

The type isolate of a rare *Phaeoacremonium* species as a cause of human eumycetoma

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ABSTRACT

An isolate of unknown identity that had been identified as causing eumycetoma was retained in an international culture collection, and eventually became the nomenclatural type isolate of the rarely encountered *Phaeoacremonium sphinctrophorum*. The case featured an indurated, painless, swollen lesion on the dorsum of the foot that had developed in a Canadian resident who had previously been a farmer in Laos. Resection alone was curative.

1. Introduction

Eumycetoma refers to a subcutaneous fungal infection of the skin and underlying soft tissue, most often involving one foot and causing a tumor-like swelling occasionally accompanied by sinuses and grainy discharge [1]. The onset is insidious and gradual, and the clinical course is variable. Untreated, it has been known to progress and cause significant morbidity. There is no official incidence and prevalence of the disease in humans due to its insidious nature, its rarity and its elevated prevalence in developing countries. The highest numbers of reported mycetoma cases are from Mexico and Sudan where the prevalence has been estimated to be over 1:100,000, becoming somewhat less in other countries in Africa and South America [2]. Sporadic cases have been reported from Europe, North America and other parts of the world. In 2016, mycetoma (including actinomycetoma and eumycetoma) was recognized as a neglected tropical disease by the World Health Organization (WHO). Rising immigration rates in recent decades have caused the disease to be more prevalent in the developed world. Fungi are the causative agent in approximately half of mycetoma cases [2]. At least 39 species of molds have been identified as causative agents of eumycetoma in humans. Some have a geographic distribution limited by climate and other ecological factors [3]. Advancements in laboratory technology in recent years have facilitated the recognition of novel causative agents.

Phaeoacremonium sphinctrophorum was first described in 2006 by Mostert et al. [4] during revision of cultures held at the Westerdijk

Fungal Biodiversity Institute (Utrecht, the Netherlands). It was represented by two samples from human subcutaneous infections, both from 1988. One was from the USA, and one from Canada. Clinical case reports were never published. The only isolation of this species from environmental reservoirs is from bamboo in Thailand [5]. In 2016, a clinical case of *P. sphinctrophorum* infection was reported [6]. It was a eumycetoma of the foot with white grainy discharge in an immunocompetent patient. Disease duration was 11 years with no remarkable history of trauma; there was partial response to itraconazole therapy. We wish to report clinical details of a second eumycetoma case involving one of the isolates of *P. sphinctrophorum* that was used for the taxonomic description of the species. It is, in fact, the isolate ex-type (taxonomic anchor strain, similar in status to a bacterial type strain) of the species, CBS 337.90.

2. Case

A 45-year-old Laotian farmer who had immigrated to Canada presented to the clinic with a two-year history, beginning in Laos, of a painless cyst on his right foot. He did not recall any trauma. He had noticed a gradually enlarging nodule on the dorsum of his right foot to a point where he had difficulty getting his shoe on. He was taking no immunosuppressive drugs and had no immunosuppressive illnesses or comorbidities. The well-defined, firm, minimally tender subcutaneous nodule was approximately 2cm in diameter. There was no suppuration,

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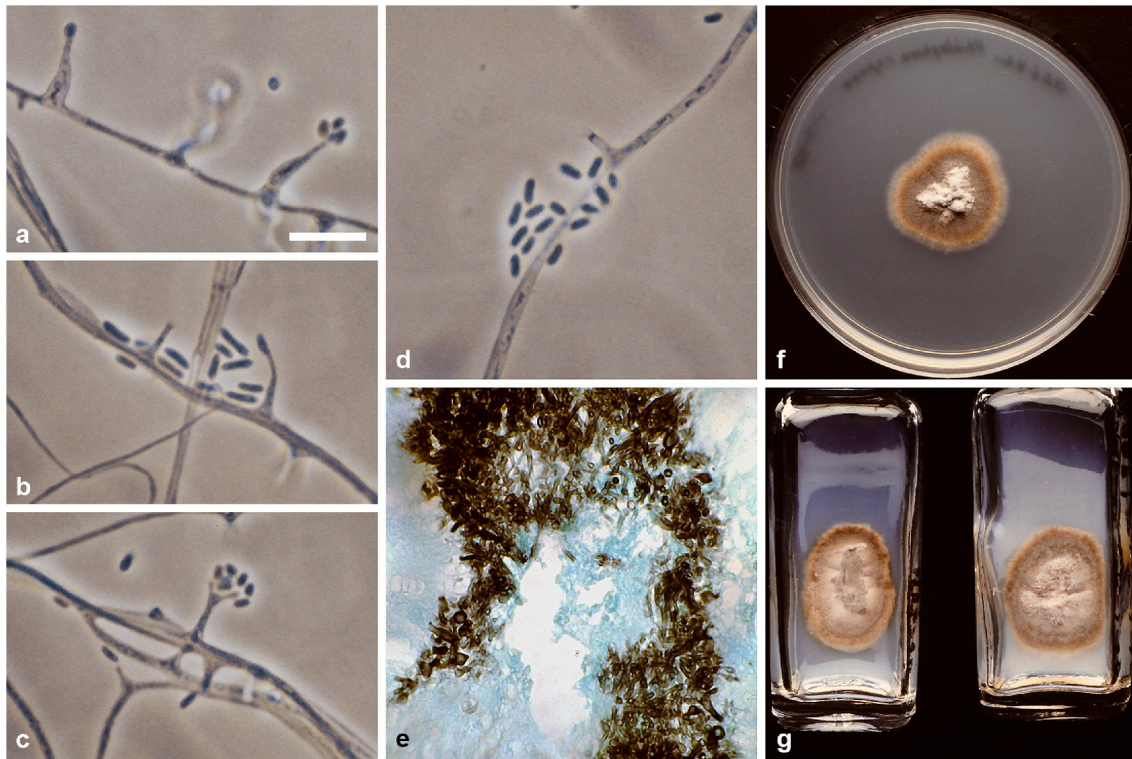


Fig. 1. *Phaeoacremonium sphinctrophorum* isolate CBS 337.90; structures from 7-day-old colony on modified Leonian's agar, 25 C. Scale bar 10 μ m applies a-d. a. broad-based phialides. b. narrow-based phialides. c. forked phialide. d. adelophialide (reduced phialide). e. Histopathology (Gomori Methenamine Silver) of clinical lesion. f. Colony on modified Leonian's agar, 7 d, 25 C. g. Colonies on vitamin-free (l) and thiamine-supplemented (r) casamino acids agar, 7 d, 25C, showing autotrophy.

erythema or ulcer. There was no striking lymphangitis, nor regional or groin adenopathy. His general physical exam was unremarkable. Blood testing including liver function tests and bilirubin were within normal range. Plain X-Ray showed no bone involvement. The nodule was presumptively diagnosed as a neurofibrosarcoma and a total excision was performed. Histopathology (Fig. 1) revealed fungal elements consistent with a eumycetoma, showing loosely organized white fungal grains. No antifungal agents were prescribed. Three months after the excision the exam of the foot was normal, and no relapse was seen. The patient was subsequently lost to follow up.

2.1. Mycology

The fungus grew out in 5–7 d as brownish colonies on blood agar and Littman oxgall agar (Fig. 1). On subculture to modified Leonian's agar, it produced colours ranging from umber to sepia. It has since been described and illustrated in detail by Mostert et al. [4]. In brief, colonies on Malt Extract Agar grow 6–15 mm in 10 d at 25 C, beginning pale brownish and maturing umber to sepia, as mentioned. Phialides are formed at terminal and lateral positions in the aerial and submerged mycelium, mostly monophialidic, occasionally forked at the apex, finely warty, brown to pale brown, and varying in length from 2 μ m in basally contiguous adelophialides to 21 μ m in basally septate discrete phialides, with bases often expanded in short phialides and conspicuously constricted in longer ones. Conidia mostly oblong-ellipsoidal to obovoid, (2.5–)3–4 \times 1.5–2 μ m (average 3 \times 1.5), borne in mucoid heads.

Even though the patient was ultimately not treated with antifungals, an exploratory test of antifungal susceptibility was done by M.J. Rinaldi at the Fungus Testing Laboratory, University of Texas Health Science Center, San Antonio, TX. Procedures were according to provisional guidelines available at the time (1991), prior to formal standardization [7]. Minimum Inhibitory Concentrations (MICs) at 48 h were 0.29 μ g/ml for amphotericin B, 0.1 μ g/ml for ketoconazole, \leq 0.018 μ g/ml for

itraconazole, and 10 μ g/ml for fluconazole. Minimum Fungicidal Concentrations (MFCs) at 48 h for the four drugs were 0.58 μ g/ml, 3.2 μ g/ml, >10 μ g/ml and >80 μ g/ml, respectively. Newer antifungal agents were not available at the time for testing.

3. Discussion

The fungus genus *Phaeoacremonium* is an uncommon but regularly seen causative agent of eumycetoma in humans [8]. *Phaeoacremonium* inoculum is acquired from the environment, where many species cause diseases of woody plants, often characterized by discoloration of the stem interiors. *Phaeoacremonium* species form an ecological consortium with the unrelated *Phaeoconiella chlamydospora* in causing the notorious Esca or Petri grapevine diseases worldwide. Various species are isolated from other woody plant hosts, where their status as disease agents or commensal endophytes may be uncertain [3].

The morphologic identification of specific *Phaeoacremonium* species is unreliable and the correct identification of the species is challenging. Thus, some of the cases in the literature have been reported under the generic name *Phaeoacremonium* only, and some of the isolates involved are likely to have been misidentified. In recent years, methods for molecular identification have been developed. Most are DNA phylogenetic techniques based on sequencing the internal transcribed spacers (ITS 1 and 2) and the 5.8 S rRNA gene, and β -tubulin, actin and calmodulin gene regions [3,4]. According to the review and revised taxonomy published by Gramaje et al in 2015 [3], 46 species of *Phaeoacremonium* have been identified by DNA sequencing, and 11 of these have been reported as causing disease in humans. *Phaeoacremonium parasiticum* and *P. krajdinii*, both of which have been isolated from grapevines and other plants, are causal in the majority of human infections.

Recent years have seen an increase in the number of reported clinical cases of *Phaeoacremonium* infection due to a combination of improved taxonomic discernment, improved laboratory techniques and a rise in

the immunocompromised population. The source of the infection in humans is presumably inoculation of a phaeohyphomycotic cyst or mycetoma under the skin via splinter of an infected plant, by traumatic implantation of soil particles or rarely by inhalation [3]. The most common clinical manifestation is eumycetoma, but *Phaeoacremonium* can cause other clinical manifestations, especially in the immunosuppressed host. These include onychomycosis [9], endophthalmitis [10], endocarditis [11] myositis [12], tenosynovitis [13], central nervous system infection [14], respiratory tract infection [15] and disseminated disease [16]. Some of the patients with the above manifestations were immunosuppressed; most were transplant patients [16] with a few cases suffering from underlying malignancies or receiving treatment with infliximab [17]. Others had no known risk factors for opportunistic infections.

There are three reported cases of *P. sphinctrophorum* causing human infections. All three featured a subcutaneous infection. No more is known about one case (source of isolate CBS 694.88 from subcutaneous cyst, Hawaii, 1988). In the recent case reported by Bustamante et al. [6], a frank mycetoma with draining sinuses was seen. The current case presented as a blind cyst but was likely a eumycetoma in its early stages. All reported cases were in men. The fact that two of the cases were in immunocompetent young men (there is no information about the third patient), points to the possibility of non-opportunistic pathogenicity. No preceding trauma was known in either case. The geographic distribution of Peru, Hawaii and Laos (collected in Toronto, but the disease process started in Laos) shows the potential worldwide prevalence of this species, at least in tropical regions.

The clinical presentations of the two detailed cases were dramatically different. While one had a relatively benign course with a single lesion that did not recur after simple excision, the other had multiple unresectable nodules and grainy discharge with partial response to antifungal therapy. This could be related to the difference in time of diagnosis with the latter being diagnosed 11 years after onset versus 2 years in the first. This points to the potential hazards of late diagnosis. An indolent subcutaneous infection at first resembling a slowly enlarging cyst may develop over time into a classic mycetoma.

Though antifungal susceptibility has not been studied for either case isolate at modern standards, the Peruvian case [6] showed clinical response to itraconazole, while the current case showed *in vitro* response to this drug. Badali et al. [18] found that eight other *Phaeoacremonium* species, including *P. krajdenui*, the species most closely related to *P. sphinctrophorum*, were resistant to itraconazole and most susceptible to voriconazole.

Current recommendations for a nodule as observed in our patient, include total excision for culture and histopathological diagnosis, followed by DNA-based identification of the etiologic agent, and susceptibility testing. In the case of *P. sphinctrophorum*, ensuring clearance of the fungus after surgery, if needed, could be attempted with itraconazole or voriconazole, with a preference for the latter based on genus-wide MIC results.

Declaration of competing interest

There are no conflicts of interest.

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