JOURNAL OF CLINICAL MICROBIOLOGY, May 1990, p. 1009–1015
0095-1137/90/051009-07$02.00/0

Vol. 28, No. 5

Microbiology and Potential Virulence of *Sporothrix cyanescens*, a Fungus Rarely Isolated from Blood and Skin

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Received 28 August 1989/Accepted 18 January 1990

***Sporothrix cyanescens* has been recovered from blood and a finger lesion at several medical centers in the United States. The morphology and physiology of these and three additional isolates were studied. *S. cyanescens* was distinguished from *S. schenckii* and *S. fungorum* by white to lavender colonial pigmentation and from *S. schenckii* also by the formation of secondary conidia. All isolates of *S. cyanescens* grew well at 37°C, were cycloheximide susceptible, strongly urease positive, and benomyl resistant, failed to hydrolyze starch, and were inhibited by sodium chloride in vitro at a concentration of ≥12%. Study of *S. cyanescens* in a murine model by using intravenous inoculation failed to demonstrate an invasive pathogenic potential. The validity of the transfer of *S. cyanescens* to the new genus *Cerinosterus* Moore is discussed.**

The genus *Sporothrix* comprises a large number of species, many of which have teleomorphs in the genus *Ophiostoma* (formerly included in the genus *Ceratocystis*). The dimorphic fungus *Sporothrix schenckii* is a well-known pathogenic member of this genus. *Sporothrix cyanescens* was described by de Hoog and de Vries in 1973 (5) on the basis of six isolates from human skin and air samples. Of three isolates from human specimens, one was suspected to be the cause of mycosis of human skin and two were isolated from skin of the scalp and beard areas. In 1985, *S. cyanescens* was isolated on a single occasion from a blood specimen from two lymphoma patients undergoing antineoplastic chemotherapy (J. L. Harris, D. F. Dunbar, L. Sigler, A. Chapman, and L. Boucher, Abstr. Annu. Meet. Am. Soc. Microbiol. 1987, F83, p. 402). One patient, who had a long and complicated medical history, received no antifungal drugs and remains alive. The other patient, whose medical history was relatively unremarkable except for the lymphoma, responded well to amphotericin B but died of other complications. In neither of these two cases was there any documented evidence of fungus infection or involvement in patient mortality. However, its occurrence in specimens from normally sterile body sites, its isolation from debilitated individuals, and its thermotolerance suggested that *S. cyanescens* has the potential to be an opportunistic pathogen in the compromised host.

Following the report of these isolations from lymphoma patients, we have identified 11 additional isolates of *S. cyanescens* from blood culture specimens and 1 isolate from a finger lesion. The increased frequency of recovery of this mold, which has occurred in widely separated areas of the United States, raises further questions as to the potential pathogenicity of *S. cyanescens*. Although *S. cyanescens* is seldom reported, it is likely that this little-known fungus goes unrecognized in many laboratories. In this study, we describe the morphology and physiology of human and environmental isolates of *S. cyanescens* in comparison with isolates of *S. schenckii* and a morphologically similar species, *Sporothrix fungorum* (5). Because the pathogenic potential of *S. cyanescens* is not known, we examined the virulence of one isolate from a finger lesion in experimental animals.

MATERIALS AND METHODS

Cultures examined. A total of 14 clinical isolates of *S. cyanescens* referred to or isolated by the Texas Department of Health, Austin, the New York State Department of Health, Albany, and the Centers for Disease Control, Atlanta, Ga., were deposited in the University of Alberta Microfungus Collection (UAMH) along with the ex-type strain (culture derived from the type specimen; 19) and 2 environmental isolates (Table 1). Several isolates of *S. schenckii* and *S. fungorum* were also examined (Table 1).

Morphology and thermotolerance. Isolates were preserved at UAMH by freezing agar slants at −20°C (2) and by lyophilization and storage at 4°C. Fourteen day-old stock cultures were obtained from either the frozen stock or lyophil ampoule by subculture onto Pablum cereal agar without antibiotics (CER; 17) and stored at 4°C. These stocks were used for inoculating all media. The gross colony morphology of each isolate at 25°C was examined on Phytone yeast extract agar (PYE; BBL Microbiology Systems, Cockeysville, Md.), Sabouraud dextrose agar (SAB; Difco Laboratories, Detroit, Mich.), and potato dextrose agar (PDA; Difco) at 25°C. A portion of growth from CER (approximately 1 mm^2) was transferred to the center of the petri dish, and diameters of colonies and gross morphological features were examined at 7-day intervals for 4 weeks. Color terms were determined using the color standards of Kornerup and Wanscher (11). Thermotolerance was determined by measuring colony diameters on PDA at 37 and 42°C after 7 and 14 days. Ability to convert to a yeast phase at 37°C was determined by inoculating brain heart infusion agar slants (Difco) covered with 2 to 3 drops of brain heart infusion broth (Difco). Microscopic mounts were prepared at 7 and 21 days and examined to determine if budding cells were produced. Microscopic morphology at 25°C was exam-

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TABLE 1. *Sporothrix* strain numbers and sources of isolates

Strain	Source[a]
S. cyanescens	
UAMH 3276	Air; Rabat, Morocco; J. Chabert (5140J)
UAMH 3277	Air; Rabat, Morocco; J. Chabert (5189)
UAMH 3677	Ex-type, human skin; Groningen, The Netherlands; T. F. Visser (31285)=CDC B-1868=CBS 357.73
UAMH 5108	Case 1 (blood, male, 64 yr, malignant lymphoma [C stage, IV], Michigan), I. Weitzman (strain 64)
UAMH 5374	Case 2 (blood in thioglycolate broth [Difco], female, 61 yr, lymphocytic lymphoma and metastasis to bone marrow, now deceased, Texas), J. Harris, =BY6-1038
UAMH 5972	Blood (Septi-Chek system), adult female, elevated temp on steroid therapy, New York; M. Kemna, =M756-87
UAMH 6053	Blood (Dupont Isolator), elderly male diabetic, bladder tumor, kidney removed, now deceased, Texas; J. Harris, =BY8-566
UAMH 6054	Blood (Dupont Isolator), newborn male, respiratory distress, suspected sepsis, on antibacterial antibiotics 7 days, Texas; J. Harris, =BY8-579
UAMH 6107	Blood, Connecticut; A. Padhye, =CDC B-4620
UAMH 6108	Blood, A. Padhye, =CDC B-4621
UAMH 6120	Blood, A. Padhye, =CDC B-4507
UAMH 6164	Blood, A. Padhye, =CDC B-4683
UAMH 6281	Blood (Septi-Chek system), male, 70 yr, respiratory distress, chest pain, now deceased, New York; M. Kemna, =M883-87
UAMH 6285	Case 3 (finger lesion, male, greenhouse worker, 72 yr, New York); M. Kemna, =M70-88
UAMH 6326	Blood (Septi-Chek system), child, routine blood culture on admission, Maine; M. Kemna, =M727-87
UAMH 6327	Blood, Virginia; A. Padhye, =CDC B-4509
UAMH 6482	Blood, male, 54 yr, history of bronchiectasis since teens, no antifungal therapy, Texas; J. Harris, =BY8-1203
S. fungorum	
UAMH 3678	Ex-type, old fruit body *Fomes* sp., Federal Republic of Germany; W. Gams, =CDC B-1870=CBS 259.70
S. schenckii	
UAMH 3613	Arm lesions, male physician, Alberta, Canada; M. Kelm (MU7887)
UAMH 3614	Forearm lesions, female, 43 yr, Alberta, Canada; M. Kelm (MU8380)
UAMH 5829	Arm lesion, male, tree nursery worker, 23 yr, Alberta, Canada; P. Kibsey (MY838)
UAMH 5870	Left hand lesion, male, 60 yr, Saskatchewan, Canada; H. Congly (1631M)

[a] Collections: CDC, Centers for Disease Control, Atlanta, Ga.; BY, Texas Department of Health, Austin; M, New York State Department of Health, Albany; CBS, Centraalbureau voor Schimmelcultures, Baarn, The Netherlands.

ined by 6- to 10-day-old slide culture preparations using CER or by tease mounts in lactofuchsin or glycerin jelly.

Physiological tests. The isolates were evaluated for their responses to the following tests: (i) urea hydrolysis by using Christensen's urea broth (8) read at 4, 7, and 14 days; (ii) resistance to cycloheximide at a concentration of 0.4 mg/ml by recording colony diameters on Mycosel agar (BBL) at 14 days; (iii) tolerance to sodium chloride-amended medium (9); (iv) starch hydrolysis by the method of Weitzman et al. (25); and (v) tolerance to the fungicide benomyl (21). For testing of salt tolerance, inoculum (1 mm^2) was transferred to SAB agar supplemented with 3 or 5% (wt/vol) NaCl. Similar inoculum was prepared from CER stocks of isolates showing diameters of ≥5 mm on SAB with 5% NaCl after 14 days and was used to inoculate SAB amended with 7, 10, 12, or 15% NaCl. Colony size was recorded after 14 days, and colonies with diameters ≤3 mm were considered to be strongly inhibited at that concentration of salt. *Exophiala werneckii* (*Phaeoannellomyces werneckii*) (UAMH 3962), a strongly salt-tolerant species (9), was used as a control for this test. Benomyl resistance was measured by recording growth rates after 14 days on unamended modified Melin-Norkrans medium (15) compared with Melin-Norkrans medium amended with benomyl in acetone (2 μg/ml). Colonies with diameters of ≤5 mm were considered to be susceptible to benomyl.

Virulence studies. A single isolate (UAMH 6285) was selected for the study of virulence in a murine model. Inoculum was prepared from growth on a PDA slant incubated at 30°C for 7 days. Surface growth was harvested in sterile 0.85% saline by using sterile wooden applicator sticks and was filtered through a sterile Kimwipe (Kimberly-Clark, Roswell, Ga.). The suspensions were sedimented by centrifugation, washed once in sterile saline, and suspended to give 2.5×10^8 cells per ml consisting of single conidia.

Male NYLAR outbred mice weighing 18 to 20 g were used. One group was treated with 250 mg of cortisone acetate (Sigma Chemical Co., St. Louis, Mo.) per kg prepared as an aqueous suspension and given subcutaneously on the day before inoculation. Each of four normal mice and cortisone-treated mice received 5×10^7 cells in 0.2-ml volumes in a lateral tail vein. Animals were monitored once a day for 64 days. At 31 and 41 days after inoculation, one mouse from each group (normal and cortisone treated) was sacrificed by CO_2 overdose and lungs, livers, spleens, kidneys, brains, testes, and inguinal lymph nodes were cultured as described previously (22). On day 64, the remaining mice were sacrificed and the above organs were pooled from two animals within each group and cultured. Representative organs from selected mice were fixed in neutral buffered Formalin and embedded in paraffin, and adjacent sections were stained with either Gomori-Grocott methenamine silver or hematoxylin and eosin. A clinical isolate of *S. schenckii* (M665-88) was used as a virulence control.

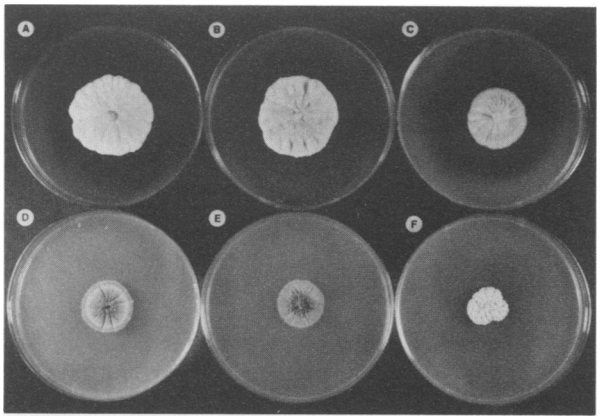

FIG. 1. Colonies of *Sporothrix* strains on PYE after 2 weeks at 25°C. (A to C) *S. cyanescens* UAMH 6281, 6326, and 6108, respectively (note that diffusing pigment darkens medium); (D and E) *S. schenckii* UAMH 5829 and 3613, respectively; (F) *S. fungorum* UAMH 3678.

RESULTS

Colony characteristics and growth rates at 25°C. *S. cyanescens* is a moderately rapidly growing fungus, forming flat, furrowed, or slightly raised, velvety to powdery, occasionally glabrous colonies which are initially white to creamy white (3A2-4A3). The most distinctive feature is the development, usually within 2 weeks, of a grey-blue (16B2) to lavender (17A2) surface color, usually accompanied by a red or blue diffusing pigment and a reverse color ranging from creamy white (4A3) to dark violet (19F5) or burgundy (12E7). Coloration of the colony and development of a diffusing pigment was most pronounced on PYE and PDA (Fig. 1A to C and 2). No lavender surface color developed in any colony on SAB within 14 days, and the diffusing pigment was absent for most strains (Fig. 3). The isolates were variable in their production of the lavender color, sometimes developing it on one medium but not on another, and in their production of diffusing pigment, e.g., UAMH 6108 (Fig. 1C and 2B). They were also variable in their growth rates, with diameters ranging from 21 to 40 mm on PYE, 16 to 36 mm on PDA, and 16 to 30 mm on SAB after 2 weeks (Table 2). A pronounced musty odor was detectable in cultures after 2 to 3 weeks of growth, especially when several isolates were incubated together in a small growth chamber or when the petri dishes were opened for subculture.

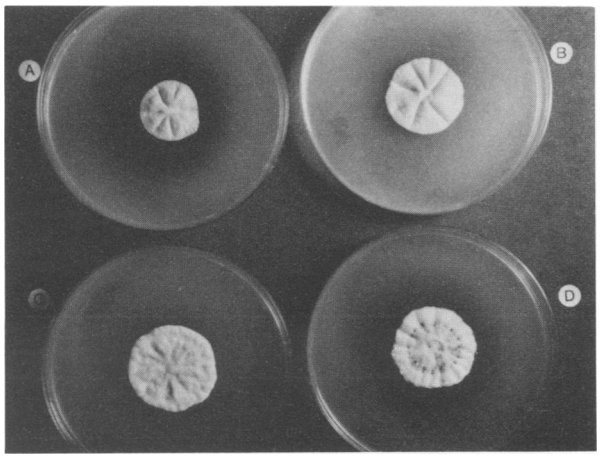

FIG. 2. Colonies of *S. cyanescens* on PDA after 2 weeks at 25°C. (A) UAMH 3677; (B) UAMH 6108; (C) UAMH 5374; (D) UAMH 6326. Note strong diffusing pigment in panel A and no pigment in panel B.

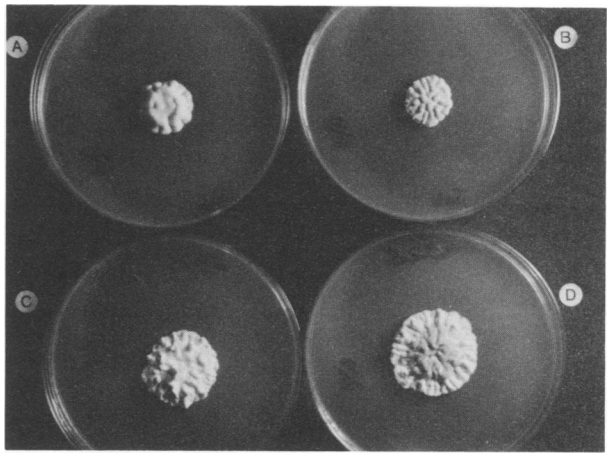

FIG. 3. Colonies of *S. cyanescens* on SAB after 2 weeks at 25°C showing slower growth, cerebriform appearance, and absence of diffusing pigment. (A) UAMH 3677; (B) UAMH 6108; (C) UAMH 5374; (D) UAMH 6326.

The colonies of *S. fungorum* were slower growing (Fig. 1F), ranging from 12 to 16 mm in diameter on the different media after 2 weeks (Table 2), but similar in topography and texture. However, they remained white on all media and failed to develop a diffusing pigment or pronounced reverse color. The growth rates of *S. schenckii* were similar on all media, with mean diameters of colonies ranging from 18 to 21 mm (Table 2). Colonies were initially glabrous and smooth or furrowed and pale creamy white, gradually dark-

TABLE 2. Comparison of *S. cyanescens* with *S. schenckii* and *S. fungorum* by growth rate and physiological tests[a]

Growth rate and physiology	*S. cyanescens*	*S. schenckii*	*S. fungorum*
Mean colony diam (mm)			
PYE (25°C)	26	19	16
SAB (25°C)	22	18	16
MYC[b] (25°C)	0	19	12
PDA (25°C)	29	21	12
PDA (37°C)	29	14	0
PDA (42°C)	13[c]	0	ND
Urea hydrolysis			
Day 4	+	−	−
Day 7	+	+–weak	−
Day 14	+	+	−
Benomyl tolerance	+	−	+
Salt tolerance	12–15	7	>15
Starch hydrolysis	−	−	−

[a] Number of strains tested: 17 *S. cyanescens*; 4 *S. schenckii*; and 1 *S. fungorum*. Mean colony diameter was measured at 2 weeks. ND, Not determined. Salt tolerance was measured as the percent MIC of NaCl in amended SAB (wt/vol) at 2 weeks. Colonies with diameters ≤3 mm were considered to be strongly inhibited.

[b] MYC, Mycosel agar.

[c] Mean diameter for 13 isolates (4 did not grow).

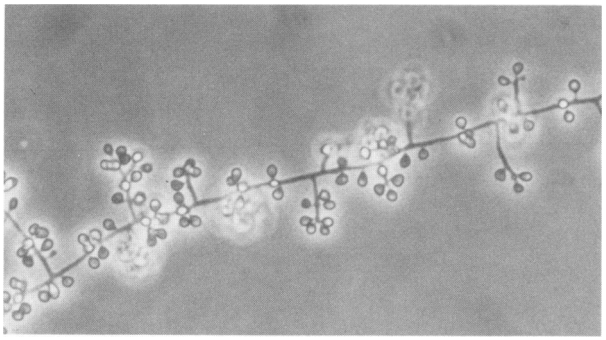

FIG. 4. UAMH 5870, showing solitary conidia borne sympodially in rosette arrangement and sessile conidia typical of *S. schenckii*. Magnification, ×610.

ening within 14 days on PYE (Fig. 1D and E) or PDA to dark brown or black when more aerial mycelium formed. Colonies on SAB tended to remain glabrous and paler, but some brown pigmentation occurred by 28 days.

Physiological studies. The physiological features are compared in Table 2. The species differed in their tolerance to cycloheximide, benomyl, and sodium chloride and in their urease activity. *S. cyanescens* was susceptible to cycloheximide, showing no growth on Mycosel agar, whereas *S. schenckii* and *S. fungorum* were resistant. Both *S. cyanescens* and *S. fungorum* grew at the same rate on benomyl-free and benomyl-amended medium, but *S. schenckii* was strongly inhibited. *S. cyanescens* was urease positive, reacting strongly by 4 days. *S. schenckii* was weakly urease positive by 7 days and strongly positive by 14 days, but *S. fungorum* failed to hydrolyze urea. *S. schenckii* was inhibited by 7% sodium chloride, whereas *S. fungorum* was tolerant of 15% salt. The majority of isolates of *S. cyanescens* were strongly inhibited by salt concentrations at 12 or 15%. One isolate (UAMH 6107) was inhibited at a concentration of 7%, and two other isolates (UAMH 6108 and 6281) were inhibited at 10%. None of the species hydrolyzed starch.

Microscopic morphology at 25°C. In *Sporothrix* species, small, usually apiculate conidia are borne sympodially from narrow denticles, which are often arranged in a rosette pattern (3). The conidiogenous cells occur in an intercalary position or at the ends of short, erect, narrow (usually 1- to 3-μm wide) conidiophores. In the majority of species, including *S. schenckii*, only a single conidium is borne on each denticle (Fig. 4). This species is further distinguished from most other species by the formation of small, solitary dark brown conidia which are formed directly on the sides of the hyphae (3, 18). As these conidia develop, colonies begin to turn brown.

In *S. cyanescens* and *S. fungorum*, the primary conidia proliferate to form one or more secondary conidia and neither produces the solitary dark brown conidia. Although the microscopic morphology of these species has been described in detail elsewhere (5), we provide a brief description and illustrations of both species.

The terminal conidiogenous cells of *S. cyanescens* are frequently rather inflated (up to 5 μm across) and bear several flattened denticles; the intercalary conidiogenous cells consist of one or two lateral denticles or are inflated and bear several denticles. Conidia are borne in two series; primary conidia (4 to 9 by 1.5 to 3.0 μm) are hyaline, ellipsoidal, smooth, and bear 1 to 3 pyriform secondary

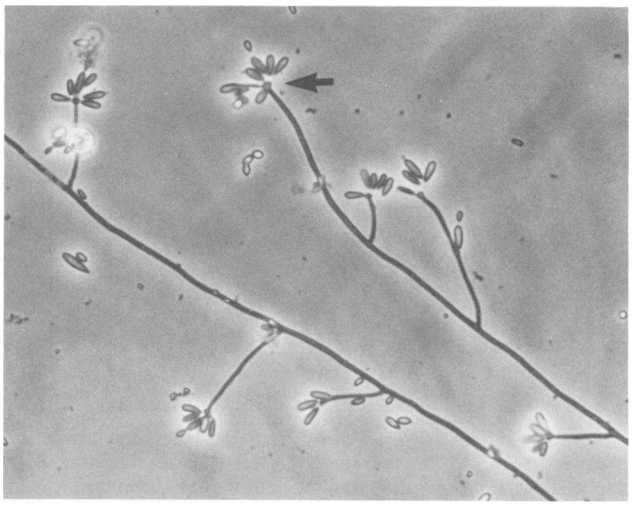

FIG. 5. *S. cyanescens* (UAMH 6054) in slide culture preparation on CER. The rosette pattern of primary conidia bearing secondary conidia may be seen in gently handled slide cultures. Note inflated conidiogenous cells (arrows). Magnification, ×580.

conidia (2.5 to 5 by 0.8 to 2 μm) on small denticles (Fig. 5 and 6). Primary conidia up to 25 μm were reported in the original description, but we have not seen conidia of this length. Most isolates sporulate rapidly, and the primary conidia detach readily from the denticles. When detached from the parent mycelium, the chains of conidia resemble budding yeast cells (Fig. 6).

S. fungorum can be differentiated by its long conidiophores and small globose secondary conidia (1.5 to 2.5 μm wide) (Fig. 7).

Growth rates and microscopic morphology at 37 and 42°C. The growth rate of *S. cyanescens* at 37°C on PDA was similar to that at 25°C (Table 2), whereas *S. schenckii* grew

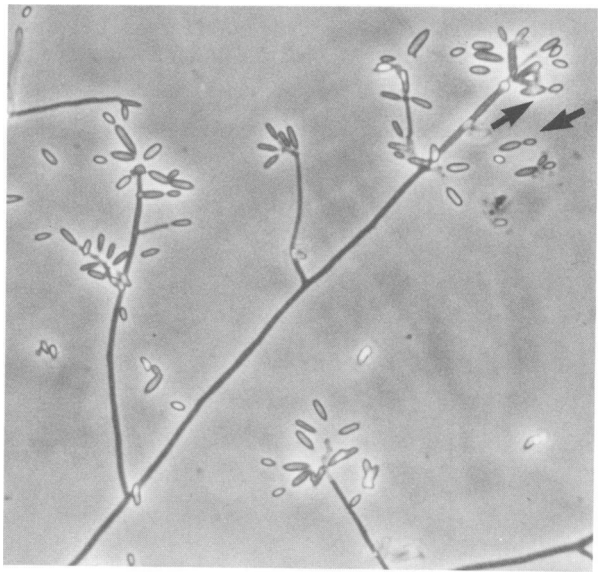

FIG. 6. *S. cyanescens* (UAMH 5972). Primary and secondary conidia are readily detached, but when they remain together, they bear a striking resemblance to budding yeast cells (arrows). Magnification, ×610.

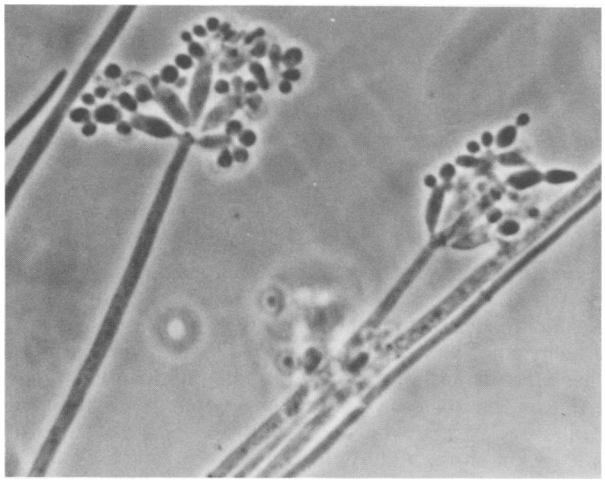

FIG. 7. *S. fungorum* (UAMH 3678). Conidia are borne in two series from long conidiophores. Ellipsoidal primary conidia bear small globose secondary conidia. Magnification, ×1120.

slightly slower and *S. fungorum* was completely inhibited at 37°C. At 42°C on PDA, *S. cyanescens* attained diameters of 9 to 19 mm, but four strains did not grow. *S. schenckii* did not grow.

When grown on brain heart infusion agar at 37°C, *S. schenckii* colonies were mucoid and microscopic examination showed typical yeast cells. *S. cyanescens* colonies were moist, waxy, and soft. Microscopic mounts showed hyphae and many conidia in chains of two, appearing similar to budding yeast cells (Fig. 8).

Virulence studies. All mice appeared to be normal and without signs of infection throughout the 64-day observation period. Samples from one normal mouse at day 31 yielded one colony of *S. cyanescens* on each of two cultures from liver homogenate. At this same time, samples from the cortisone acetate-treated mouse yielded six and eight colonies of *S. cyanescens* from duplicate cultures of kidney homogenate. All cultures were negative from the remaining animals sampled on days 41 and 64. No fungi were seen in stained sections of lung, liver, spleen, testes, or brain from a normal mouse sampled at day 31.

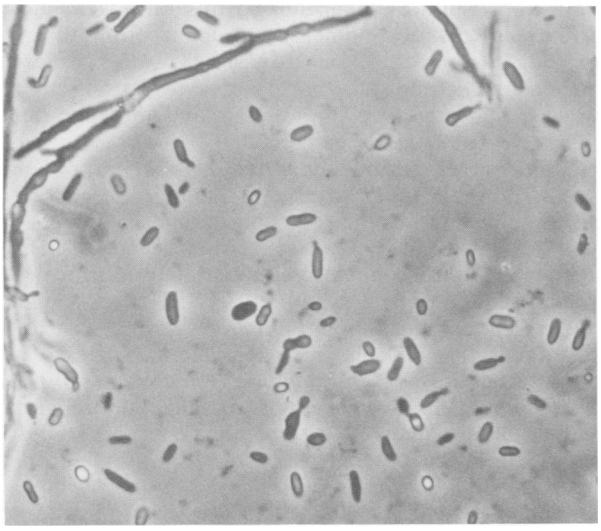

FIG. 8. *S. cyanescens* (UAMH 3677) after growth on brain heart infusion agar at 37°C for 21 days showing hyphae and detached conidia with a yeastlike morphology. Magnification, ×610.

DISCUSSION

Identifying features, key to the species, and taxonomic relationships. Several contrasts can be made between *S. cyanescens* and the well-known pathogen *S. schenckii*. Secondary conidia are not formed on the primary conidia of *S. schenckii* as they are in *S. cyanescens*. Pigmentation may develop in *S. schenckii*, but it is melaninlike and cell bound, occurring with development of the lateral, darkly pigmented conidia. In contrast, *S. cyanescens* colonies develop a distinct lavender color usually accompanied by a diffusing pigment. The lavender pigmentation is not expressed on SAB but is evident on media such as PYE or PDA. *S. cyanescens* is thermotolerant, with most strains able to grow at 42°C, susceptible to cycloheximide, and strongly urease positive by day 4. These features differentiate it from both *S. schenckii* and *S. fungorum*. While the microscopic morphology of the latter is similar to *S. cyanescens*, *S. fungorum* is unlikely to be encountered in a clinical setting since it is known so far to come only from decaying fleshy fungi. *Sporothrix catenata* (4), which has been isolated from calf skin, differs from *S. cyanescens* in that conidia form in longer chains and the colonies remain white.

Key to the species.

1. Solitary conidia produced in rosettes at apices of slender conidiophores; sessile darkly pigmented conidia usually present. *S. schenckii*
1. Secondary conidia produced. 2
2. Colonies white to lavender, red or blue diffusing pigment usually present, cycloheximide susceptible. *S. cyanescens*
2. Colonies remaining white on all media, no diffusing pigment, cycloheximide resistant. *S. fungorum*

A comparative study of *Sporothrix* glycoproteins (12) also demonstrated differences between the species. Rhamnose was present in *S. schenckii* and absent in *S. cyanescens*. Relative to other species, the latter had a high proportion of galactose and glucose and a low proportion of mannose.

Similar findings were obtained by Weijman and de Hoog (23) in a study of cell wall carbohydrates of a large number of *Sporothrix* species. They considered L-rhamnose or xylose in cell walls to be significant markers of teleomorph affinity and divided the genus into three sections. Rhamnose-containing species suggested a link to the ascomycete family Ophiostomataceae since *S. schenckii* and several species of the genus *Ophiostoma* (formerly included in the genus *Ceratocystis*) demonstrated this component, whereas non-rhamnose-containing species were linked to the ascomycetous yeasts. The argument was further supported by the high mannose content of the latter species and ultrastructural evidence of a simple septum with Woronin bodies in *S. schenckii* (20). The only xylose-containing species, *Sporothrix luteoalba*, was considered to be basidiomycetous, and this connection was confirmed by demonstration of a dolipore septum with a nonperforate pore cap (20) and a subsequent study (14) linking this species to teleomorphs in the genus *Cerinomyces* (members of the family Dacrymycetaceae). *S. cyanescens* was unusual in its lack of both L-rhamnose and xylose, its low mannose content, and its simple dolipore septum without a pore cap (20, 23). On the basis of these findings, Weijman and de Hoog placed *S.*

cyanescens in *Sporothrix* section *Luteoalba* along with *S. luteoalba*. While they recognized the heterogeneity of the genus *Sporothrix*, they argued against its subdivision, since the sections could not be distinguished by morphological features. Recently, Moore (16) transferred both species (with *S. luteoalba* as the type species) to the new genus *Cerinosterus* for species with a *Sporothrix* morphology, xylose in the cell wall, and dolipore septa.

That *S. cyanescens* is linked to the basidiomycetes is supported by ultrastructural evidence, strong urease activity, and benomyl tolerance. Previous studies (21) have shown that the addition of the fungicide benomyl to agar media may be useful to indicate teleomorph affinity since most ascomycetous, but not endomycetous, fungi are severely restricted by benomyl at concentrations of 2 μg/ml. In our study, *S. schenckii* was inhibited but *S. fungorum* (teleomorph *Stephanoascus* [6]) and *S. cyanescens* were resistant.

Although the teleomorph of *S. cyanescens* is unknown, Khan and Kimbrough (10) reported that reduced dolipores without pore caps occurred most commonly in some members of the families Filobasidiaceae and Ustilaginaceae (smuts). According to Kwon-Chung (13), members of the family Filobasidiaceae have yeast anamorphs (in the genera *Cryptococcus* and *Rhodotorula*), are nonfermentative, assimilate inositol, hydrolyze urea, and produce soluble starch. Haploid stages of smuts have not been well described, but studies are in progress (1). In culture, some species are mycelial rather than yeastlike and then the microscopic morphology may be very similar to that of *Sporothrix* spp. One of the main distinguishing features is the propensity of the vegetative hyphae to lose their cytoplasmic contents while cytoplasm becomes concentrated in the sporulating regions (L.S., unpublished data). This feature is readily observed in slide culture preparations of species of the genus *Ustilago*, such as *U. hypodytes* (7). As noted by Boekhout (1), smutlike fungi are commonly isolated from air, blood, and other substrates, but our ability to identify them with any degree of accuracy is impeded by lack of detailed studies of their in vitro characteristics.

A connection between *S. cyanescens* and these families of heterobasidiomycetes is tenuously based on septum structure. *S. luteoalba* and *S. cyanescens* appear to differ in septum structure, cell wall constituents, morphology, and connections to teleomorphs. Since we find no evidence to suggest that these species are related, the appropriateness of the taxonomic transfer proposed by Moore (16) requires further consideration. We therefore opt to continue use of the name *S. cyanescens* for now.

Pathogenicity. Since 1985, we have studied 13 isolates of *S. cyanescens* from blood culture specimens and 1 from a skin lesion. Case histories of patients for whom we were able to obtain detailed information (Table 1) demonstrated that this fungus was recovered primarily from individuals who were immunocompromised or debilitated. Weitzman (24) discussed the significance of saprophytic fungi as agents of infection in immunocompromised patients. She cautioned that saprophytes which are repeatedly isolated from a normally sterile site must be given consideration as a possible disease agent, especially if there is corroborating direct microscopic evidence in the primary specimen or evidence of invasion by histopathology.

Two of our patients (Table 1, cases 1 and 2) had lymphocytic lymphoma, for which they received chemotherapy. The male patient was admitted to the hospital many times for problems including recurrent *Pseudomonas* pneumonia and severe peripheral vascular disease and for a colectomy, a bypass graft, and amputation of the left leg below the knee. During one admission, *S. cyanescens* was recovered from two blood culture specimens drawn on 2 successive days. The patient received no antifungal therapy and survives, although he has had several subsequent admissions to the hospital for various complications. The female patient developed fungemia with fever, and two of four blood culture specimens, one from a central venous catheter and one drawn from the left forearm, grew *S. cyanescens*. She responded to amphotericin therapy but subsequently died of hemorrhagic complications. In neither of these cases did we find documented evidence of active invasion by *S. cyanescens*; the implication of infection was based primarily on isolation from normally sterile sites and observation of the thermotolerance of this fungus. A third case involved a greenhouse worker who developed a strictly localized necrotic lesion on the right index finger following a prick from a blackberry briar. When treatment with antibacterial antibiotics showed no improvement, he was treated with potassium iodide, but he refused to continue therapy. *S. cyanescens* was isolated from the necrotic lesion. No biopsy was performed, and the results of the direct examination are unknown.

S. cyanescens appears to be a skin commensal which may be introduced into the blood by a venous catheter or during collection of the specimen. The frequency of its occurrence on skin has yet to be evaluated; the use of cycloheximide-containing media for isolation of fungi from dermatologic specimens would preclude the routine isolation of this mould from skin scrapings. Although we initially suspected contaminated blood culture systems as a possible source of the fungus, the use of several different types of systems (Table 1) argued against that possibility.

Tests for pathogenicity conducted previously and in this study suggest that *S. cyanescens* has low invasive potential. Several of the originally described strains were inoculated on the skin of guinea pigs (5). After 6 weeks, no lesions had developed at the inoculation sites on the test animals. Our strain (UAMH 6285) did not behave as a virulent fungus in our model using either normal or cortisone-treated mice. In studies using normal mice, clinical isolates of *S. schenckii* produced 100% mortality over a 12- to 24-day period post-inoculation. Cultures taken during this time were grossly positive (colonies too numerous to count) from all organs sampled. In contrast, strain UAMH 6285 of *S. cyanescens* produced neither mortality nor observable signs of infection and was eliminated from both normal and cortisone-treated animals by 41 days after inoculation.

Our findings are further supported by the report of Kurata (12) that only *S. cyanescens* was unreactive among 11 species studied in skin tests on sporotrichosis patients.

The habitat of *S. cyanescens* has not been well defined. Catalogues of the Commonwealth Agricultural Bureaux International Mycological Institute, Kew, United Kingdom, and the Centraalbureau voor Schimmelcultures, Baarn, The Netherlands, list isolates from sources such as air, plant material from *Eucalyptus pauciflora*, *Eucalyptus transcontinentalis*, and *Vitis vinifera* in Australia and India, and pomegranate in Israel. At the completion of this study, the senior author encountered *S. cyanescens* growing on the embryo of *Prunus persica* var. *nucipersica* (nectarine).

In summary, we conclude that, although *S. cyanescens* grows well at body temperature, it does not appear to be virulent. Since the fungus has been encountered in clinically significant situations, we have presented information to alert

the diagnostic laboratory of the features for its identification and differentiation from the pathogenic *S. schenckii*.

ACKNOWLEDGMENTS

We thank L. Boucher, formerly of Seaway Hospital, Trenton, Mich., for providing information on one of the cases in this study; A. A. Padhye, Centers for Disease Control, Atlanta, Ga., for providing several cultures; T. J. Walsh, National Cancer Institute, Bethesda, Md., for interpreting the experimental histopathology; R. T. Moore for bringing *Cerinosterus* to our attention; and G. Rankin for technical assistance.

Part of this work was supported by grants to L. Sigler from the University of Alberta Support for the Advancement of Scholarship Fund and by Natural Sciences and Engineering Research Council operating grant A3755.

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