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Evolution of black yeasts: possible adaptation to the human host

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Abstract

Ascomycetous black yeasts show adaptations to a wide array of environmental conditions. Dothideaceous black yeasts are mostly found on plant leaves, while among herpotrichiellaceous species there are numerous opportunists on humans.

Factors which are of ecological significance include the presence of melanin and carotene, formation of thick cell walls and meristematic growth, presence of yeast-like phases, presence of additional forms of conidiogenesis, thermo- and osmotolerance, adhesion, hydrophobicity, production of extracellular polysaccharides, siderophores and acidic or alkaline secondary metabolites.

The potential pathogenicity of a species is partly determined by its natural ecological niche. Dothideaceous black yeasts are osmotolerant rather than pathogenic. Herpotrichiellaceous black yeasts probably have low competitive ability and are found in rather special niches as secondary saprophytes, e.g., on bacterial mats, on other fungi or in poor environments. Some species possibly utilize animal vectors for dispersal.

General characteristics of black yeasts

The term 'black yeasts' indicates those melanized groups of fungi of which several representatives are able to reproduce by unicellular growth. This property is found in a few genera of basidiomycetes related to the Filobasidiales (De Hoog 1979). The majority of black yeasts, however, belong to divergent orders of ascomycetes (De Hoog & McGinnis 1987).

An ascomycetous 'black yeast' may or may not have a yeast-like phase, depending on the ecological niche it inhabits. For example, certain species which occupy dynamic environments such as intermittently drying pools of water, reproduce as a yeast as long as submersed growth is possible. When conditions change, a more hydrophobic, hyphal stage may be formed with erect conidiophores, holding the conidia up and away from the substrate for air dispersal. Other, related species which occupy non-aqueous ecological niches possess a thallus that is hydrophobic in all stages of development. As a consequence, closely related members of 'black yeasts' may be highly dissimilar morphologically. Thus, if the 'black yeasts' are taken as a taxonomic rather than a morphogenetic group, yeast-like growth is not a prerequisite for membership in the group.

A few species are known to have teleomorphs which mostly belong to the Pseudosphaeriales, an order of bitunicate ascomycetes. The main teleomorph genera are *Capronia* (family Herpotrichiellaceae; Müller et al. 1987) and *Discosphaerina*, *Guignardia*, *Dothiora*, *Pringsheimia* and *Sydowia* (family Dothideaceae; Sivanesan 1984).

Taxonomy at the family level: main groups of black yeasts and their medical significance

The teleomorph relationships in 'black yeasts' (Herpotrichiellaceae vs. Dothideaceae) are supported by characters of the anamorphs including thallus development and karyology (Takeo & de Hoog 1991). In addition, these families are delimited on the basis of their coenzyme Q systems. Members of Dothideaceae, as well as anamorphs classified in *Aureobasidium* and *Hormonema* have Q-10(H_2) (Sugiyama et al. 1987). Q-10 has been found in most *Exophiala* species, but species of *Capronia* (Herpotrichiellaceae), the teleomorph genus of *Exophiala*, are variable for this character (Yamada et al. 1989). *Aureobasidium* and related organisms are mostly phyllosphere fungi, living in environments rich in sugars (Cooke 1959). Their abundance is promoted by acid rain (Helander & Rantio-Lehtimäki 1990) and it has been suggested that environmental factors are responsible for their increased occurrence as opportunistic plant pathogens, particularly on conifers (Funk 1985). In contrast, opportunistic fungi on humans are nearly exclusively found among members of the Herpotrichiellaceae and associated anamorphs.

On the basis of the above parameters, the genus *Hortaea* is regarded as being close to the plant-inhabiting genus *Hormonema*. However, earlier workers (McGinnis et al. 1985) classified *Hortaea werneckii* in *Exophiala* because it caused a human mycosis known as 'tinea nigra'. Tinea is a superficial disorder characterized by blackish stains on the palm of the hand (Rippon 1988). De Hoog & Gerrits-van den Ende (1992) found *H. werneckii* to exhibit a complicated life cycle, apparently suited for survival in salt water as well as under conditions of drought. It was therefore assumed that the fungus may naturally occur in tidal pools subject to intermittent drying and that patients are infected while playing at the beach. Only subjects having elevated salt levels on their hands would develop a 'tinea'. De Cock (1993) and Uijthof et al. (1993) distinguished seven populations in *H. werneckii* on the basis of mitochondrial and nuclear DNA patterns, respectively. These populations included strains from nature as well as strains from 'tinea'. An evolutionary adaptation to life on human skin therefore seems unlikely. Rather, an ecological similarity may be supposed between salty keratin and evaporating seawater. The fungus should therefore be regarded as osmotolerant (Tanaka et al. 1990) rather than humanpathogenic, as are the remaining species of the Dothideaceae.

Clinically relevant herpotrichiellaceous black yeasts

Herpotrichiellaceous black yeasts are etiologic agents of rather divergent human mycoses. Mycoses after traumatic inoculation are independent of the immune status of the patient. Characteristic clinical pictures are chromoblastomycosis, phaeohyphomycosis, phaeomycotic cysts and mycetomes. Inhalation mycoses may take a rather different course, partly dependent on the general condition and immune status of the patient. Main agents of systemic mycoses are *Cladosporium trichoides* and *Exophiala dermatitidis*. The latter species occurs subclinically in patients with cystic fibrosis or with a previous history of pulmoneous disorder; a transient pneumonia may be noted (Barenfanger et al. 1989). In the case of severe impairment of natural immunity, e.g., after major surgery, neurotropic dissemination may occur which is often fatal (Matsumoto et al. 1984).

Natural niches and virulence factors

There are only a few fungal groups which cause such frequent and dissimilar human mycoses as seen in black yeasts, without being truly animal-dependent. The apparent ease with which herpotrichiellaceous black yeasts cause opportunistic infections favours the hypothesis that these species share factors, needed for growth and dispersal in their natural niche, which also facilitate survival inside the vertebrate body. The latter is exemplified by the chronic character of mycoses after traumatic inoculation. With a general ability to survive inside animal tissue, only relatively few additional factors are needed for causing specific types of mycoses.

Members of Herpotrichiellaceae are secondary saprophytes, i.e., dependent on material that has been partly digested by first invaders (Munk 1956). They seem to have little competitive ability and need to be isolated using selective media (De Hoog & Haase 1993). They have solved this problem along different lines: either by choice of uncommon, specific substrates, or by choice of habitats that suppress competitors. In the latter type, two habitats are noted, viz. waters poor in nutrients, or extreme habitats such as stony surfaces. Careful screening of relevant habitats using selective methods has revealed that black yeasts actually are fairly common (Dixon et al. 1980; Nishimura et al. 1987; de Hoog & Haase 1993).

Survival under Extreme Conditions

Meristematic growth, i.e., with isodiametric cellular extension and indeterminate wall thickening is a general reaction of the organism to various kinds of stress. It is the prevalent type of reproduction in *Botryomyces, Phaeotheca* and *Phaeosclera*. Representatives of these and similar genera are frequently found on leathery plant leaves or on desert rocks (Durrell 1964; Titze & de Hoog 1991). Small cavities may be formed in stony surfaces which each harbour small, persistent fungal colonies (Braams 1993). There are several hitherto unclassifiable, meristematic strains in the CBS collection which either were derived from human subcutaneous infections or from extreme environments (de Hoog, unpublished). The respective fungi survive extreme drought, high temperatures and UV irradiation due to their thick, melanized cell walls (Wheeler & Bell 1988) and slow growth with endoconidiation (Sigler et al. 1981). All species produce carotene when incubated in light (Geis & Szaniszlo 1984). These components neutralize oxygen radicals (Moore et al. 1982) and thus are relatively resistant to oxygenic action of host phagocytes. The roles of these virulence factors has been discussed by Dixon & Polak-Wyss (1991). The fungi are able to alter their microenvironment by producing extracellular acidic or alkaline metabolites. *In vitro*, *Exophiala* strains are sometimes gradually transformed into a granular form if subjected to their maximum growth temperature (Naka et al. 1987; Tintelnot et al. 1991) or by growth at a low pH (Szaniszlo et al. 1976). The same was found in *Fonsecaea pedrosoi* (Alviano et al. 1992), a species in which chlamydospore formation is known to be stimulated on blood agar (Silva 1957). Stress concentrations of salt stimulate production of muriform cells and chlamydospores in various pathogenic *Cladosporium* species (de Hoog, unpublished).

Submerged growth

Most herpotrichiellaceous black yeasts are able to grow at low oxygen tensions. Several species are found in seawater (e.g., *E. salmonis, E. pisciphila* and *E. psychrophila*). *E. dermatitidis* might occur in poor, sweet or slightly brackish waters, judging from its occurrence in humidifiers, biofilters and sink plumbing (de Hoog & Haase 1993). The annellidic cells abundantly produce yeast-like growth, the cells easily disseminating in water due to their hydrophilic extracellular polysaccharides.

Types of dispersal

Most *Exophiala* species show strong morphological plasticity determined by environmental conditions. The fungal 'giant cell' enables them to change rapidly from yeast, which disperses by water, to filamentous growth, and *vice versa* (Geis & Jacobs 1985). Hydrophobic, sympodial conidia (anamorph genus *Rhinocladiella*) serve dispersal by wind. Several species, among which *Fonsecaea pedrosoi, Cladosporium carrionii, Exophiala dermatitidis* and *E. spinifera* produce additional phialidic collarettes. The sticky balls of small conidia are not easily dispersed in water, but rather suggest adherence to an arthropod or vertebrate vector. In the human host, the ability to bud may enable a fungus to disseminate haematogenously; with hyphal growth alone, the fungus usually remains localized.

Growth in poor substrates

High levels of siderophore production may indicate natural occurrence in substrates which are poor in iron. Species able to cause disseminated mycoses are more active producers than species which remain superficial (de Hoog & Haase 1993). *Exophiala dermatitidis*, when disseminated, shows marked neurotropism (Matsumoto et al. 1984). This may be caused by a slightly higher level of free iron in the central nervous system than in serum. *Exophiala* species are frequently found in environments with slightly raised or rising electrolyte levels, such as biofilters used in water purification plants, humidifiers for air-conditioning systems, drain-pipes and condensed water (Nishimura et al. 1987). Slightly raised salt levels determine the occurrence of *E. dermatitidis* in the lungs of patients with cystic fibrosis (Kusenbach et al. 1992). Sodium chloride concentrations over 5%, however, are not tolerated, even by species of *Exophiala* which live in ocean water (Kane & Summerbell 1987; de Hoog, unpublished). Most black yeasts are able to assimilate nitrate and nitrite, with the exception of *E. dermatitidis* (de Hoog & Haase 1993). Apparently this species needs an organic nitrogen source despite its striking occurrence in waters low in nutrients.

Thermotolerance

Cladosporium trichoides and *Exophiala dermatitidis* are able to tolerate 40 °C and above. *C. trichoides* may need this ability because it has a dry thallus and occurs in soil. *E. dermatitidis*, however, has a submerged life cycle, including dispersal in yeast form by aerosols. The combination of these two factors is suggestive for occurrence either in heated waters, or an intermittent vectoring by warm-blooded animals.

Conclusion

The factors which promote virulence in the human host actually serve the fungus' survival in its natural ecological niche. Dothideaceous black yeasts, among which is *Hortaea werneckii*, generally are strong competitors and are easily isolated from osmotic substrates. The clinical picture 'tinea nigra' is explained by osmotolerance rather than by pathogenicity. Herpotrichiellaceous black yeasts probably have low competitive ability. They are found in rather special niches as secondary saprophytes, e.g., on bacterial mats, on other fungi or in poor or extreme environments. The latter niche explains their chronic occurrence in human tissue after traumatic inoculation. The combination of a totally submerged life cycle, thermotolerance and phialides in *Exophiala dermatitidis* might point to a possible utilization of an animal vector for dispersal.

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