Phaeoacremonium krajdenii, a Cause of White Grain Eumycetoma

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We describe the first case of white grain pedal eumycetoma caused by Phaeoacremonium krajdenii in a 41-year-old man from Goa, India. Based on histological examination of biopsy tissue showing serpentine granules, a culture of the granules yielding phaeoid fungal colonies, and morphological characteristics and sequence comparison of the partial β-tubulin gene with the ex-type isolate of P. krajdenii, the causal agent was identified as P. krajdenii.

CASE REPORT

A 41-year-old patient from Goa, India, visited a dermatologist in December 2000 with complaints of a single nodular, painful swelling over the dorsum of his foot that had persisted for 1 year. About 18 years earlier, he had developed a swelling and multiple discharging sinuses over the dorsum of his right foot. At that time, he was treated surgically by a local surgeon, and based on a histological examination, he was diagnosed as having a mycetoma of the foot. He was treated with cotrimoxazole and sulfamethoxazole for a period of 6 months without improvement. However, following surgery, the swelling of the foot had subsided. After about 10 years, new nodules and swellings had developed over the operated scar. At this time, he consulted a dermatologist and was referred to one of us (B.M.H.) for mycological examination.

Over the scars of the previous surgery, a single small, nodular lesion measuring 6 by 6 mm was observed. The nodule was aseptically punctured, and a pale white granule, 0.5 to 2.0 mm in diameter, was aspirated. Direct examination of one-half of the granule in potassium hydroxide showed numerous hyalines, septate hyphae, and a few thick-walled cells. The other half of the granule was cultured on Sabouraud glucose agar containing chloramphenicol (Sab+C) and incubated at 25 to 30°C. White to off-white fungal colonies became evident after 8 to 10 days. Colonies slowly became darker and velvety and were olivaceous grayish brown. A provisional diagnosis of white grain mycetoma was made. The mycetoma consisted of a necrotic core where eosinophilic material surrounded the fungal elements in the granules. A biopsy sample was taken and based on a histological examination, he was diagnosed as having a mycetoma of the foot. He was treated with ketoconazole tablets (400 mg/day) for 4 months. A surgical redebridement was contemplated after about 4 months of treatment, with the hope that the spread of the infection would be restricted by then. However, the patient could not be found for the follow-up.

The slides of the biopsy tissue were stained with hematoxylin and eosin (H&E) and Gomori’s methenamine silver (GMS) stains. Sections of the skin biopsy sample stained with H&E showed swelling, draining sinuses, and intradermal mycetoma. The mycetoma consisted of a necrotic core where eosinophilic material surrounded the fungal elements in the granules, a presentation referred to as the Splendore-Hoeppli reaction (Fig. 1B and C). The granules measured 0.5 to 2.0 mm in diameter and were irregular in shape. The fungal elements in the core were not pigmented in H&E-stained sections. Around the cores of the granules, there were abundant epithelioid macrophages, plasma cells, and eosinophils as well as occasional neutrophils. Outside the mycetoma, there was scattered inflammation with an abundance of eosinophils. Epidermal psoriasiform hyperplasia covered the mycetoma site. The slides stained with the GMS procedure showed intertwined hyphal elements consisting of septate, branched hyphae with various lengths, 2.0- to 3.0-μm diameters, and thick-walled, vesiculate chlamydospores (Fig. 1D).

A portion of the biopsy tissue containing granules was cultured on Sab+C and Sab+C containing cycloheximide and based on a histological examination, he was diagnosed as having a mycetoma of the foot. He was treated with ketoconazole (400 mg/day). However, the patient could not be found for the next 2 years, new lesions appeared on the plantar aspect of the foot (Fig. 1A).

After his return in October 2000, a biopsy was performed. A portion of the biopsy tissue was sent for histopathological examination, and another portion was cultured. The specimen yielded the same fungus as had been obtained previously. Both a biopsy tissue block and a subculture were sent to one of us (A.A.P.) at the Centers for Disease Control and Prevention (CDC) for additional studies. The X-ray examination of the foot had shown no bone involvement but revealed soft-tissue swelling. Results for routine clinical chemistry, hematological investigations, and serology for human immunodeficiency virus were within normal limits. The patient was treated with itraconazole tablets (400 mg/day) for 4 months. A surgical redebridement was contemplated after about 4 months of treatment, with the hope that the spread of the infection would be restricted by then. However, the patient could not be found for the follow-up.

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FIG. 1. (A) Mycetoma on the right foot of the patient showing discharging sinuses on the dorsal aspect of the foot. (B) Tissue section showing a serpentine granule of *Phaeoacremonium krajdenii*. Stain, H&E. Magnification, ×483. (C) Magnified portion of the granule showing a Splendore-Hoeppli reaction. Stain, H&E. Magnification, ×772. (D) Portion of the granule showing intertwined hyphal elements and vesiculate chlamydospores. Stain, GMS. Magnification, ×772. (E) Slide culture of *P. krajdenii* (CDC B-6093 is CBS 110361) showing mono- and polyphialides of cylindrical to elongate-ampulliform shapes and ellipsoidal to allantoid conidia. Magnification, ×772. 1, 2, and 3, first, second, and third types of phialides as described in the text; arrows, collarettes.
(Sab+C+C) prepared in-house. The morphology of the isolate was studied on potato dextrose agar (PDA) (Difco Laboratories, Detroit, MI) and malt extract agar containing 2% malt extract (Oxoid Ltd., England, United Kingdom) and 1.5% agar (Difco Laboratories, Detroit, MI). The cultures were incubated at 25°C and 37°C in the dark. The initial colonies on Sab+C and PDA were moist, creamy to off-white, and slightly raised in the center. They became grayish brown to dark gray after 8 to 10 days at both temperatures. Growth was not inhibited by cycloheximide. The colonies after 2 weeks were velvety, flat, and olivaceous brown and measured 18 to 20 mm in diameter at 25°C and 10 to 12 mm in diameter at 37°C. No growth was observed at 40°C.

The slide cultures on PDA after 2 weeks of growth at 25°C were mounted in lacto-fuchsian medium. Microscopic mounts from the malt extract agar culture were made after 2 to 3 weeks of incubation at 25°C from the aerial mycelium as well as from the agar surface. The hyphae were septate, verrucose, medium brown, and 2 to 3 μm wide. Warts were observed on many vegetative hyphae. The conidiophores were short, unbranched, occasionally constricted at the basal septa, 15 to 45 μm long, and 1.5 to 2 μm wide. The apical cells of the conidiophores produced one or two phialides each. The phialides were monophialidic (i.e., with one fertile opening) at first but often became polyphialidic (i.e., they developed additional fertile openings on short, forking side branches). Phialides differed in shape. Those of the first type were cylindrical, wide at the base, tapering toward the apex, 4 to 11 μm long, and 1 to 2 μm wide. The second type was elongate ampulliform, attenuated at the base, 7 to 15 μm long, and 1.5 to 2 μm wide. The third type was subcylindrical, elongate ampulliform at the base, 13 to 22 μm long, and 1 to 2 μm wide. Numerous phialides at maturity gave rise percurrently to new phialides in a process referred to as phialide rejuvenation. When this occurred, the older phialides were often inflated at the base and had one to five septa. The collarettes at the tips of the phialides were flaring, narrow, 1 to 3 μm long, and 1 to 2 μm wide. The conidia were subhyaline, oblong ellipsoid to allantoid (sausage shaped), and 3 to 5 μm long by 1 to 2.5 μm wide (Fig. 1E). The phialides on the agar surface were cylindrical and were 2 to 11 μm long and 1 to 2 μm wide. The conidia on the agar surface were allantoid to oblong ellipsoid, 4 to 6.5 μm long, and 1 to 1.5 μm wide. The isolate hydrolyzed allantoin. It was studied in more detail at the Centraalbureau voor Schimmelmilities (CBS), Utrecht, The Netherlands, and was accessioned in the CDC and CBS collections as CDC B-6093 and CBS 110361, respectively.

Genomic DNA extraction was performed on CBS 110361 by using the isolation protocol of Lee and Taylor (10). Approximately 560 bp at the 5' end of the β-tubulin gene was amplified using primers T1 (14) and B2b (5). The PCR amplification and sequencing were performed as described by Mostert et al. (13). A consensus sequence was computed from the forward and reverse sequences with SeqMan from the Lasergene package (DNASTAR, Madison, WI). The sequence obtained was compared with the β-tubulin sequence of the ex-type sequence of Phaeoacremonium krajdenii (CBS 109479, GenBank accession no. AY579330) with Sequence Alignment Editor version 2.0a11 (16) and the percent DNA similarity calculated. The β-tubulin sequence of CBS 110361 was 99.8% similar (1 base variation) to the sequence of the ex-type isolate of P. krajdenii over a total continuous length of 556 bases. The close DNA similarity of CBS 110361 to the ex-type isolate confirmed this isolate as P. krajdenii.

At present, there are 28 species known to cause eumycotic mycetoma. Thirteen of these species cause white grain mycetoma, and 15 species are known to cause black grain mycetoma (11).

The majority of described human Phaeoacremonium infections have been subcutaneous abscesses and cysts or chronic or acute mycotic arthritis (6, 8, 12, 15). Cases are reported with approximately equal frequencies in immunocompromised and immunocompetent hosts. Disseminated infections, fungemia, and endocarditis have been reported only occasionally (7, 18).

In the course of a recent taxonomic revision of the genus Phaeoacremonium (13), eight species were recognized as causing human infection. They were Phaeoacremonium alvsi, Phaeoacremonium amstelodamense, Phaeoacremonium gris-eorum, Phaeoacremonium krajdenii, Phaeoacremonium parasticticum, Phaeoacremonium rubrigenum, Phaeoacremonium tardicrescens, and Phaeoacremonium venezuelense. P. parasitica, the type species of the genus, was originally described as Phialophora parasitica (3). At present, two of the eight recognized opportunistic Phaeoacremonium species have been reported to cause white grain eumycetoma. In the first reported mycetoma case involving what later transpired to be a Phaeoacremonium isolate, a fungus causing white grain eumycetoma in a Venezuelan patient was identified as Cephalosporium serrae (1), an arcane synonym for the fungus now known as Verticillium nigrescens (17). Crous et al. (3) reidentified the case isolate, CBS 651.85, as Phaeoacremonium inflatipes. In a molecular and morphological reexamination, the isolate was shown not to be a member of P. inflatipes and was rediagnosed as the ex-type isolate of the new species P. venezuelense (13).

An earlier reported case of white grain eumycetoma caused by a Phaeoacremonium isolate involved a 30-year-old Indian woman living in the United Kingdom who developed mycetoma in her right foot after visiting India (9). The causal agent was identified as Phialophora parasitica. The isolate could not be reexamined because it was nonviable. The CBS collection contains a P. krajdenii isolate, CBS 633.93, that was stated to be from a mycetoma from a 31-year-old male examined in Norway. This case, to our knowledge, was not reported in the scientific literature. The isolate was originally deposited as Phialophora repens, a name that was frequently misapplied to isolates of undescribed Phaeoacremonium species in the years from 1970 to 2000. Genuine P. repens is not involved in human infection (13).

The present case, then, represents the first reported case of white grain mycetoma caused by P. krajdenii. An interesting feature of this case was that the granules in the tissue were surrounded by eosiophilic material exhibiting a Splendore-Hoeplli reaction. Such an intensely eosiophilic reaction has also been reported surrounding white grains of Pseudallescheria boydii (2). Both of these eumycotic agents with melanized hyphae or conidia produce whitish grains in tissue. Melanin production in Phaeoacremonium species is facultative, while melanin pigment is limited to conidia in P. boydii.

The present case is also the first to be reported with the
recently published name P. krajdenii. Two previous cases involving this species have been published with the name P. repens; for both, the case isolates in CBS were reexamined and sequenced as described by Mostert et al. (13) and shown to be P. krajdenii. Both cases were very similar to the present one, but the lesions examined were tentatively classified as subcutaneous phaeohyphomycotic cysts rather than mycetoma, despite the presence of grain-like "microcolonies" or "hyphal masses" in the lesions. One involved a lesion on the scalp of a lepromatous-leprosy patient from what is now the Democratic Republic of Congo (12), while the other involved a lesion on the hand of a patient in Japan who had no discernible predisposing factors other than mild diabetes mellitus (8). In the Japanese report, Hironaga et al. (8) commented that the lesion, which was first noticed by the patient only 2 months prior to presentation, contained hyphal aggregates that "looked like the grains of eumycotic mycetomas but did not manifest the Splendore-Hoeppli phenomenon sometimes seen in [these] mycetomas." These authors also commented that draining sinuses, a feature essential to the classification of a lesion as a mycetoma, were not seen. The present case, featuring a lesion that developed for many years, was more typical of a classic mycetoma, with well-developed granules, a marked Splendore-Hoeppli effect, and draining sinuses, as can be seen in Fig. 1A. It would appear, then, that subcutaneous cases caused by P. krajdenii will at first manifest as phaeohyphomycotic cysts with organized grain-like hyphal clusters in granulomatous tissue but will later mature as classic white grain eumycetomas.

The majority of Phaeoacremonium species are plant pathogens (3, 4, 13) and endophytes infecting woody plants. Grape diseases named Petri disease and esca, apoplexy, or black meagens (3, 4, 13) and endophytes infecting woody plants. Grape but will later mature as classic white grain eumycetomas. "organized grain-like hyphal clusters in granulomatous tissue krajdenii P. krajdenii; for both, the case isolates in CBS were reexamined and that developed for many years, was more typical of a classic mycetoma, were not seen. The present case, featuring a lesion in GenBank under accession number AY57933.

Nucleotide sequence accession number. The nucleotide sequence for CBS 110361 described in this study was deposited in GenBank under accession number AY57933.

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REFERENCES

1. Alborno, M. B. 1974. Cephalosporium sanae, agente etiológico de miceto-
3. Crous, P. W., W. Gams, M. J. Wingfield, and P. S. Van Wyk. 1996. Phaeoacremonium species have also been isolated from soil (3, 13). Other sources of inoculum could include dust and indoor water sources (13). Medically important Phaeoacremonium species can easily be distinguished from species not involved in human disease by their abilities to grow at 37°C and 40°C. Even though the identification of medically important Phaeoacremonium spp. can be very difficult based on cultural and micromorphological characters alone, the definitive identification of these species is facilitated by recently developed identification procedures involving the combined or separate use of β-tubulin sequence data and reevaluated morphological data. As outlined by Mostert et al. (13), an online keying system allowing the use of both morphological and molecular characters can be accessed via the CBS website by clicking on “The Phaeoacremonium database” at http://www.cbs.knaw.nl/databases/index .htm.