Comments on the Differentiation of a Gliomastix Isolated from Sputum versus Sporothrix schenckii*

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The criteria for the identification of *Sporothrix schenckii* are carefully described in standard text books on medical mycology. In these, however, it is assumed that the laboratory worker is dealing with clinical material from which he will isolate and identify *S. schenckii*. In nature there also occur other fungi which may resemble *S. schenckii* and the differential identification of these may present difficulties. This is particularly so since sporotrichosis has become a rare infection in Europe and opportunities to examine the causative fungus may be limited to dealing with stock cultures. The problem can be compounded by the diversity of cultural and morphological features exhibited by *S. schenckii* and by the variety of clinical manifestations of the disease. Conant et al. (1971) discuss the classification of the disease under 6 headings including cutaneous and systemic forms. Although pulmonary sporotrichosis is relatively rare, Conant et al. (1971) consider that it “almost certainly occurs more often than it is diagnosed.” Consequently the careful examination of fungal colonies in cultures of sputum may at times involve protracted procedures to ensure that a significant pathogen is not being lightly discarded as an unidentified contaminant.

This communication has been prepared to demonstrate the procedures used to differentiate a fungal culture from *S. schenckii* and in so doing to stress some of the lesser known criteria of the latter fungus.

Throughout the investigation, the culture in question was compared with 2 cultures of *S. schenckii*.

Materials and Methods

Culture 1167: a subculture on an opaque white medium of a glabrous black fungus. This had been isolated, among many other different fungal colonies, from the sputum of a 44 years old patient. The macroscopic and microscopic morphology at this stage suggested a possible identification as *S. schenckii* but intraperitoneal inoculation of male rats had been negative. In July 1974 this culture was no longer viable but a subculture on Sabouraud dextrose agar, showing a wrinkled cream growth with brown to black areas, was used for subsequent investigations.

Cultures of Sporothrix schenckii

2. B-, a recent isolate from a case of cutaneous sporotrichosis, received from Dr. E. Belfort, Instituto de Medicina Tropical, Universidad Central de Venezuela, Caracas, Venezuela.

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Media

1. Sabouraud glucose agar, with and without actidione (cycloheximide) and chloramphenicol, in plates and slopes.
2. Beerwort agar.
3. Potato glucose agar.
4. Czapek agar.
5. Lactritmel agar (Borelli 1962, cited with modifications, Smith & Rush-Munro 1971).*
6. Francis cystine glucose blood agar.
9. 1% yeast extract.

Cultures were incubated at 26° and at 37° C as indicated. Slide cultures were prepared according to the standard technique of Riddell (1950). Lactophenol, with and without cotton blue, was used as mounting fluid. It is important that the dye be omitted when the natural pigmentation of a fungus is to be observed.

Results

Actidione sensitivity

The culture 1167 was markedly inhibited by actidione, 0.05%, showing only minimal growth at the site of the inoculum after 1 month's incubation at 26° C.

S. schenckii is not sensitive to the antibiotic and grows equally well on all media in which it is incorporated. For example, although D. T. M. agar was originally designed for recognition of dermatophytes, S. schenckii grows well on it but the colour of the mediums remains yellow with the initial growth, changing to red after 10—14 days. Resistance to actidione is not stressed in all text books as a characteristic of S. schenckii.

Rate of growth

A colony of Sporotrix schenckii measured approximately 55 x 60 mm after 1 month's incubation at 26° C on a plate of Sabouraud glucose agar without antibiotics. On the same plate a colony of 1167 measured approximately 15 x 20 mm (Fig. 1 A). There was a slight zone of inhibition of the S. schenckii at it approached the opposing colony of 1167. This technique is recommended by Raper und Fennell (1965) for comparison of Aspergillus colonies but is a useful procedure for other fungi, providing that growth is not excessively rapid or luxuriant.

Growth at 37° C and Production of Yeast Phase

The 2 cultures of S. schenckii produced characteristic creamy growths of the yeast phase of the organism when grown at 37° C on Francis cystine glucose blood agar. The culture 1167 consistently produced a tough creamy yeast-like growth with many filamentous hyphae on Sabouraud glucose agar at 26° C. There was no growth at 37° C on Sabouraud glucose agar nor on Francis agar (Fig. 1 B, D).

Production of Pigment

On Sabouraud glucose agar the surface of the colony of 1167 showed concentric rings of dark brown pigment. After 6 weeks the colony was uniformly dark grey-brown with

* Formula (modified): Skim milk powder 14 g
Wheat flour 14 g
Pure honey 7 g
Agar 14 g
Water 1000 ml

Sterilize for 10 minutes at 110° C

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Figure 1:

A. Plate Culture: Sabouraud Glucose Agar (S.G. A.) 1 month 26° C left: *Sporothrix schenckii*, strain "B-" right: *Culture 1167*;

B. Tubes 1—6: *Culture 1167*
1. Original culture with black, glabrous colony
2. Subculture on S.G. A.
3. Confluent yeast-like growth on S. G. A.
4. Single colony on S. G. A.
5. Colony on potato glucose agar with diffusible pigment
6. Colony on lactritmel agar showing extensive pigment production
7. *Sporothrix schenckii*, strain "B-" lactritmel agar
8. *Sporothrix schenckii*, strain H-29, lactritmel agar;

C. *Culture 1167*: Slide culture S.G. A. showing parallel hyphae and, centre, early conidiophores suggesting *S. schenckii* (lactophenol cotton blue × 400);

D. *Culture 1167*: Yeast-like morphology, from tube 2 (S. G. A. 26° C) (Gram stain × 1,000);

E. *Culture 1167*: Mount from Czapek agar with mature phialides and phialospores (Amann’s lactophenol × 400).
a fine aerial mycelium. After 8 weeks a slight brown pigment could be detected in the medium. The original glabrous black colony on the white medium showed no diffusible pigment.

On beerwort agar there was a wrinkled yellowish growth darkening to black at the edges with no obvious diffusible pigment.

On potato glucose agar a restricted zone of dark brown pigment could be seen diffusing into the medium beneath and around the colony after 14 days. On lactritmel agar there was a wide spread zone of diffusible dark brown pigment after 14 days. Approximately half of the total length of the sloped medium was stained brown (Fig. 1 B).

*S. schenckii* may produce grey, brown or black colonies. The 2 strains used were creamy white at time of the investigation but a recent subculture of “B-” on beerwort agar had produced a dark grey powdery colony. This subculture maintained the grey colony on lactritmel agar with a black reverse but no diffusible pigment. Ajjello et al. (1963) report a yellowish pigment in the medium of some cultures of *S. schenckii*.

**Growth Stimulation**

The growth of *Sporothrix schenckii* is stimulated by thiamine. This is conveniently demonstrated using a casein basal medium with and without thiamine (Ajjello et al. 1963). In the present investigation an alternative medium (Yeast carbon base for nitrogen assimilation tests), with and without 2 drops of a 1% yeast extract, was used. Both cultures of *S. schenckii* showed marked enhancement of growth after 7 days at 26°C with surface pigmentation of the culture B-.

The slower growing culture 1167 showed a slight stimulation by yeast after 7 days with production of a brown pigment in the medium.

**Microscopic Morphology**

Both cultures of *S. schenckii* on Sabouraud glucose agar at 26°C produced characteristic pyriform conidia (sympodulospores) in rosette formation on the conidiophores arising from the hyphae. The culture "B-" produced triangular pigmented spores on potato glucose agar and lactritmel medium.

The culture 1167 showed an apparent diversity of structures on Sabouraud glucose agar with small yeast-like cells predominating in the early stages. The elongated yeast cells showed multilocular budding and germ-tube formation. Slide cultures on Sabouraud glucose agar showed a dense network of hyphae with the peripheral outgrowths often in parallel groups of 2 to 5. Short lateral hyphae terminating in a single cell suggested conidiophores. Yeast-like cells and rounded chlamydospores were also present (Fig. 1 C).

On Czapek agar growths of *S. schenckii* showed characteristic conidia and conidiophores. The culture 1167 showed conidia (phialospores) emerging from the conidiophores (phialides) and retained in masses. The morphology was thus suggestive of *Cephalosporium* (Fig. 1 E).

The nature of the colony, the production of dark zones, the final black colour of the original, the aggregated hyphae and the diffusible pigment indicated that *Gliomastix* was a more probable identification. Mounts from the pigmented colony on lactritmel agar showed the phialides and conidia to be pigmented yellow and in masses (as from the original black colony) these were dark yellow-brown.

**Discussion**

The aggregated hyphae and pigmentation supported the identification of *Gliomastix Guégen* rather than *Acremonium* (Link) Saccardo or *Cephalosporium* Corda, but the Baarn List of Cultures (1972) refers *Gliomastix* to *Acremonium*.
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Gliomastix is still retained by Gilman (1959), Barron (1968), Smith (1971) and Kendrick & Carmichael (1973) and hence this designation is used here.

In view of the diversity of fungal isolates from the culture and of the definitive differentiation from Sporothrix schenckii (or its possibly perfect stage Ceratocystis stenoceras [Robak] Moreau) a species identification of the Gliomastix was not considered necessary. Species of this genus are common in soil and may be encountered as air-borne contaminants.

Summary

A fungal colony isolated from a sputum specimen (isolated in conjunction with other fungi) showed some cultural and morphological features suggesting a possible identification of Sporothrix schenckii. The cultural procedures followed to differentiate and identify the fungus are described. The isolate was identified as a species of Gliomastix Guégen.

Key-words: Gliomastix, Sporothrix, growth rate, actidione inhibition, diffusible pigment.

Zusammenfassung

Eine Pilzkolonie, die in einer Sputumprobe gemeinsam mit anderen Pilzen isoliert worden war, zeigte bei Differenzierung kulturelle und morphologische Eigenschaften, die auf das mögliche Vorliegen von Sporothrix schenckii hindeuteten. Die anschließend in 2 Laboratorien durchgeführten Untersuchungen zur Differenzierung und Identifizierung des Pilzstammes werden beschrieben und unter den Gesichtspunkten der Routinediagnostik des mykologischen Labors erörtert. Der Stamm wurde als Angehöriger der Spezies Gliomastix Guégen identifiziert.

Bibliography


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