A skin infection mimicking chromoblastomycosis by a Capnodialean fungus

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Most black fungi that are repeatedly involved in human infection belong to the order Chaetothyriales. Capnodialean melanized fungi often thrive in extreme environments like rock surfaces and hypersaline microhabitats. They are able to grow meristematically with very thick cellular walls, resembling muriform cells of agents of chromoblastomycosis. In this report we describe a member of the order Capnodiales causing a chromoblastomycosis-like infection in human skin. However, in tissue the fungus presented with toruloid hyphae and intercalary chlamydospore-like conidia with transversal septa, rather than with muriform cells. Judging from ITS rRNA sequences, the fungus is related to, but clearly different from, the genera Catenulostroma and Pseudotextularia; members of these genera are environmentally and only rarely occur on human hosts.

Keywords Black yeasts, meristemistic fungi, Capnodiales, Catenulostroma, cutaneous mycosis, itraconazole, rock fungi, extreme habitats

Introduction

Melanin is one of the major virulence factors in opportunistic black yeast. It is a complex of dihydroxy-naphthalene (DHN) polymers produced in the cytoplasm and anchored with lipids, proteins and carbohydrates in the cell wall [1]. The lipidic moiety is responsible for induction of granuloma in the host tissue, while the peptide portions function as ion chelates, competing with the serum proteins. The carbohydrate portion plays an important role in cell to cell contact, enhancing the interaction of etiologic agent and host tissue [2]. DHN polymers are able to neutralize oxidants released from phagolysosomes of the neutrophils during phagocytosis [3].

Melanins are particularly abundant in members of the ascomycete orders Dothideales (relatives of Aureobasidium) and Capnodiales (relatives of Cladosporium), which comprise numerous species colonizing dry, exposed surfaces in often extreme environments [4]. However, despite their heavily melanized nature and contrary to expectations, only very few human infections are caused by these fungi. The great majority of etiologic agents of chromoblastomycosis and other skin and disseminated mycoses belong to a less intensely melanized order of black fungi, the Chaetothyriales (relatives of Exophiala) [5]. Consequently, presence of additional virulence factors in the latter order must be hypothesized.

The present paper describes a severe, chromoblastomycosis-like skin infection caused by an uncommon melanized fungus. The identification of the isolate was challenging because it showed poor sporulation and its rDNA internal transcribed spacer (ITS) sequence did not reveal any close match other than to some
Catenulostroma and Pseudotaeniolina species, which are hypothesized members of Capnodiales.

Case report

On 24 July 2004 a 46-year-old male farmer from Colider city (Mato Grosso state, Brazil) presented to the Infectiology Ambulatory of Julio Müller University Hospital, showing a lesion on the right inferior limb. According to the description of the patient, the lesion had developed over a five year period. Physical examination showed nine individual, verrucose, vegetative, painless and non-itching lesions on the right leg. There were no lymphatic commitments or satellite lesions (Fig. 1A,B). Direct examination from a biopsy specimen showed the presence of black septic hyphae. Cultures grew slowly at 28°C and 37°C, forming black filamentous colonies. In view of species identification the strain was grown on inverted, compartmented plates with Sabouraud's glucose agar enriched with 2% yeast extract and containing 0.5% chloramphenicol at 25°C. Slide cultures were incubated for 2 to 3 weeks and observed in lactophenol cotton blue. Microscopy revealed thick-walled, olivaceous-brown hyphae which locally produced some darker, inflated, terminal or intercalary clumps of cells, or chlamydospore-like conidia (Fig. 2C,D). The isolate (MT 658/CBS = dH 16993) was enlisted in the reference collection of the Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands.

 Morphologically the species was judged to be a Taeniolina species on the basis of preponderantly meristicatic and catenulate growth. In view of molecular identification, DNA was extracted following the protocol of Möller and Peltola [6]. The ITS domains were amplified using the primers ITS1 and ITS4 according White et al. [7]. PCR was carried out containing 0.75 units Taq DNA polymerase in 10 mM Tris-HCl, 50 mM KCl, 2 mM MgCl₂, 0.2 mM of each nucleotide (Promega Corporation, Madison, USA), 0.6 µM of each primer (Bioneer Corporation, Alameda, CA, USA). Amplification cycles were 30 s at 95°C for denaturation, 57°C for 30 s for annealing and 30 s at 72°C for extension. Subsequently, 5 µl of each amplified product was electrophoresed on 6% polyacrylamide gels and stained with silver nitrate, to confirm the amplification. Direct sequencing of PCR products was done after the purification with the Qiaquick kit (Qiagen, Maryland, USA), following the manufacturer's recommendations. Sequencing was performed using a MEGA sequencer (Amersham/Pharmacia, Piscataway, USA) after labeling with BigDye Terminator Cycle Sequencing Ready Reaction. Consensus sequence was created using the package BioEdit (v. 7.1). The sequence was compared to those available in GenBank, using the Blastn program (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and in dedicated black yeast research databases maintained at CBS. Specifically, the sequence was compared with 44 ITS rDNA sequences of (predicted) members of Teratosphaeriaceae (Capnodiales). Trees were constructed using PAUP v.4.0b10, with 500 bootstrap replications (Fig. 2). No close match with any known species was found. A sister group was represented by Penidiella rigidiphora at 6.5% of difference, and Catenulostroma abietis, Pseudotaeniolina globosa and Catenulostroma chromoblastomycosum differing 14.9%, 17.4% and 20.8%, respectively.

The patient responded to treatment with itraconazole 200 mg/day during two years. Clinical improvement was noted, but no follow-up was allowed.

Discussion

The lesions present in the patient's leg were dry, suggestive of chromoblastomycosis. At the hospital a biopsy intervention was performed and the material submitted to fresh examination and culture, confirming growth of a black fungus. Although the clinical appearance was very similar to lesions classified as chromoblastomycosis, the characteristic muriform cells, the hallmark of this disease [8], were absent.

Molecular investigations (Fig. 2) revealed that the isolated fungus had affinities to Teratosphaeria-like species (family Teratosphaeriaceae, order Capnodiales [11]) rather than to any of the known agents of chromoblastomycosis which belong to the order Chaetothyriales. Teratosphaeriaceae compose a group of extremotolerant ascomycetes recently segregated from Mycosphaerella [9]. Species mostly present with heavily melanized anamorphs which tend to grow under environmentally stressed conditions on leathery plant leaves and on rock, occurring particularly in semiarid climates; all have the ability to survive in osmotic or dry habitats [4]. Within this group, no close match of our fungus with any of the described species was found. All higher branches were unresolved. For the comparative analysis nevertheless ITS sequences were used, because of the relatively large number of sequences deposited in GenBank and in other accessible databases.

The approximate sequence comparison (Fig. 2) provided an overview of the fungus' relatives, although these all were remote. Sequence data of other Taeniolina species are as yet not available. Penidiella rigidiphora was a nearest neighbor at 6.5% ITS sequence diversity. Other anamorph genera, morphologically comparable

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Fig. 1 Maximum Parsimony tree based on rDNA ITS sequences of 44 members of *Teratosphaeriaceae*, constructed with PAUP v.4.0b10, with 500 bootstrap replications (values >70 are shown with the branches). AF 309603 and CPC 11551 are taken as outgroup. Red stars indicate strains originating from human hosts.

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in heavy melanization and a tendency towards meristematic growth, are Friedmanniomycetes and Catenulostrroma [9,10], most species being derived from rock or leathery leaves in temperate or arctic climates. Species of Catenulostrroma and Pseudotaeniolina are found in a more distant position. All have a similar ecology involving dry plant surfaces in semi-arid climates, on pine leaves or on rock. Some strains in this group were derived from human hosts (stars in Fig. 2). In Catenulostrroma abietis one of the strains studied had been obtained from a human skin infection (unpublished data), while Pseudotaeniolina globosa was once isolated post mortem with unclear etiology [11]. Catenulostrroma chromoblastomycesum is known from a single strain originating from Zaire in equatorial Africa [10] and deposited in the CBS culture collection with the annotation ‘from human chromoblastomyces’. However, no proper clinical description is available, and there is no indication whether biopsies were taken and whether characteristic features of chromoblastomyces were present, particularly muriform cells [8]. We therefore regard the clinical classification of this species as doubtful. The few, poorly established human cases ascribed to (anamorph) members of the family Teratosphaeriaceae suggest that the occurrence of strains in human tissue is strictly coincidental, possibly enhanced by general extremotolerance of the fungi concerned. The term ‘chromoblastomyces’ is not appropriate for the present case report since this term is restricted to a well-delimited disease, caused by a small series of pathogens belonging to the order Chaetothyriales [8]. In the literature we regularly encounter cases listed as ‘chromoblastomyces’ which do not fulfill the criteria of the disease. Another example is a Chaetomium funicola skin infection [13] which lacked unambiguous muriform cells in tissue. In the past, any verrucose skin lesion presenting brownish structures in tissue could be uncritically classified as ‘chromoblastomyces’. The term is now restricted to species capable to form muriform cells in host tissue, cause local acanthosis, and with chaetothyrialean etiology. The cases listed above therefore should be regarded as doubtful.

Fig. 2  (A, B) Verrucous lesions on the right leg of patient presenting to the Julio Müller University Hospital (Mato Grosso, Brazil). (C) Hyphae and meristematic cells of the isolated fungus; ×400. (D) Catenulate terminal cells resembling conidia; ×1000.
Due to the lack of sporulation, it was difficult to attribute any anamorph genus name to the present fungus. Modern molecular classification is preferably linked to teleomorph nomenclature, generic names comprising broad monophyletic groups within a single family. Anamorph generic names are meant to serve ecological purposes, e.g., describing independent parts of a fungal life cycle represented by different synanamorphs which enable colonization of divergent habitats, equally within the limits of a single teleomorph family or order. The predicted teleomorph of our fungus is *Teratosphaeria* (*Teratosphaeriaceae, Capnodiales*). The strain observed was isolated from a patient living in the central region of Brazil, characterized by an equatorial, hot and humid climate with three months of dryness between June and August, where meristematic capnodialean fungi are likely to occur abundantly.

Correct fungal identification is important for etiology and epidemiology of the disease. Also, unrelated cases may incorrectly be added due to clinical similarity. In Brazil, in a large study involving 325 patients with skin lesions with confirmed histopathology, the etiological agents were identified by culture from 77 samples only [14]. These data show the lack of information related to skin disease in subtropical areas and the need of establishing proper diagnostics.

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