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Scedosporium inflatum, an Emerging Pathogen

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The salient morphologic and physiologic characteristics of 18 isolates of *Scedosporium inflatum*, a newly reported human pathogen, were compared with those of the morphologically similar fungi *Scedosporium apiospermum*, *Scopulariopsis brevicaulis*, and *Scopulariopsis brumptii*. The formation by *S. inflatum* of annelloconidia in wet clumps at the apices of annellides with swollen bases was found to be the most useful characteristic in differentiating this potential pathogen.

Malloch and Salkin (6) described a new species of *Scedosporium* Saccardo as *Scedosporium inflatum* Malloch et Salkin in 1984. The dematiaceous fungus had been initially isolated from a bone biopsy specimen obtained from a 6-year-old boy who had developed a deep subcutaneous lesion on the sole of his right foot. While morphologically and physiologically similar to *Scedosporium apiospermum* (Sacc.) Castellani et Chalmers, *S. inflatum* was established as a distinct species owing to its more rapid growth on standard nutrient agar media and the formation of annellides with swollen bases. In addition, unlike *S. apiospermum*, a teleomorph is unknown for *S. inflatum*.

During the past year, we have evaluated 18 isolates of *S. inflatum*: 15 associated with infections in humans and other animals and 3 recovered from soil. Several of these isolates were originally incorrectly identified as other molds such as *S. apiospermum*, *Lomentospora prolificans* Hennebert et Desai, *Sporothrix schenckii* Hektoen et Perkins, and *Wardomyces humicola* Hennebert et Barron. Because of the confusion surrounding the identification of *S. inflatum* and because the fungus has been isolated from subcutaneous infections, clinical microbiologists need to become aware of this opportunistic pathogen and its salient features.

The objectives of this investigation were to provide criteria for the accurate identification of *S. inflatum* and to evaluate its in vitro susceptibility to several antimycotic agents.

MATERIALS AND METHODS

Test organisms. While each of the four laboratories which participated in this collaborative investigation maintained a collection of all test isolates, to eliminate possible confusion for the readers, the procedures described, unless otherwise stated, were those conducted at one of our facilities (University of North Carolina at Chapel Hill). Test isolates (Table 1) were maintained on potato-dextrose agar slants (PDA; prepared from raw components by M.R.M.) and stored in a standard freezer at −70°C. When required for morphologic and physiologic studies, a portion of a frozen culture was chipped off, aseptically transferred to a PDA slant, and incubated at 25°C for 14 days.

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Morphology by light microscopy. PDA and V-8 juice agar slide cultures (10) were prepared for each test isolate when initially received. Slide cultures were thus used to evaluate the purity of test isolates, as well as to study conidial morphology and ontogeny. Observations of the cultures were made after 2 to 4 weeks of incubation at 25°C with a Zeiss Universal microscope equipped with bright-field, differential-interference-contrast, and phase-contrast optics. Stock cultures described above were used in a limited number of repeat developmental studies. Colony morphologies were examined after incubation for 14 days at 25°C on PDA.

Morphology by fluorescence microscopy. As conducted at the Wadsworth Center for Laboratories and Research, the cover slip from a 14-day-old PDA (Difco Laboratories, Detroit, Mich.) slide culture of each *S. inflatum* isolate was aseptically removed, rapidly passed through the flame of a Bunsen burner to heat fix the adhering growth, and mounted on a drop of an 0.05% solution of Cellufluor (Polysciences, Inc., Worthington, Pa.) in 10% KOH. This working solution of the nonspecific fluorescent stain was prepared by first dissolving 0.1 g of purified Cellufluor in 10 ml of distilled water and then adding 0.5 ml of this solution to 9.5 ml of a 10% KOH solution. A precipitate which formed upon the addition of the Cellufluor to the KOH solution was removed by filtering the working solution through a polysulfone filter (0.45-μm pore size). The mounted cover slips were examined with a Nikon Lobophot microscope equipped with an epifluorescence attachment. For this study, the Nikon U filter tube containing a UV excitation filter (330 to 380 μm) and a barrier filter (420 μm) along with a 515W eyepiece side absorption filter were used in combination to achieve maximum fluorescence.

Morphology by scanning electron microscopy. Five test isolates, *Scopulariopsis brumptii* Salvanet-Duval (NCMH 1793), *Scopulariopsis brevicaulis* (Sacc.) Bainier (NCMH 2513), *S. apiospermum* (NCMH716, NCMH1510), and *S. inflatum* (NCMH2671), were used in studies conducted at the North Carolina State University School of Veterinary Medicine. The fungi were grown in 100-mm plastic petri plates containing 20 ml of PDA (Difco) for 2 to 40 days at 30°C. To fix the colonies, petri plates were inverted and opened in a fume hood, 1 drop of 2% aqueous osmium tetroxide was added to the inside of the top of each plate, and the plates were reassembled in an inverted position.

TABLE 1. Epidemiologic characteristics of test isolates

Organism and strain no.[a]	Source	Host	Geographic location
Scedosporium apiospermum			
NCMH128	Brain abscess	Human	Chapel Hill, N.C.
NCMH234	Sputum	Human	Palo Alto, Calif.
NCMH235	Heart	Human	Palo Alto, Calif.
NCMH716	Kidney	Dog	Germany
NCMH1019	Mycetoma (foot)	Human	Alabama
NCMH1025	Eye	Human	New Jersey
NCMH1026	Maxillary sinus	Human	Unknown
NCMH1510	Sputum	Human	Chapel Hill, N.C.
NCMH2131	Mycetoma	Human	Medellin, Colombia
NCMH2498	Unknown	Human	Unknown
NCMH2889	Bursa fluid	Human	Salt Lake City, Utah
NCMH2890	Wrist	Human	Salt Lake City, Utah
NCMH2891	Bone marrow	Human	Seattle, Wash.
NCMH2892	Unknown	Human	Minneapolis, Minn.
NCMH2893	Foot	Human	Mesa, Ariz.
NCMH2895	Unknown	Human	Portland, Oreg.
Scedosporium inflatum			
NCMH1283	Bone biopsy (foot)	Human	Oakland, Calif.
NCMH2032	Draining sinus	Cat	Davis, Calif.
NCMH2277	Synovial fluid (knee)	Human	St. Louis, Mo.
NCMH2365[b]	Bone biopsy (foot)	Human	Farmington, Maine
NCMH2442	Eye scrapings	Horse	Louisville, Ky.
NCMH2623	Surgical specimen (knee)	Human	Oakland, Calif.
NCMH2671	Eye scrapings	Horse	Raleigh, N.C.
NCMH2686	Bone biopsy	Human	Pensacola, Fla.
NCMH2698	Surgical specimen (knee)	Human	Boston, Mass.
NCMH2703	Surgical specimen (hip)	Human	Ventura, Calif.
NCMH2704	Wound on finger	Human	California
NCMH2705	Sputum	Human	California
NCMH2708	Peritoneal cavity	Human	Adelaide, Australia
NCMH2850	Surgical specimen (foot)	Human	Los Angeles, Calif.
NCMH2928	Wound on ankle	Human	Atlanta, Ga.
NCMH2869	Soil	*Schefflera* sp.	Toronto, Canada
NCMH2870	Soil	*Schefflera* sp.	Toronto, Canada
NCMH2871	Soil	*Ficus benjamina*	Toronto, Canada
Scopulariopsis brevicaulis			
NCMH2513	Nail scrapings	Unknown	Durham, N.C.
NCMH2897	Mastoid sinus	Human	Minneapolis, Minn.
Scopulariopsis brumptii			
NCMH1614	Sputum	Human	Quebec, Canada
NCMH1731	Sputum	Human	Chapel Hill, N.C.
NCMH1793	Unknown	Human	Long Island, N.Y.
NCMH2165	Sputum	Human	Chapel Hill, N.C.
NCMH2378	Cutaneous tissue	Human	Chapel Hill, N.C.
NCMH2730	Bronchial washing	Human	Lansing, Mich.

[a] NCMH, North Carolina Memorial Hospital, Chapel Hill.
[b] Subculture derived from the isolate used to prepare the type specimen (6).

After 5 min of exposure, the bottom half of each plate was removed and several small portions of growth were cut from the center of the colony and transferred to 2 ml of McDowell and Trump fixative (7) in 1-dram (3.9-ml) vials. The air within the vials was aspirated to create a partial vacuum to cause the samples to sink to the bottom of the fixative solution. Samples were stored for 2 h to 1 week at 4°C (7). After the material was fixed, the fixative was decanted off and the samples were rinsed twice (15 min per rinse) in 0.1 M phosphate buffer and then put into 1% osmium tetroxide in 0.1 M phosphate buffer for 1 h at room temperature. The samples were washed twice in distilled water (5 min per wash), passed through a graded ethanol series up to 100% ethanol, critical point dried with liquid CO_2, mounted on aluminum stubs with colloidal silver, and sputter coated with gold-palladium before being examined in a JEOL JSM 35CF scanning electron microscope operated at 15 kV.

Physiologic studies. To evaluate cycloheximide susceptibility, a small portion of growth of each isolate was aseptically removed from a stock culture, transferred to the surface of a Mycosel agar slant (0.5 mg of antifungal agent per ml of medium; BBL Microbiology Systems, Cockeysville, Md.) in a culture tube (20 by 150 mm), and incubated at 30°C. Growth was assessed after 7 and 14 days.

To ascertain growth-temperature relationship, a portion of growth of each isolate was again aseptically removed from a stock culture, transferred to the surface of a Sabouraud glucose agar slant (Difco) in a culture tube (20 by 150 mm), and incubated at 37°C. Growth was assessed at 7 and 14 days.

In vitro antifungal susceptibility studies. All test isolates were subcultured onto potato flakes agar slants (11) and grown for 2 weeks at 25°C for susceptibility studies conducted at the University of Texas Health Science Center at San Antonio. An inoculum suspension of annelloconidia was prepared by flooding each 2-week-old slant with 5 ml of

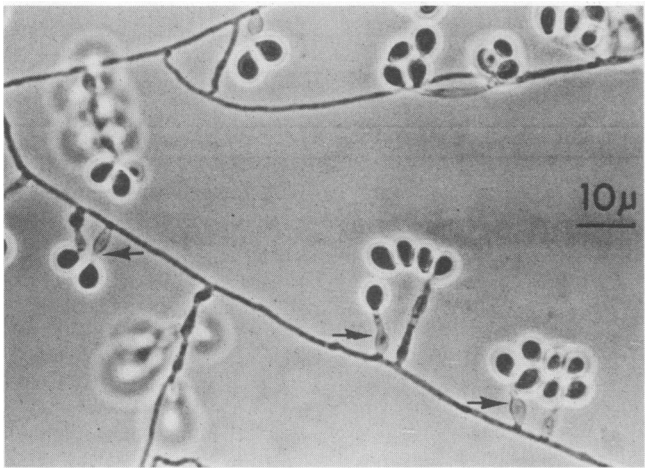

FIG. 1. Annelloconidia of *S. inflatum* formed in clusters at the apices of annellides with swollen bases (arrows).

sterile, distilled water and scraping the surface with a sterile Pasteur pipette. A portion of the conidial suspension was then transferred aseptically with a sterile pipette to yeast morphology broth test medium (YMB; Difco) and mechanically agitated, and the concentration of conidia was assessed with an AO Bright-Line hemacytometer (American Optical Corp., Buffalo, N.Y.). The final conidial concentration was adjusted to 10^4/ml and verified by streaking 0.001 ml of the suspension onto 100-mm petri plates containing Sabouraud glucose agar (20 ml per plate) for direct colony counts.

Each antimycotic agent investigated in these studies was prepared in a dilution series at 10× concentration and then stored at −70°C. Amphotericin B (50 mg; Fungizone I.V.; E. R. Squibb & Sons, Princeton, N.J.) and miconazole (10 mg; Monistat I.V.; Janssen Pharmaceutica, Piscataway, N.J.) were first dissolved in 10 and 1 ml, respectively, of sterile distilled water and then diluted with sterile distilled water. Ketoconazole (50 mg; Nizoral; Janssen Pharmaceutica) was first dissolved in 1 ml of 0.2 N HCl and then diluted with sterile distilled water.

A serial dilution series of itraconazole (50 mg; Janssen Pharmaceutica) was obtained by stirring the antimycotic agent into 10 ml of hot polyethylene glycol (75°C) for up to 4 h and then diluting this stock solution with polyethylene glycol. Fluconazole (10 mg; Pfizer Central Research, Groton, Conn.) was dissolved with gentle heating in 5 ml of sterile distilled water and then serially diluted with sterile water. A 0.1-ml portion of each dilution within the 10×-concentrated series of each antimycotic agent was stored at −70°C until ready for use.

A 0.9-ml sample of the 10^4 annelloconidial inoculum suspension in YMB medium of each test isolate was transferred aseptically with a sterile pipette to tubes containing 0.1 ml of each of the 10×-concentrated dilutions of each antimycotic agent. This effectively created the following test ranges of antimycotic agents (micrograms per milliliter): amphotericin B, 18.47 to 0.14; miconazole, 20 to 0.31; ketoconazole, 12.8 to 0.0125; itraconazole, 10 to 0.018; and fluconazole, 80 to 1.25. A 1-ml sample of the inoculum suspension without antimycotic agents was used as the growth control for all studies. Isolates were incubated at 35°C and visually inspected for growth after 48 h to determine the MIC of each antimycotic agent. Owing to their poor development at 35°C, the MICs for isolates of *S. brumptii* were determined at 25°C. The MIC for all isolates was defined as the first concentration in which no growth was noted. Triplicate tests were conducted with each isolate, and the MICs presented represent an average value derived from these multiple studies. *Paecilomyces varioti* Bainier (ATCC 36257) was tested concurrently as the control organism in all susceptibility determinations.

RESULTS AND DISCUSSION

Colonies of *S. inflatum* on PDA are rapid growing, flat, moist, spreading, olive-grey to black, arachnoid to woolly, with radial cracks at the center. Cottony sectors often develop with age. The conidiophores are hyaline to pale brown, unbranched, variable in length, and bear at their apices the conidiogenous cells. The latter are annellides which form singly along the vegetative hyphae and in small clusters of two to five at the ends of the conidiophores (Fig. 1). The annellides of *S. inflatum* are distinctive in that the bases are swollen and range in size from 1.5 to 3.0 μm (average, 2.5 μm) in diameter (Fig. 1). In contrast, the annellides of *S. apiospermum* are cylindrical and occur solitarily (Fig. 2) (1, 5, 8). The conidia of *S. inflatum* are hyaline to olivaceous, smooth, thin-walled, and ovoid and range in size from 2.0 to 5.0 by 3.0 to 13.0 μm (average, 3.4 by 5.3 μm). They are formed serially and collect in wet clumps at the tip of the conidiogenous cells (Fig. 1). The fertile portion of the annellide elongates as conidia are produced at the apex, resulting in the formation of successive ringlike areas of proliferation known as annellations (Fig. 3). In contrast to the evenly spaced, symmetrical annellations formed by *Scopulariopsis* spp., those of *Scedosporium* spp. are nonsymmetrical (Fig. 4). While conidiogenous cells of *S. inflatum* may occasionally proliferate sympodially, the irregular annellations and observation of laterally attached conidia may give the false impression that this is a common method of conidiogenous cell development.

In addition to the typical annelloconidia formed from percurrently proliferating annellides, *S. inflatum* NCMH 2698 formed chlamydosporelike structures. These globose, thick and rough-walled, light brown, one-celled, solitary conidia develop from the apices of cylindrical conidiophores which are laterally situated along the length of the vegetative hyphae (Fig. 5). They may be readily differentiated from the annelloconidia by their larger size, i.e., 5.5 to 8.0 μm (average, 6.6 μm) in diameter, thicker walls, and the pres-

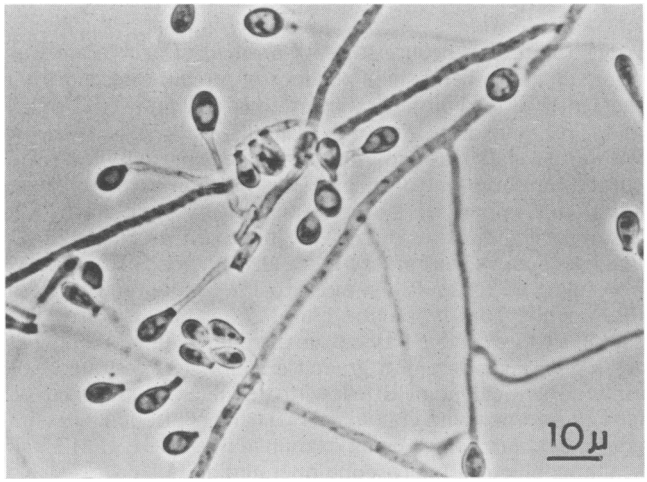

FIG. 2. Annelloconidia and annellides of *S. apiospermum*.

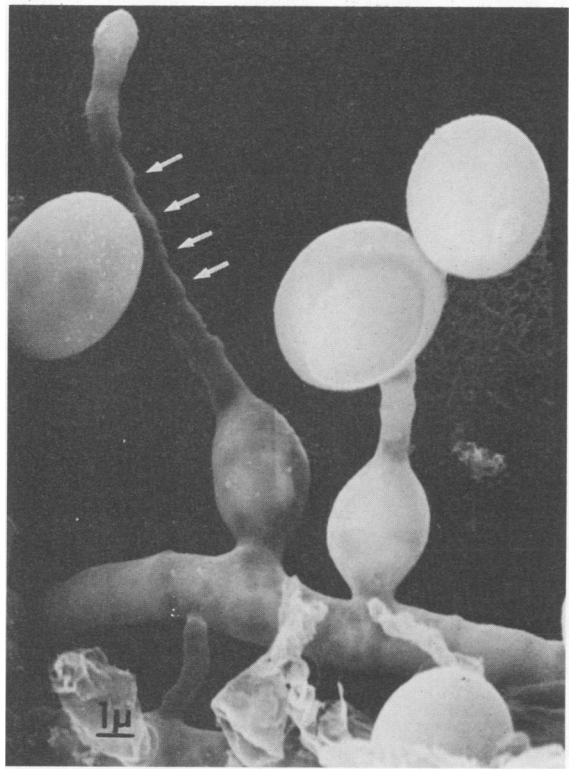

FIG. 3. Elongate fertile portion of an annellide of *S. inflatum* showing successive ringlike areas of proliferation (arrows).

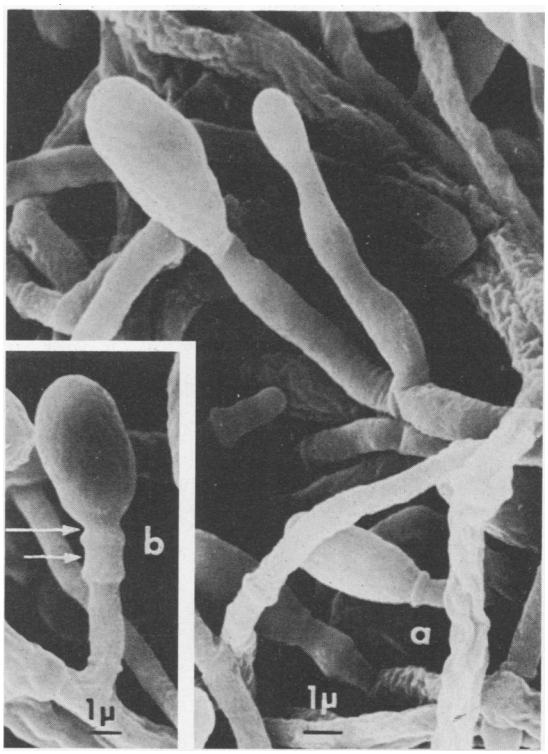

FIG. 4. *S. apiospermum*. (a) Developing annelloconidia on cylindrical annellides; (b) annellide with irregular-length annellations (arrows).

ence of annullar frills at the points where they break free of the conidiophores.

S. brumptii is a dematiaceous fungus that has been reported as the etiologic agent in a case of pulmonary hypersensitivity (4) and as the possible cause of mycetoma of the foot (2). The mold, like other members of the genus *Scopulariopsis*, forms annelloconidia at the apices of annellides (Fig. 6 and 7). Since the annellides of *S. brumptii* have swollen bases, the fungus might be confused with *S. inflatum* (Fig. 8). However, the arrangement of the conidia in wet masses at the apices of the annellides of *S. inflatum* as compared with the formation of conidia in dry chains by *S. brumptii* clearly differentiates the two organisms. In addition, the evenly spaced, smooth annellations of *S. brumptii* are quite different from the irregular, roughened annellations of *S. inflatum* when examined in Cellufluor mounts by fluorescence microscopy (Fig. 9).

Our attempts to induce the formation of the teleomorph of *S. inflatum* have not been successful, and consequently, whether the fungus will be associated as an anamorph of the genus *Pseudallescheria* Negroni et Fischer as is *S. apiospermum* remains to be determined. In addition, unlike *S. apiospermum*, a *Graphium* Corda synanamorph was not observed with any of the isolates of *S. inflatum*.

All isolates of *S. inflatum* grew on Sabouraud glucose agar at 37°C, but none (except NCMH2871) were capable of developing on Mycosel. In contrast, the isolates of *S. apiospermum* and that of *S. brevicaulis* grew at the elevated incubation temperature and on Mycosel. While all isolates of *S. brumptii* grew at 37°C, they were variable in the ability to grow on cycloheximide-containing media. Thus, neither of these two physiologic criteria can be used to differentiate *S. inflatum* and *S. brumptii*.

It has been reported that isolates which are found through in vitro susceptibility tests to be inhibited by <2, <6, and <7 μg of amphotericin B, ketoconazole, and miconazole per ml, respectively, may be considered, owing to the pharmacodynamics of the antimycotic agents, to be susceptible irrespective of the testing method (9). Utilizing these criteria, all isolates of *S. inflatum* (except NCMH2703 with miconazole) were found to be resistant to these antimycotic agents (in vitro MICs of >18.0, >12.0, and >20.0 μg of amphotericin B, ketoconazole, and miconazole per ml, respectively). In addition, all isolates were resistant to fluconazole (MIC, >80.0 μg/ml), and 10 of the isolates were also resistant to itraconazole (MIC, >10.0 μg/ml). However, four isolates

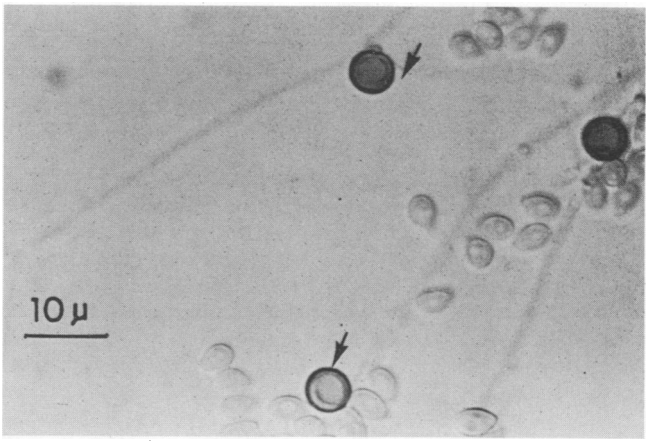

FIG. 5. Chlamydospores (arrows) of *S. inflatum*.

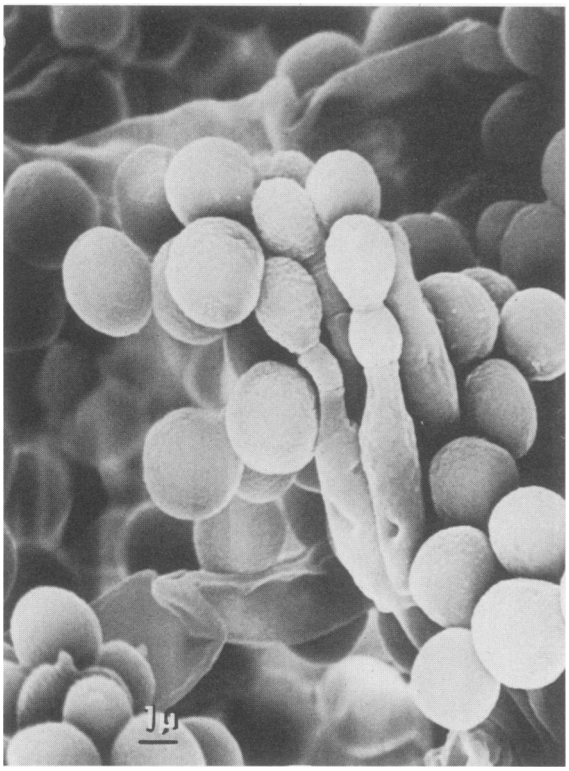

FIG. 6. Annellides and annelloconidia of *S. brumptii*.

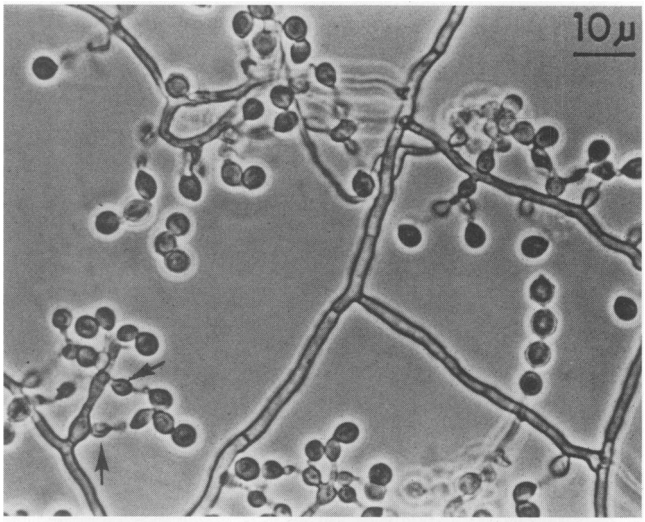

FIG. 8. Chains of annelloconidia of *S. brumptii* forming on annellides with swollen bases (arrows).

(NCMH1283, -2623, -2671, and -2704) were susceptible to itraconazole (MIC, ≤0.018 μg/ml). Data from the present investigation and published susceptibility data for *S. apiospermum* and its teleomorph *Pseudallescheria boydii* (Shear) McGinnis, Padhye, et Ajello (9) indicate MICs of amphotericin B, ketoconazole, and miconazole which range from 1.56 to >100, 0.25 to 8, and <0.016 to 4 μg/ml, respectively. Therefore, *S. inflatum* would appear to be more resistant to these three antimycotic agents than *S. apiospermum*. While all isolates of *S. apiospermum* were as resistant to fluconazole as were those of *S. inflatum*, six of the seven isolates studied were far more susceptible to itraconazole (MIC, <2.5 μg/ml). Only NCMH2894 was susceptible to >10.0 μg of this antimycotic per ml.

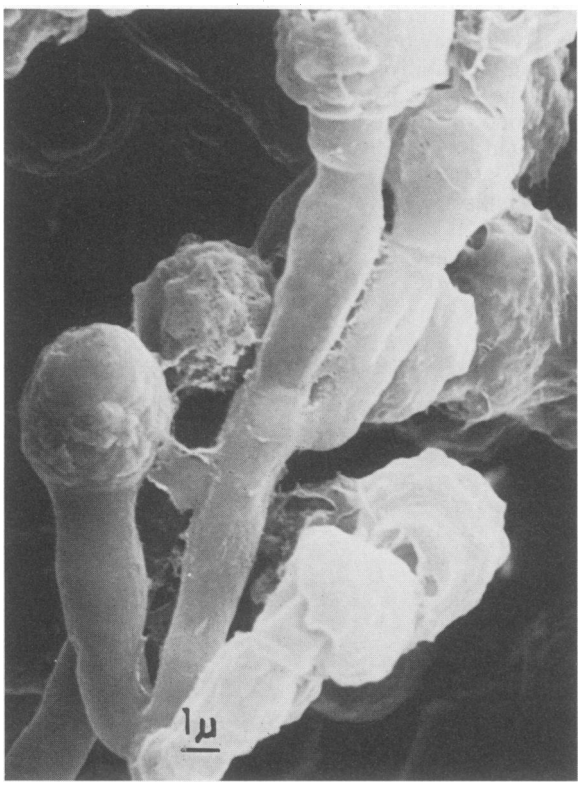

FIG. 7. Annellides and annelloconidia of *S. brevicaulis*.

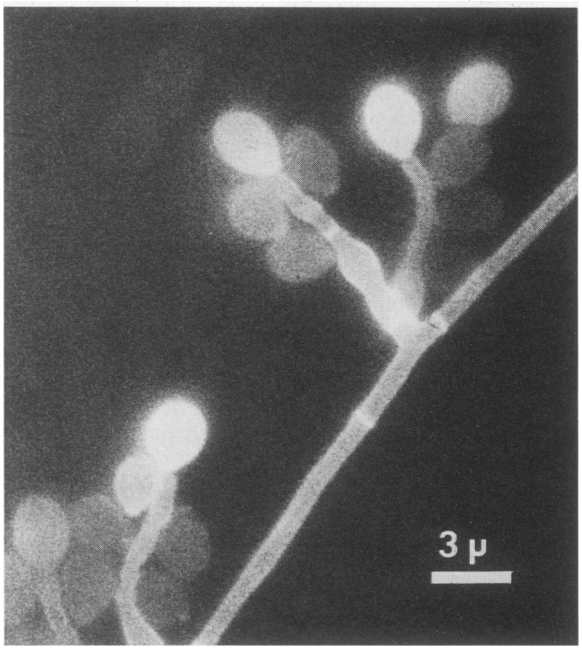

FIG. 9. Cellufluor-stained mount of *S. inflatum* showing irregular annellations.

Unfortunately, no standardized procedures have been established for antifungal susceptibility testing, and there have been few studies of the correlation between the in vitro susceptibility of a pathogenic fungus to an antifungal agent and the in vivo efficacy of the agent in the treatment of the infection. However, the resistance of *S. apiospermum* to amphotericin B was reported to correlate with the lack of efficacy of the antimycotic agent in the treatment of a subcutaneous infection (3). Similarly, the resistance of *S. inflatum* to polyene and imidazole antifungal agents correlates with treatment failures involving these antimycotic agents (personal communication, C. Wilson, Children's Hospital, Boston, Mass.; isolate NCMH2698). It appears that *S. inflatum* is a potentially highly virulent and aggressive pathogen which may be difficult to control because of, in part, its resistance to available antifungal agents.

On the basis of the results described, the following simple key can be used in differentiating clinically encountered isolates of the four taxa:

A. Annelloconidia formed singly and collecting in clumps at the apices of the annelides.

B. Annellides long and tapering toward apices, bases swollen, colony fast growing on PDA, chlamydospores occasionally present.

Scedosporium inflatum

BB. Annellides cylindrical, colony slow growing on PDA, chlamydospores absent.

Scedosporium apiospermum

AA. Annelloconidia formed singly in dry chains at the apices of the annellides.

B. Annellides long and tapering toward apices, bases swollen, conidia brown to black in mass, colony slow growing on PDA, grey.

Scopulariopsis brumptii

BB. Annellides cylindrical or with slightly swollen bases, conidia subhyaline in mass, colony fast growing on PDA, tan.

Scopulariopsis brevicaulis

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