

REVIEW ARTICLE

The spectrum of opportunistic filamentous fungi present in the CBS culture collection

Das Spektrum opportunistischer Fadenpilze in der CBS-Sammlung von Pilzkulturen

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Summary. The mould collection of the Centraalbureau voor Schimmelcultures, Baarn, The Netherlands, was screened for isolates originating from warm-blooded animals. The range of species indicates that distribution of clinically relevant, pathogenic or opportunistic strains over the fungal kingdom is non-random. Some opportunistic fungi possess adaptations to life under hostile environmental conditions, enabling them to survive inside the human body. Presence of melanin or carotene seems to be an important virulence factor. Opportunistic fungi which sporulate in submersion are able to disseminate or cause severe local mycoses when the aspecific immune system of the host is impaired. Mycoses caused by a few dimorphic fungi, mostly in their natural ecological niche living in association with vertebrates, are promoted by specific immune deficiencies.

Zusammenfassung. Die Pilzsammlung des Centraalbureau voor Schimmelcultures, Baarn, Niederlande, wurde auf Herkunft der Isolate untersucht. Die Verteilung klinisch relevanter Stämme über das Pilzreich ist nicht willkürlich. Opportunistische Pilze sind an extreme ökologische Bedingungen angepaßt und können deswegen im menschlichen Körper überleben. Melanin und Carotin werden als wichtige Virulenz-

Faktoren betrachtet. Einige opportunistische Pilze disseminieren oder verursachen ausgedehnte Läsionen, wenn das unspezifische Immunsystem geschädigt ist. Nur wenige, meistens dimorphe Pilze, welche in ihrer natürlichen ökologischen Nische an Wirbeltiere gebunden sind, werden erst infektiös bei geschwächter spezifischer Immunabwehr des Wirtes.

Introduction

The mould collection of the Centraalbureau voor Schimmelcultures comprises about 28,000 living strains, some of which date back to the previous century. Over a period of 85 years CBS staff members have tried to obtain living strains of each species which can be grown in culture, with the aim of displaying the diversity of the fungal kingdom. In most cases up to five strains per species were enlisted. Only few species were represented by a larger series of taxonomically similar strains.

Enlistment of human pathogenic strains was mostly judged by workers in the medical mycology section. Thus casually pathogenic strains of species which were otherwise known as saprophytes were also accepted. In addition, through the CBS identification service a wide array of clinically relevant fungi is seen. This provides an opportunity to establish the distribution of clinically significant strains and species over the fungal kingdom. Thus may be verified whether (a) some fungal groups contain pro-

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portionally more opportunists than others, or (b) opportunists show a random distribution. The latter option would support the suggestion that immunodeficient patients may be colonized by practically any, otherwise saprophytic, fungus [1]. Alternatively (a), even immunocompromised hosts must be regarded as difficult substrata for fungal growth, and thus would require fungi well-equipped with virulence factors.

Materials and methods

Strains analysed were those listed in the CBS List of Cultures [2]. Actinomycetes and most yeasts were excluded. Numbers of strains from living, warm-blooded vertebrates were counted (815) as well as those from other sources (22,372). Vertebrate sources of isolation were recorded according to the following categories: superficial, subcutaneous, sinusoid, pulmoneous and systemic. If information was insufficient, the location was listed as "miscellaneous". No distinction was made between pathogenic and opportunistic fungi. The following main taxa or groups were distinguished: Zygomycota, Basidiomycota, Eurotiales, Onygenales, remaining hyaline Ascomycetes, remaining melanized Ascomycetes, hyaline Hyphomycetes, melanized Hyphomycetes, Coelomycetes. Fungi were attributed to these categories according to the 'Dictionary of Fungi' [3].

Results

Of the 23,187 strains screened, 3.5% (815 strains) originated from living warm-blooded vertebrates including humans. Figure 1 shows that Eurotiales, Onygenales, melanized Hyphomycetes and, to a lesser extent, Zygomycetes and non-melanized Hyphomycetes contain a greater than average number of strains from vertebrate origin. Figure 2 gives an overview of the prevalent locations of these strains. Most of the medical Zygomycetes have been derived from sinuses, while the Eurotiales mainly originate from pulmoneous and sinusoid locations. The Onygenales contain two main groups, viz. superficial fungi (dermatophytes) and species causing systemic mycoses (*Histoplasma*, *Coccidioides* etc.). Hyaline hyphomycetes and particularly melanized hyphomycetes are often involved in subcutaneous mycoses. The hyaline filamentous yeast genus *Geotrichum* is often found in lungs.

The main genus of Zygomycota containing clinically relevant strains is *Rhizopus*, while in the

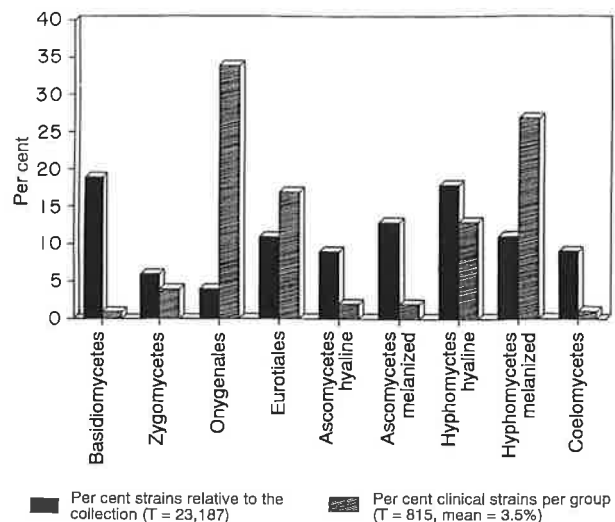


Figure 1. Percentages of clinical strains deposited in CBS collection relative to total number of strains.

Eurotiales *Aspergillus* is preponderant. Among the hyphomycetes and filamentous yeasts, the hyaline genera *Acremonium*, *Geotrichum* and *Phialemonium* and the melanized genera *Cladosporium*, *Exophiala*, *Phialophora* and *Pseudallescheria* are of particular significance (Table 1). The melanized group shows a somewhat wider taxonomic diversity, 33% of the human- and animal-associated strains belonging to genera other than the ones listed ('miscellaneous'; Table 1).

Discussion

The spectrum of fungi with recognized clinical relevance is gradually expanding [e.g. 4, 5]. The array of species deposited in the CBS culture collection demonstrates this diversity, but does not reflect the mycological flora currently identified in clinical laboratories. Mostly the more uncommon and peculiar strains were sent to the CBS for identification and thus had a larger chance of enlistment. The growing awareness of clinicians for fungal infections has a further impact on the number of opportunistic species registered. On the other hand, nearly all species ever reported to be involved in mycoses are represented in the collection, though many of the opportunists are from non-vertebrate sources. The small number of strains per species maintained in the collection sometimes hampered a clear establishment of some tendencies we witness in our identification service. True pathogens are recognizable by the fact that nearly all strains of that species are of vertebrate origin, while opportunists were generally derived from sources other than vertebrates.

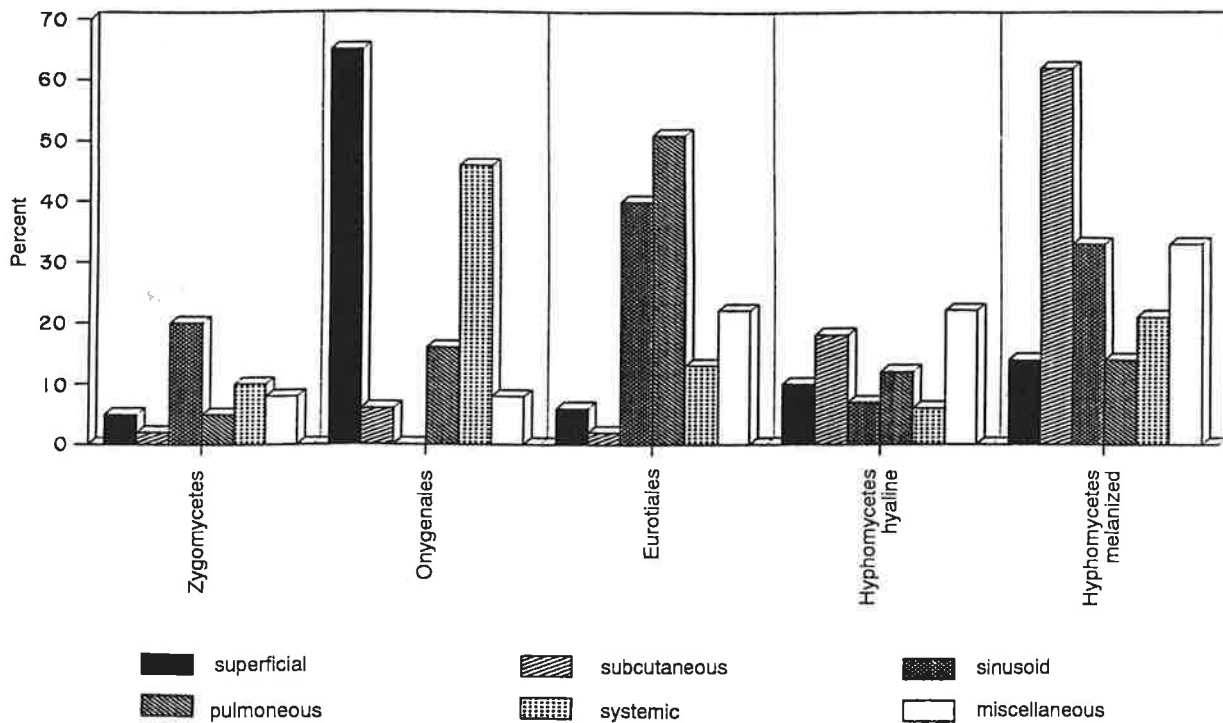


Figure 2. Origin of clinical isolates belonging to major fungal groups. Numbers are relative to total number of isolates from each source.

Table 1. Major genera of opportunistic fungi per main group with number of medical isolates	
Hyaline Hyphomycetes (T=107)	(49) <i>Acremonium</i> (21) <i>Geotrichum</i> (12) <i>Phialemonium</i> (6) <i>Beauveria</i> (6) <i>Fusarium</i> (5) <i>Scytalidium</i> (8) miscellaneous
Melanized Hyphomycetes (T=179)	(46) <i>Exophiala</i> (23) <i>Cladosporium</i> (20) <i>Phialophora</i> (12) <i>Pseudallescheria</i> (9) <i>Fonsecaea</i> (6) <i>Hortaea</i> (4) <i>Madurella</i> (59) miscellaneous

The distribution of the more recent, clinically relevant strains over the fungal kingdom is clearly non-random; consequently option (b), concerning the view that practically any fungi might cause mycoses in the immunocompromised host (see 'Introduction'), is refuted. The occurrence of opportunistic mycoses is partly determined by a decreased immune response of the host, but also by properties of the respective fungi. Pathogenicity and opportunism require several virulence factors. Different combinations of factors are responsible for differences in pathogenesis. Therefore, clinical prevalences found during the screening of CBS strains are supposed to reflect

differences in sets of virulence factors in the analysed taxa.

Virulence factors

Thermotolerance. Ability to grow at temperatures above 35 °C is a basic requirement for any potential pathogen of warm-blooded animals. Of the Eurotiales, which produce conidia which are easily inhaled, the thermotolerant members of the genus *Aspergillus* comprise a rather wide spectrum of clinical isolates. In contrast, opportunists are rarely found among the equally ubiquitous, but mostly mesophilic, genus *Penicillium*; clinically relevant CBS isolates nearly all belong to a single, pathogenic species, *P. marneffei*. Thermotolerance is also found in species currently regarded as saprophytes or plant pathogens, such as *Alternaria*.

Nitrogen and vitamin requirements. Most saprophytes are able to utilize nitrate, while numerous pathogens require more complex substrates. Dermatophytes and their allies utilize keratin as a source of nitrogen and are often unable to synthesize a number of vitamins. Several species of yeasts occur endogenously in nutrient-rich mucous membranes. Among the fungi occurring in dung, manure or guano, a relatively large number of pathogens and opportunists is noted (e.g. *Cryptococcus*, *Histoplasma*, *Pseudallescheria*). In addition, fungi growing on chitinous substrates, viz. entomogenous and mycoparasitic fungi, comprise

some opportunists on mammals (e.g. *Beauveria*, *Engyodontium*, Entomophthorales).

Osmotolerance. Several vertebrate pathogens, such as *Coccidioides immitis*, are able to survive in environments with low water activity, which compensates for their lack of competitive ability [6]. *Aspergillus* species other than *A. fumigatus* and *A. flavus* are particularly found in ears; this may be explained by their osmophilic nature. Several species normally occurring in substrates with raised electrolyte contents are found on humans as a second substrate of choice (Table 2). Locations are either cutaneous (*Hortaea*, *Phialophora*) or pulmoneous (*Arachniotus*, *Exophiala*).

Microaerophily. Tissue invasion requires propagation under restricted availability of oxygen. Most monomorphic filamentous fungi produce sterile hyphae in tissue, and thus remain restricted and localized. *Pseudallescheria boydii* is able to sporulate in submersion and grows under anaerobic conditions [7]. Thus, it may disseminate haematogenously. The importance of the species as an agent of systemic mycoses is increasing, while in the past it was mainly known from mycetomes.

Yeast-like growth. Most opportunistic fungi remain localized after infection, causing single-organ mycoses characterized by non-sporulating, hyphal growth in tissue. It may be speculated that only those species that have the possibility of haematogenous or lymphogenous dissemination are able to cause sepsis. This is, outside the 'classical' agents of systemic mycoses, particularly known in yeasts and dimorphic fungi such as black yeasts (*Exophiala* [8]) or *Sporothrix* [9], in fungi with arthroconidia (*Geotrichum* [10]) and in fungi that are able to sporulate under microaerobic conditions (*Pseudallescheria* [7]; see previous section).

Pigmentation. Presence of melanin and carotene is advantageous to life in hostile natural ecological niches; resistance to conditions of drought or UV-

irradiation is remarkable [11]. Melanin and carotene are able to neutralize oxygen radicals, enabling the fungus to overcome a major factor of aspecific defence. This might explain why entirely hyaline hyphomycetes, i.e. with little or no melanin or carotene, are somewhat under-represented (Fig. 1).

Classification of opportunistic fungi

Basidiomycota. Very few human-associated Basidiomycetes are known (Fig. 1), except for some yeasts (*Cryptococcus*, *Malassezia*; not shown). Spores of the thousands of species of the order Agaricales (mushrooms) are commonly massively inhaled, but are only occasionally found in sputum and rarely cause various systemic disorders (Fig. 2). The latter fungi are mostly identified as *Coprinus* spp. Also common air-borne, plant pathogenic rusts and smuts are clinically insignificant. Only the smut anamorph genus *Tilletiopsis* is found relatively often.

Zygomycota. Representatives of the insect-pathogenic order Entomophthorales (*Conidiobolus*, *Basidiobolus*) can provoke specific, clearly recognizable clinical pictures. The remaining medically significant taxa, belonging to the order Mucorales, cause mutually similar disorders. Most Mucorales are inhaled and may cause sinusitis or fulminant pulmoneous foci in patients with ketoacidosis or impaired immunity [12]. The strong iron-dependence of such fungi explains their marked growth in blood vessels [13].

Eurotiales. This order contains species with massive production of dry, air-borne conidia (e.g. *Aspergillus*, *Penicillium*). Propagules are consequently easily inhaled, and are mainly found in sinus, trachea and lung (Fig. 2). Thermotolerant species, such as *A. fumigatus* and *A. flavus*, are major agents of pulmoneous mycoses in debilitated patients. Osmophilic *Aspergillus* species, such as *A. niger*, are particularly found in ears. Cases of invasive aspergillosis are restricted to patients with neutrophilic granulocyte dysfunction, whereby the portal of entry is invariably pulmoneous or sinusoid. *Penicillium marneffei* causes systemic mycosis due to its ability to grow inside macrophages. The species seems to have developed this ability as an adaptation to growth inside bamboo rats [14].

Onygenales. This order of Ascomycetes contains the dermatophytes (*Epidermophyton*, *Microsporum*, *Trichophyton*) as well as the 'classical' causative

Table 2. Species isolated from salty or animal substrates

Species	Number of isolates		
	salt	animal	other
<i>Arachniotus littoralis</i>	4	1	—
<i>Exophiala dermatitidis</i>	2	14	—
<i>Hortaea werneckii</i>	2	10	2
<i>Phialophora littoralis</i>	2	2	1

agents of systemic mycoses (*Blastomyces*, *Histoplasma*). Teleomorphs are hitherto unknown in *Coccidioides* and *Emmonsia*, but these genera are frequently speculated to be of Onygenalean affinity. The order exhibits a marked tendency towards specialization on the vertebrate host. This is reflected in the CBS collection by the fact that in numerous taxa all strains held have come from human or animal material. Nearly all species of the order are able to degrade keratin, such as skin, feathers or hair [15]. Thirty-four per cent of the CBS strains were isolated from live hosts. The dermatophytes are restricted to superficial locations (Fig. 2). Despite their vertebrate association, they are unable to grow subcutaneously. The highly specialized, 'classical' agents of systemic mycoses (*Coccidioides*, *Histoplasma* etc.) possess specific mechanisms to overcome phagocytosis, such as formation of giant cells or intracellular growth within macrophages. In their natural ecological niche they are associated with animals by which they are dispersed within endemic areas [6]. In humans they mostly cause asymptomatic infections, but endogenous reactivation may take place in debilitated hosts or in patients with impaired T-cell function [15].

Remaining Ascomycetes, including Hyphomycetes. This group contains the majority of the opportunistic species. Despite the apparent absence of specialization on warm-blooded animals in many groups, individual strains are frequently involved in subcutaneous and also in systemic mycosis (Fig. 2). This is particularly noted in those species which contain melanin (Dematiaceae, black yeasts) or carotene (*Acremonium*, *Fusarium*). The portal of entry of melanized fungi is mostly subcutaneous after trauma with contaminated material. Dematiaceae (*Drechslera*, *Curvularia*) occur as pathogens on grasses, but can be inhaled and may cause sinusitis [17], commonly noted in cattle as 'helminthosporiosis'. Only few melanized fungi enter via the lungs. This has been supposed for *Cladosporium trichoides* [18], *Exophiala dermatitidis* [19] and *Pseudallescheria boydii* [20]. When systemic, these fungi are all neurotropic, possibly due to their somewhat hygrophobic nature which causes a predilection for lipid-rich nervous tissues.

Host response

Fungal infections in the immunocompetent host are controlled by cellular, specific and aspecific immune systems, humoral immunity being less significant. Albeit the two immune systems are highly interactive, immunodepression favours some fungi more than others, depending on which

mechanism is impaired. Thus, the significance of each of the two cellular immune systems in defence against fungi can be surmised statistically from clinical strains in which information on the underlying immune deficiency was given. CBS collection data confirm that only few fungi are associated with specific immune deficiency (e.g. AIDS), while cases of aspecific immune deficiency (e.g. myeloid leukaemia, neutropenia) show a much wider spectrum of fungi, otherwise mostly known as saprophytes. Some examples are listed in Table 3. Most opportunistic saprophytes are monomorphic. The pathogens generally have an alternating life cycle, comprising a passage through animals [13, 21] or they live in close association with animals (Table 3).

Aspergillus fumigatus and *Penicillium marneffeii* on the one hand and *Trichosporon* and *Cryptococcus* on the other are rather close taxonomically, the latter two also sharing similar antigens [22]. Nevertheless, they are controlled by different immune systems. Therefore, it may be speculated that the capacity to take advantage of deficiencies in either aspecific or specific immunity is a relatively small step from an evolutionary point of view.

Major types of mycosis

Superficial mycoses. Fungi of this group are either keratinophilic (dermatophytes) or osmotolerant (black yeasts). Lipophilic fungi are mainly found among basidiomycetous yeasts. Agents of superficial mycoses form expanding lesions in the outer keratinous layers. Invasive growth is limited, the fungus provoking heavy inflammation when entering deeper skin layers. In the CBS files on these strains generally no reference is made towards any underlying disease or immune deficiency.

Subcutaneous mycoses. Etiologic agents are traumatically introduced with contaminated material. The fungi concerned normally occur as saprophytes in nature. If they possess adaptations

Table 3. Fungi associated with deficiencies in host defence

Aspecific immunity:	Specific immunity:
<i>Aspergillus</i>	<i>Blastomyces</i>
<i>Candida</i>	<i>Candida</i>
<i>Geotrichum</i>	<i>Coccidioides</i>
<i>Pseudallescheria</i>	<i>Cryptococcus</i>
<i>Scytalidium</i>	<i>Histoplasma</i>
<i>Sporothrix</i>	<i>Penicillium</i>
<i>Trichosporon</i>	
Zygomycetes	

to survival under stress conditions, such as heat or drought, they may also be able to survive inside host tissue and cause chronic, localized mycoses. Aspecific host reactions are violent, e.g. suppuration and fibrosis in mycetomes, hyperkeratosis in chromomycoses or collagen encapsulation in mycotic cysts. In the case of impaired aspecific immunity, the invaded area may increase considerably or the fungi may disseminate.

Pulmonary and sinusoid mycoses. Among the fungi that are inhaled, few are able to enter the blood stream of immunocompetent hosts and thus few may be regarded as true pathogens. Among these are the 'classical' systemic fungi. Endogenous reactivation and dissemination takes place particularly in the case of impaired specific immunity. Other fungi colonize and invade sinus, trachea or lungs only in the case of marked underlying diseases or impaired aspecific immunity, especially ketoacidosis, leukaemia or cystic fibrosis.

Disseminated mycoses. Dissemination is restricted to those species which are able to form propagules in submersion. This is the case in dimorphic fungi ('classical' systemic fungi, yeasts, black yeasts, *Sporothrix*), but also in fungi lacking a yeast-like phase (*Geotrichum* and *Pseudallescheria*). Depending on the species, septic dissemination can take place after impairment of either specific or aspecific immunity in species which form yeast cells or arthroconidia or sporulate in submersion.

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