**Tilletiopsis minor**: a New Etiologic Agent of Human Subcutaneous Mycosis in an Immunocompromised Host

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We describe herein the isolation of *Tilletiopsis minor* from a subcutaneous cyst of a 70-year-old immunocompromised male. The diagnosis was based on repeated isolation of the fungus, observation of hyphal elements in tissue sections, the ability of the mold to grow at or near body temperature, and the achievement of a complete cure following surgery and antifungal therapy.

Fungi previously considered to be saprobes or plant pathogens are increasingly being implicated as etiologic agents in human disease due to widespread use of cytotoxic and immunosuppressive drugs, indwelling catheters, transplantation, etc. (5, 9). Subcutaneous mycoses are caused by a variety of environmental fungi that become implanted following skin trauma. The initiation and progression of disease depend upon the state of immunity of the host and the ability of the fungus to survive in vivo. Lesions may remain localized or disseminate to surrounding tissues, requiring surgical intervention and/or antifungal therapy (5, 9, 10). In this paper we describe isolations on two occasions, 3 months apart, of *Tilletiopsis minor* from a cystic lesion of an immunocompromised patient.

A 70-year-old white male retiree presented in March 1996 with pain, swelling, and redness in the right distal forearm of 2 months’ duration. He did not recall having received any trauma to that arm. The patient had undergone a splenectomy in 1994 for autoimmune hemolytic anemia and received 60 mg of prednisone per day, which was later tapered to 10 mg every other day. The cyst was surgically excised and drained. The patient received a course of broad-spectrum antibiotics. No direct examination or Gram stain was performed on the first drainage specimen. The excised specimen yielded a pure fungal culture. However, the etiologic significance of this isolate was not clear due to inconclusive identification. Three months later, the patient returned with a recurrence of the cyst at the same site. Surgical debridement was performed, and the debrided material was submitted for histopathological analysis and culture. The gross specimen consisted of two irregular, rubbery, tan-brown, soft tissue fragments measuring 2.5 by 2.0 by 0.8 cm. Microscopically, the specimen consisted of fragments of fibrovascular and fibrotendinous tissues. It showed a granulomatous tissue response with multinucleated giant cells, multifocal abscess formation, necrosis, and focal cystic degeneration (Fig. 1a). Acid-fast staining for mycobacteria was negative. Periodic acid-Schiff and Gomori’s methenamine silver staining revealed the presence of short, moniliform hyphal elements (Fig. 1b). Colonies on Sabouraud dextrose agar were soft, creamy, and yeastylike. Since a fungal etiology was suspected, the patient received 100 mg of fluconazole (Diflucan) twice daily for 2 weeks. The lesions healed completely, and there had been no recurrence of the lesion in the last 15 months.

Two reference cultures were received by the Laboratories for Mycology at the Wadsworth Center. The laboratory’s routine procedures were used to delineate the morphological and physiological characteristics of these cultures (7). Examination of calcofluor mounts of teased portions of growth from cultures revealed the presence of thin, curved conidia borne on branching, septate, hyaline hyphae. Subcultures of these iso-
lates grew on Sabouraud dextrose agar between 25 and 35°C, with remarkably reduced growth at 37°C. Young colonies (3 to 5 days) were soft, creamy, and yeasts and developed satellite colonies, thereby suggesting the production of ballistospores (forcibly ejected basidiospores). The colonies turned dark, tough, and cartilaginous in 3 weeks (Fig. 2). There were no significant differences in colony morphology and sporulation at different incubation temperatures.

Potato dextrose agar slide cultures at 30°C showed delicate, septate, hyaline hyphae after 3 days of incubation, and these hyphae became dark with age. The conidia were hyaline and curved, measuring 8 to 9 μm by 2 to 3 μm (Fig. 3 and 4). Budding yeastlike cells and ballistospores were also seen in slide cultures. The sickle-shaped ballistospores were borne on sterigmata arising from a fine mycelium. Three-week-old cultures showed globose to clavate-shaped terminal or intercalary chlamydospores measuring 7 to 15 μm in diameter. The biochemical assimilation tests were done according to the procedures described by Lodder (6). The mold isolates assimilated

potassium nitrate, maltose, lactose, ethanol, phenylalanine, and ammonium sulfate. The two isolates did not assimilate mannitol or asparagine. These molds liquefied gelatin in 7 days at 30°C, produced urease, and split arbutin. The isolates were tentatively identified as T. minor based on their macro- and microscopic morphologies, biochemical reactions (Table 1), and the physiological characteristics described by Boekhout (1) and Gokhale (3). Species identification was confirmed by comparison of morphological and physiological characteristics with those of T. minor ATCC 10764 (CBS-543.50) and T. washing-tonensis ATCC 36489 (CBS-5444.50). The isolates have been deposited in the Fungus Culture Collection of the New York State Department of Health under accession numbers M-265/96 and M-325/96.

In vitro tests of susceptibility to amphotericin B (Sigma, St. Louis, Mo.), fluconazole (Roerig/Pfizer Inc., New York, N.Y.), and itraconazole (Janssen Biotech N.V., Olen, Belgium) were performed according to a macrobroth procedure adapted from the reference method for yeasts (2). Both isolates were inhib-

![FIG. 2. Colony appearance of T. minor cultured on Sabouraud dextrose agar at 30°C. (a) Seven-day-old yeastlike colony with small satellite colonies at the periphery of the culture plate, consistent with ballistospore formation. (b) Twenty-one-day-old culture with fused satellite colonies forming a tough, dark, cartilaginous colony.](http://jcm.asm.org/)

![FIG. 3. Sporulation by T. minor on potato dextrose agar at 30°C. (a) Delicate, hyaline hyphae bearing curved conidia, from a 3-day-old culture. (b) Dark hyphae with scattered curved, conidia and globose chlamydospore (arrow) after 21 days of growth. Magnifications, ×400.](http://jcm.asm.org/)
FIG. 4. Scanning electron micrograph of T. minor spores. (a) A bunch of curved conidia and a single ballistospore are seen. (b) Shown is a curved ballistospore attached to a sterigma arising from a septate hypha. Bars, 1 μm.
ited by amphotericin B (MIC, 0.06 μg/ml), fluconazole (MIC, 8 μg/ml), and itraconazole (MIC, 0.5 μg/ml).

The evidence that T. minor was the etiologic agent of the cystic lesion includes the following: (i) isolation of the fungus on two occasions from clinical specimens, (ii) observations of hyphal elements in tissue sections, (iii) the mold’s ability to grow at or near body temperature, and (iv) improvement of the patient’s condition as a result of surgery and antifungal therapy. A search of online English literature did not reveal any previous report of an association of T. minor with human disease.

The patient in this report is an avid gardener, and it is conceivable that T. minor was accidentally implanted during gardening and/or that the patient acquired this infection while raking leaves during the fall season. T. minor has been recovered in culture from dry leaves (8). The patient was receiving low-dose corticosteroids, which leads to a reduction of circulating T cells and B cells, and as a result he probably had an increased susceptibility to fungal infections (4). Since the antifungal therapy was given at a subtherapeutic dose and for a shorter duration, it is conceivable that the surgical intervention was curative in this case.

Derx (1930) first described and named the genus Tilletiopsis while examining plant leaves because it resembled species of Tilletia (8). The white or cream-colored colonies of members of the genus Tilletiopsis distinguish it from Sporobolomyces species, which form salmon- or pink-colored colonies. Tilletiopsis strains also resemble Entyloma species in their colony morphology, but ballistospores of Entyloma do not bud in a yeast-like manner (8). Two species, T. washingtonensis and T. minor, are recognized based on the size of the conidia, colony color, and nutritional tests (1, 3, 8). Recently, Boekhout (1) examined the taxonomic position of the genus Tilletiopsis along with other ballisticonidium-forming yeasts and fungi and grouped them in the family Sporobolomycetaceae (1).

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REFERENCES

| TABLE 1. Biochemical tests for differentiation of Tilletiopsis strains |
|-----------------|-----------------|-----------------|-----------------|
| Biochemical test | Isolate 1 (M265-96) | Isolate 2 (M325-96) | T. minor (ATCC 10764) | T. washingtonensis (ATCC 36489) |
| Lactose         | ±               | ±               | +               | –               |
| Mannitol        | –               | –               | –               | +               |
| Ammonium sulfate| +               | +               | ±               | –               |
| Phenylalanine   | +               | +               | –               | –               |
| Asparagine      | –               | –               | ±               | +               |
| Arbutin         | +               | +               | –               | –               |

*+, positive; –, negative; ±, result varied.*