc. prolificans* (Table 2). However, when the more sensitive test in liquid medium was applied, growth proved to be strain dependent in *P. boydii* (Table 2). In contrast to Dixon & Polak-Wyss [21], the species were not found to differ in urease test results.

Morphologically, the inflated bases of conidiogenous cells are an important feature to recognize *Sc. prolificans*. Sometimes, however, single lateral conidia are prevalent and therefore the availability of additional physiological tests is useful. Salkin *et al.* [22] contested the identity of *Lomentospora prolificans* Hennebert & Desai and *Scedosporium inflatum* Malloch & Salkin, as supposed by Guého & de Hoog [10] using DNA/DNA reassociation experiments, on the basis of as yet unpublished differences in restriction fragment length polymorphisms. However, the physiological patterns of the

Table 1. Fermentation products (in mM) of *Pseudallescheria*, *Graphium* and *Scedosporium* spp. after 14 days of anaerobic growth, average of duplicate tests proportional to µg of protein produced

Strain	H_2	CO_2	Ethanol	Acetate	Butyrate	Lactate	Succinate
P. boydii variant 1							
IP 1698.87	tr	0.29	0.27	0.05	tr	0.13	0.02
IP 1945.90	tr	0.27	0.27	0.05	tr	0.10	0.02
CBS 101.22	-	0.17	0.16	0.07	tr	0.15	0.02
CDC A-495	tr	0.22	0.19	0.06	tr	0.42	0.07
P. boydii variant 2							
CBS 987.73	-	0.20	0.17	0.10	tr	0.05	0.01
CBS 695.70	-	0.26	0.21	0.09	0.01	0.27	0.03
CBS 297.38	tr	0.35	0.35	0.03	tr	0.16	0.02
9.1	tr	0.23	0.20	0.08	tr	0.14	0.02
1.2	tr	0.19	0.19	0.08	tr	0.13	tr
P. boydii variant 3							
CBS 108.54	-	0.23	0.24	0.07	tr	0.13	0.01
IP 1742.88	tr	0.16	0.15	0.04	tr	0.04	tr
Sc. prolificans							
467.74	tr	0.13	0.15	0.06	tr	0.12	0.01
114.90	tr	0.30	0.26	0.07	tr	0.11	tr
G. cuneiferum							
779.85	tr	0.26	0.29	0.04	tr	0.14	0.03
Sc. chartoikoon							
131.14	tr	0.15	0.14	0.08	tr	0.13	tr
357.36	-	0.37	0.20	0.29	tr	0.11	0.01
G. tectonae							
127.84	tr	0.44	0.49	0.08	tr	0.04	tr

tr, trace.

two type strains, CBS 114.90 and CBS 467.74 respectively, are remarkably similar (Table 2).

The molecular variants of *P. boydii* found by Guého & de Hoog [10] are physiologically indistinguishable. Most strains tolerate 2% NaCl and 5% $MgCl_2$. Kirk [23] obtained optimal growth in seawater (1.7–2.8% salinity). He found that cleistothecium formation was hampered and synnema production stimulated at higher salt concentrations. This underlines the slightly osmophilic nature of the synnematal morph, which develops particularly on drying parts of colonies.

All tested microascaceous species are able to grow anaerobically and produce similar small amounts of fermentation products (Table 1). No or only slight fermentation is observed in standing test tubes with Durham inserts (Table 2). Submerged growth is strictly hyphal and mostly without any macroscopical trace of melanin; sporulation is relatively abundant. *P. boydii* and *Sc. prolificans* are able to produce conidia in submersion *in vitro*, as well as in host tissue [12, 24, 25], which is uncommon in human pathogenic hyphomycetes [26]. A single conidium is formed on each conidiogenous cell, as observed in agar cultures under cellophane membranes. In aerobic culture, conidium formation is repetitive, conidia being produced in heads [27]. The submerged conidiation possibly enables *P. boydii* to disseminate haematogenously. The fungus often rapidly invades the central nervous system [3]. Judging from its siderophore activity, it is iron dependent. In contrast to serum, CNS tissue contains free iron, which may partly explain the neurotropic behaviour of the fungus. Siderophores are also found in the equally neurotropic black yeast *Exophiala dermatitidis* [28] and are insignificant in the superficial agent *Hortaea werneckii* [29].

Single-spore isolations, whether from ascospores or from the two types of conidia, resulted in identical, ascosporulating cultures. Lingappa & Lingappa [30] presented evidence that ascospores are more heat shock resistant than conidia. The entire thallus of the fungus is uninucleate. Croziers were not observed, asci being directly produced from ascogonia. The fungus is thus truely homothallic, each uninucleate cell being able to produce the sexual form of propagation. Cleistothecial cultures showed recurrent production of sectors with different expansion growth and lacking cleistothecia, confirming earlier observations by El-Ani [31]. Occasionally some scattered cleistothecia

Table 2. Physiological patterns of 12 strains of *Pseudallescheria boydii* and *Scedosporium prolificans*

	P. boydii							*Sc. prolificans*				
	Variant I				Variant II			Variant III				
	1251.80	1698.87	101.22	297.38	695.70	1.2	9.1	108.54	1742.88	1913.90	114.90	467.74
Fermentation												
D-Glucose	–	–	–	–	–	–	–	–	–	–	–	–
Assimilation												
D-Glucose	+	+	+	+	+	+	+	+	+	+	+	+
D-Galactose	+	+	–	+	+	+	+	+	+	+	+	+
L-Sorbose	–	–	–	w	–	–	–	w	w	w	–	–
D-Glucosamine	–	+	+	–	–	w	w	w	w	–	w	–
D-Ribose	–	+	+	+	+	–	+	+	w	–	w	
D-Xylose	+	+	+	+	+	+	+	+	+	+	+	+
L-Arabinose	+	+	+	+	+	+	+	+	+	+	+	+
D-Arabinose	–	w	w	w	w	–	–	w	–	–	–	–
L-Rhamnose	–	w	w	+	+	+	w	+	+	+	+	+
Sucrose	w	+	+	+	+	+	+	+	+	–	–	–
Maltose	+	w	+	+	+	+	+	+	+	+	+	+
α,α-Trehalose	+	w	+	+	+	+	+	+	+	+	+	+
m-α-Glucoside	–	–	–	–	–	–	–	–	–	–	–	–
Cellobiose	+	+	+	+	+	+	+	+	+	+	+	+
Salicin	+	+	+	+	+	+	+	+	+	+	w	w
Arbutin	+	+	+	+	+	+	+	+	+	+	+	+
Melibiose	+	–	+	–	–	–	–	–	–	w	–	–
Lactose	w	w	w	w	w	+	w	–	–	w	–	w
Raffinose	w	–	–	–	–	–	–	–	–	–	–	–
Melezitose	–	w	–	–	–	–	–	–	–	–	–	–
Inulin	–	–	–	–	–	–	–	–	–	–	–	–
Soluble starch	+	+	+	+	+	+	+	+	+	+	+	+
Glycerol	–	–	w	–	w	–	w	–	–	+	+	+
Erythritol	–	–	–	–	–	–	–	–	–	–	–	–
Ribitol	+	+	+	+	+	+	+	+	+	–	–	–
Xylitol	w	w	+	+	+	+	+	+	+	–	–	–
L-Arabinitol	+	+	+	+	+	+	+	+	+	–	–	–
D-Glucitol	w	w	w	–	w	–	+	w	w	+	+	+
D-Mannitol	+	–	w	w	w	–	+	w	w	+	+	+
Inositol	–	–	–	–	–	–	+	–	–	+	w	–
Gluc.-δ-lactone	w	–	–	–	–	–	–	–	–	–	–	–
D-Gluconate	+	–	–	–	–	–	–	–	–	–	–	–
D-Glucuronate	+	w	–	–	–	–	–	–	–	–	–	–
D-Galacturonate	–	–	–	–	–	–	–	–	–	–	–	–
DL-Lactate	–	–	–	–	–	–	–	–	–	–	–	–
Succinate	–	–	–	–	–	–	–	–	–	–	–	–
Citrate	–	–	–	–	–	–	–	–	–	–	–	–
Methanol	–	–	–	–	–	–	–	–	w	–	–	–
Ethanol	+	w	+	+	+	+	+	+	+	+	+	+
Nitrate	+	+	+	+	+	+	+	+	+	+	+	+
Nitrite	+	+	+	+	+	+	+	+	+	+	+	+
Ethylamine	+	+	+	+	+	w	+	+	+	w	+	+
L-Lysine	+	+	+	+	+	+	+	+	+	+	+	+
Cadaverine	+	+	+	+	+	+	+	+	+	+	–	w
Creatine	–	–	–	–	–	–	–	–	–	–	–	–
Creatinine	–	–	–	–	–	–	–	–	–	–	–	–
Remaining tests												
24 °C	2.3*	2.4	2.9	2.7	2.4	2.7	2.5	3.3	2.5	2.0	1.6	2.5
30 °C	3.9	4.0	4.2	4.3	3.0	3.8	3.8	3.8	4.4	2.8	2.8	2.7
36 °C	2.8	3.9	1.1	4.5	1.5	0.7	0.8	0.6	2.2	1.8	3.7	3.0
42 °C	0.3	0.1	–	1.5	0.2	0.4	–	–	–	1.0	0.5	0.7
2% NaCl	+	w	+		+	w	+	+	w	+	+	+
5% NaCl	w	–	w	–	–	–	–	–	–	w	+	+
10% NaCl	–	–	–	–	–	–	–	–	–	–	–	–
2% $MgCl_2$	+	+	+	+	+	w	+	+	+	+	+	+
5% $MgCl_2$	+	w	+	w	w	w	+	+	w	+	+	+

Table 2. (*continued*)

	P. boydii							*Sc. prolificans*				
	Variant I				Variant II			Variant III				
	1251.80	1698.87	101.22	297.38	695.70	1.2	9.1	108.54	1742.88	1913.90	114.90	467.74
10% $MgCl_2$	w	–	w	–	–	–	w	w	–	w	w	w
0.05% Cycloheximide	–	–	+	–	+	–	–	+	+	–	–	–
0.1% Cycloheximide	–	–	+	–	w	–	–	+	+	–	–	–
Mycosel	w	w	+	+	+	w	w	+	+	–	–	–
Starch production	–	–	–	–	–	–	–	–	–	–	–	–
Urease	w	+	+	+	+	+	w	+	+	+	+	+
DBB	–	–	–	–	–	w	w	–	–	–	w	–
Phospholipase	–	–	–	–	–	–	–	–	–	–	–	–
Gelatin liquefaction	+	+	+	+	+	+	+	+	+	+	+	+
Acid production	–	–	–	–	–	–	–	–	–	–	–	–
Siderophore halo	1	3	5	2	2	ent	11	5	2	ent	2	ND
pH on CAS margin	5.6	4.5	6.7	4.6	7.9	8.7	8.2	8.0	7.3	8.6	5.2	ND
pH on CAS centre	5.6	7.5	6.7	7.9	8.0	8.7	8.3	8.0	7.9	8.6	5.2	ND

*Daily expansion growth in mm.
ent, entire dish homogeneously discoloured.

were formed in a very late stage. A possible explanation is that cells are normally diploid. Local spontaneous haploidization may occur, growing out with anamorph sectors. Later the original condition may be restored by local somatic diploidization. In contrast, Corlett [32] supposed that the fungus would be a haplont; mitosis, karyogamy and meiosis would all take place within a single cell, the ascogonium.

DNA/DNA reassociations confirmed the existence of several molecular variants within the species *P. boydii* [10]. Isolates with intermediate DNA homology broadly correspond with sources of isolation and with morphology. Members of the cleistothecial variant (1) have mainly been isolated from deep locations in patients with impaired, innate cellular immunity, while the synnematal variant (2) is particularly involved in otitis or sinusitis of various species of warm-blooded animals. In present-day clinical practice, the two variants are encountered with equal frequencies. In older literature, however [24], the cleistothecial variant was much less common. Gordon [33] isolated mainly cleistothecial strains from soil, possibly corresponding to our variant 3, while strains with synnemata were rare. It may thus be supposed that variant 1 in particular, taking advantage of human natural immunodeficiency, is emerging.

In the literature, *P. boydii* is reported from substrates rich in nutrients, mostly soil [24, 34], soil enriched by manure [6], agricultural and garden soil [23, 35], potted plant soil [36] or manure [37, 38]. The isolates from water mostly came from sewer [26] or heavily polluted water or mud in stagnant ponds [2, 39]. Locations under tidal waste also fit this category [23, 40, 41]. Patients who have nearly drowned in such waters frequently develop fatal, neurotropic, disseminated mycoses [42].

In nature, *Pseudallescheria boydii* occurs in nutrient-rich, poorly aerated environments, such as polluted water. From these environments it may colonize cavities inside animal hosts, as is proven by its asymptomatic occurrence, e.g. in lizard lungs [43], where it lives on epithelial or inhaled debris. In patients with leukaemia or cystic fibrosis, biomass production may reach detectable levels. The synnematous variant is slightly osmophilic and is hence relatively often found in debris accumulated in the oropharynx.

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