COMPARISON OF STRAINS OF *SPOROTRICHUM SCHENCKII* ISOLATED FROM NATURE[1]

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ABSTRACT

HOWARD, DEXTER H. (University of California, Los Angeles) AND G. F. ORR. Comparison of strains of *Sporotrichum schenckii* isolated from nature. J. Bacteriol. **85:**816–821. 1963.—Several strains of fungi, tentatively considered to be members of the genus *Sporotrichum*, have been isolated from soil and from other sources in nature. The striking morphological similarity of these isolates to strains of *S. schenckii* led to a comparative study of their biological properties. Nine strains of *Sporotrichum* from nature were compared with twelve strains of *S. schenckii* isolated from cases of clinical sporotrichosis. The nine saprophytic isolates were indistinguishable microscopically and macroscopically from strains of pathogenic *S. schenckii* when cultures were prepared on a variety of media incubated at 28 C. Only two of the nine saprophytic isolates were able to grow at 37 C. These two strains partially converted, at this temperature, to a yeast-cell phase of growth. The blastospores comprising this phase of growth were similar to those produced by *S. schenckii* under the same circumstances. Six of the nine saprophytes were essentially avirulent for mice. The remaining three strains had a very limited capacity to produce disease in experimental animals. The isolates are currently regarded as variants of *S. schenckii*.

The saprophytic existence of *Sporotrichum schenckii* has been established by the circumstances surrounding recorded cases of sporotrichosis and by isolations of the fungus from natural sources. Carmichael (1962) recently pointed out that the use of the generic designation "*Sporotrichum*" for the etiological agent of sporotrichosis is inappropriate because *S. schenckii* "does not in the least resemble" *Sporotrichum aureum* Link, 1809, the lectotype species of the genus. He proposed rather that the fungus be named *Sporothrix schenckii* after Hektoen and Perkins (1900). We have chosen to use the designation "*Sporotrichum*" in the present paper because this is common usage. The most common clinical forms of the disease sporotrichosis are acquired by the traumatic introduction of material contaminated with the fungus into the skin and subcutaneous tissue.

De Beurmann and Gougerot (1908) were the first to observe *S. schenckii* growing in nature. These investigators isolated the fungus from the bark of a beech tree, from the leaves of a horsetail plant growing at the base of the tree, and subsequently from grains of dried oats from another source. They demonstrated that these isolates were indistinguishable from strains previously recovered from patients with sporotrichosis. The saprophytes were only slightly virulent for rats, but virulence was remarkably increased by three serial animal passages. A few years later, Sartory (1915), employing a strain he had isolated from grain, also observed that the virulence of saprophytic strains of *S. schenckii* was augmented by animal passage.

Gastineau, Spolyar, and Haynes (1941) reported that C. Emmons (Chief, Mycology Section, National Institute of Allergy and Infectious Diseases, U.S. Public Health Service) recovered *S. schenckii* from one sample of sphagnum moss incriminated as the source of infection in an outbreak of sporotrichosis among florists. The authors provided no information on other biological properties of this strain.

Brown, Weintraub, and Simpson (1947) reported on their extensive experience with *S. schenckii* isolated from timber employed as

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lagging in the gold mines of the Witwatersrand. These investigators observed the fungus *in situ* over 60 times, and obtained 31 cultures from different specimens of timber. The authors did not comment on the relative virulence of all of their isolates but did demonstrate that two of the strains produced disease in rats and in human volunteers. It was also shown that chips of wood from the mines, on which the fungus spores could be demonstrated, would produce disease when rubbed on the scarified skin of human volunteers. Simson, Helm, and Bowen (1947), in the same study, showed that some saprophytic isolates of *Sporotrichum* were nonpathogenic for humans.

More recently, Dean and Haley (1962) isolated ten strains of *Sporotrichum* sp. from soil. These organisms did not grow at 37 C nor produce observable disease in mice. The authors did not comment on the similarity of their strains to *S. schenckii*, and, unfortunately, the cultures are no longer available for study (Haley, *personal communication*).

During the course of a study on soil fungi, one of us (G. F. O.) cultured several strains of fungi which were tentatively considered to be *Sporotrichum* sp. The striking morphological similarity of these saprophytic isolates to strains of *S. schenckii* recovered from patients with clinical sporotrichosis led to the comparative observations herein reported.

Materials and Methods

Strains of fungi. Twelve strains of *S. schenckii* from cases of sporotrichosis and nine strains of *Sporotrichum* sp. from saprophytic sources were used in the study. Of the nine saprophytes, four were recovered from rat dung, four from soil, and one from wood.

Cultures from dung and wood were prepared by inoculating the materials directly onto plates of Sabouraud's glucose agar containing penicillin (1,000 units/ml), streptomycin (0.1 mg/ml), and cycloheximide (0.5 mg/ml). Soil isolations were made directly from soil collected in the field, and moistened with sterile water containing antibiotics (same concentrations) after return to the laboratory (*see* Kuehn, Orr, and Ghosh, 1961). Of the 12 strains of pathogenic *S. schenckii*, 4 were obtained from patients by culture of biopsy specimens on Sabouraud's glucose agar supplemented with chloramphenicol (0.05 mg/ml) and cycloheximide (0.4 mg/ml). The remaining strains of *S. schenckii* were stock cultures maintained in the Division of Mycology, Department of Infectious Diseases, School of Medicine, University of California, Los Angeles.

All strains were maintained on slants of Sabouraud's glucose agar, stored at 5 C.

Morphological studies. The microscopic and macroscopic morphology of the fungi were observed after growth at 28 C on Sabouraud's glucose agar, carrot plugs, and moistened wood fragments from *Eucalyptus* sp. and *Acacia* sp. Details of spore germination, spore formation, and spore arrangements were studied by agar slide culture techniques employing corn-meal agar (Howard, 1961).

Physiological studies. The ability of the fungi to grow at various temperatures was studied in cultures of glucose-peptone broth (glucose, 1%; Difco Proteose Peptone No. 3, 2%). The asparagine-glucose-salts medium employed in the nutrition experiments consisted of (per liter): L-asparagine, 1 g; glucose, 10 g; Na_2HPO_4, 3.3 g; KH_2PO_4, 1.1 g; $MgSO_4 \cdot 7H_2O$, 0.25 g; and NaCl, 0.25 g. Modifications of this basic medium will be explained in the Results section. Media used in observations on the ability of the fungi to grow in a yeast-cell phase included: 5% rabbit-blood agar, Francis' glucose-cystine-blood agar (Campbell, 1945), and potato-egg medium (Kurung and Yegian, 1954).

Virulence studies. Webster-Swiss (BRVS) albino mice from a random-bred, but long-closed, colony were employed in studying the virulence of the strains of *Sporotrichum*. These animals were originally selected for virus susceptibility and bacterial (especially *Salmonella*) resistance by Leslie Webster at the Rockefeller Institute.

Special techniques or modifications of the above procedures will be explained below.

Results

Microscopic morphology. The microscopic morphology and culture characteristics of *S. schenckii* have been observed and reported often (*see* Howard, 1961, for a partial review of the literature). The 12 strains of *S. schenckii* employed in these experiments conformed in all essential morphological details to published reports.

Our attention was originally directed to the saprophytic isolates of *Sporotrichum* because of their remarkable morphological similarity to *S. schenckii*. The mycelium consisted of septate hyphae approximately 1.4 to 2 μ in diameter. The conidiospores were pyriform to ovoid in

shape, ranging in size from 1.7 to 2.1 by 2.8 to 3.5 μ, and were borne either laterally on the hyphae (Fig. 1a) or at the distal portion of tapering conidiophores (Fig. 1c). Conidia were occasionally borne on proximal portions of the conidiophores (Fig. 1p).

The conidia were attached by means of delicate sterigmata, estimated to be 0.4 to 0.2 μ in diameter. These structures collapse and appear threadlike in stained preparations (Skinner, Emmons, and Tsuchiya, 1947; Howard, 1961). When spores were released, portions of the sterigma remained attached to the conidium and to the hyphal cell bearing it (Fig. 1s).

Conidia arose from small protuberances on the hyphal walls. The tips of these protuberances (sterigmata) enlarged until the mature spores were formed. No septa were observed between the spore and the sterigma or between the sterigma and the hyphal cell from which it arose. Thus, protoplasmic continuity exists between the conidium and the hyphal cell bearing it (Fig. 1).

Conidiospores were hyaline or dark brown. The number of pigmented spores was proportional to the depth of pigmentation of the colonies from which preparations were made. The brown to black color of cultures of *S. schenckii* is also associated with colored conidia (Davis, 1914).

Secondary conidia were produced in older portions of cultures (Howard, 1961). Terminal and intercalary chlamydospores closely resembling those described in cultures of *S. schenckii* (Davis, 1914) were observed.

Conidia usually formed a single germ tube at one pole, but bipolar germination was also seen. Germinated conidia often rounded up and enlarged as the germ tube extended. Occasionally, thick germ tubes were formed, and spores arose only a short distance from the germinated conidium.

In all morphological aspects examined, the strains of *Sporotrichum* isolated from various natural sources were indistinguishable from one another and from strains of *S. schenckii*.

Culture characteristics. The appearance of the colonies of the saprophytic isolates varied considerably on different media. The surface of the colonies on Sabouraud's glucose agar was flat, waxy, wrinkled, and somewhat moist. There were few or no aerial hyphae in young cultures but fasciculated hyphae were occasionally observed in older cultures. Abundant aerial hyphae

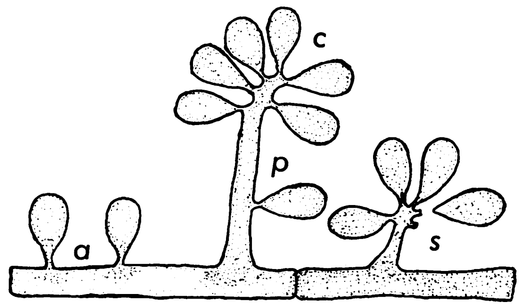

FIG. 1. *Sketch of spore arrangements of Sporotrichum sp. isolated from nature: lateral conidiospores* (a); *conidiophore and conidia* (c); *conidium borne on proximal portion of conidiophore* (p); *sterigmata* (s).

were formed on the wood fragments by several of the saprophytes and by some of the strains of *S. schenckii*. Enormous numbers of conidia were produced by the fungi grown on the relatively dry wood fragments.

Colonies were either white or various shades of brown to black. Pigmentation developed best on carrot plugs, and varied considerably from strain to strain on different media. Such variation in pigmentation is also quite characteristic of strains of *S. schenckii* (Davis, 1915).

A comparative study of the colonies of *S. schenckii* and the saprophytic isolates on the different media studied failed to reveal any significant differences.

Physiological studies. The strains of *S. schenckii* grew at 37 C in glucose-peptone broth, 5% rabbit-blood agar, Francis' glucose-cystine-blood agar, and potato-egg medium. All strains converted, or at least partially converted, at this temperature to a yeast-cell phase of growth typical for this dimorphic fungus. There were some differences in the ease of conversion of the different strains on the various media.

The results of the observations of the growth of the saprophytic *Sporotrichum* sp. in glucose-peptone broth at various temperatures of incubation are shown in Table 1. In contrast to strains of *S. schenckii*, the saprophytic isolates were remarkably heat-sensitive. Only two of the nine saprophytes were able to initiate normal growth at 36.5 C.

The two strains that grew at 36.5 C (HO 1006 and HO 1010) partially converted to a yeast-cell phase of growth at this temperature; the blasto-

TABLE 1. *Growth of Sporotrichum sp. in glucose-peptone broth at various incubation temperatures**

Strain	Source	Incubation temp† (C)				
		28	30	32	34.5	36.5
Saprophytes						
HO 1001	Rat dung	+	−	−	−	−
HO 1002	Rat dung	+	−	−	−	−
HO 1003	Soil	+	+	+	−	−
HO 1004	Soil	+	+	−	−	−
HO 1006	Soil	+	NT	NT	NT	+
HO 1008	Rat dung	+	+	−	−	−
HO 1009	Rat dung	+	+	−	−	−
HO 1010	Wood	+	NT	NT	NT	+
HO 1012	Soil	+	+	+	+	±
Pathogens						
S. schenckii (12 strains)	Sporotrichosis	+	NT	NT	NT	+

* All results were recorded after 1 month of incubation at the temperature indicated.

† Symbols: + = growth; − = no growth; ± = feeble growth; and NT = not tested (these strains were not tested at the intermediate temperature, since preliminary tests showed that they could initiate growth at 36.5 C).

spores of these strains were morphologically similar to those produced by strains of *S. schenckii*. Unfortunately, neither of these strains was readily subcultured at 36.5 C in either liquid or solid media, and it has not been possible to establish cultures of pure blastospores of either strain or culture them continuously at 36.5 C.

In these temperature experiments, the inoculum consisted of a weft of mycelium from a stock culture maintained at 5 C. It is possible that somewhat different results would have been obtained if a portion of a vigorously growing culture had been used as an inoculum. Nevertheless, it is obvious that only two of the strains of *Sporotrichum* from nature initiated growth at 36.5 C, whereas all of the strains of *S. schenckii* grew under identical circumstances. Furthermore, only two of the isolates were similar to *S. schenckii* in converting or partially converting to a characteristic yeast-cell phase of growth. Finally, these two organisms were different from *S. schenckii* in not being readily subcultured at 36.5 C.

Nutritional studies. Thiamine is required for growth by *S. schenckii* (Burkholder and Moyer, 1943; Drouhet and Mariat, 1950, 1952; Reid, 1952). Studies were made to determine whether this growth factor was required by the saprophytic isolates. Cultures were prepared on an asparagine-glucose agar medium and on the same medium supplemented with thiamine (5 μg/ml). Cultures were incubated at 28 C for 2 weeks. All 12 strains of *S. schenckii*, included as controls, and 2 of the 9 strains of *Sporotrichum* isolated from nature grew feebly or not at all on the unsupplemented asparagine-glucose agar, but produced satisfactory growth on the thiamine-containing medium. The other seven saprophytes grew equally well on both media. The two saprophytes requiring thiamine for growth were the same two that grew at 37 C. Thus, these two isolates (HO 1006, HO 1010) resembled *S. schenckii* in requiring thiamine for growth on a chemically defined medium.

Virulence for mice. Studies on virulence of the fungi were carried out as follows. Spores and hyphae were harvested in saline from Sabouraud's glucose agar cultures incubated at 28 C for 1 week. The harvest was ground briefly in a tissue grinder and resuspended in approximately 5 ml of a 5% solution of hog gastric mucin. Adult Webster-Swiss mice (6 to 8 weeks old) were injected intraperitoneally with 0.5 ml of the mucin suspensions.

Nodular peritonitis was produced in mice injected with pathogenic strains of *S. schenckii* in from 2 to 6 weeks. The lesions contained yeast cells characteristic of the parasitic phase of this fungus, and positive cultures were obtained in all instances. There was considerable variation in the extent of disease produced in the mice by the different strains of *S. schenckii*. Some strains produced early death of mice (2 weeks or less) with extensive peritoneal lesions, whereas others produced no deaths and only scattered lesions even after 6 weeks of observation. Such variation was to be expected in view of the fact that many of the strains had been maintained in stock culture for many years and it is known that pathogenic strains may show a decrease in virulence upon storage (de Beurmann and Gougerot, 1908).

None of the strains of saprophytic *Sporotrichum* killed the experimental animals during the 6-week observation period. Groups consisting of three animals were autopsied 2, 4, and 6 weeks after injection. The peritoneal cavities were examined for gross lesions, and cultures of small pieces of liver and spleen were prepared on

TABLE 2. *Isolation of Sporotrichum from tissues of experimentally injected animals*

Strain no.	No. of positive cultures*/no. of mice injected		
	Autopsied at:		
	2 weeks	4 weeks	6 weeks
HO 1001	0/3	0/3	0/3
HO 1002	0/3	0/3	0/3
HO 1003	1/3	0/3	0/3
HO 1004	1/3	0/3	0/3
HO 1006	3/3	0/3	0/3
HO 1008	0/3	0/3	0/3
HO 1009	0/3	0/3	0/3
HO 1010	3/3	0/3	0/3
HO 1012	3/3	0/3	0/3

* Cultures prepared on Sabouraud's glucose agar incubated at 28 C.

Sabouraud's glucose agar and were incubated at 28 C. The results of the culture studies are shown in Table 2.

None of the saprophytes produced the lesions characteristic of the nodular peritonitis seen in animals infected with *S. schenckii.* No observable lesions were produced in mice injected with those strains previously shown to be unable to initiate growth at a temperature above 32 C (HO 1001, HO 1002, HO 1003, HO 1004, HO 1008, HO 1009). However, two of these strains (HO 1003, HO 1004) had a limited capacity for survival in the peritoneal cavities of mice; one of the three injected mice in each instance yielded a positive culture 2 weeks after injection, but not after 4 or 6 weeks (Table 2).

Those strains capable of growth at 35 to 37 C (HO 1006, HO 1010, HO 1012) produced some evidence of an invasive capacity in mice. Small white scattered opacities were observed, 2 weeks after injection, on the capsule of the livers of all animals receiving these strains. In addition, the capsule of the liver was occasionally adherent to the diaphragm. These suspicious areas consistently yielded positive cultures. However, at 4 and 6 weeks, such lesions were only rarely observed, and all cultures even of such suspicious areas were negative (Table 2). These negative results have been repeatedly confirmed.

Discussion

We currently regard the saprophytic isolates as variants of *S. schenckii,* fully recognizing that the evidence for such a designation is by no means compelling. The remarkable morphological similarity of the organisms to *S. schenckii* does not establish identity, for the genus *Sporotrichum* Link, 1809, is not at all well defined (Dodge, 1935), and a large number of saprophytic fungi have been included in it (Carmichael, 1962; Gilman, 1957; Hughes, 1958). Furthermore, the spore arrangements in the genus are displayed by the asexual (conidial) stages of a number of ascomycetes, e.g., *Hypoxylon* and *Ophiostoma.* We have not had the opportunity of comparing our isolates with any large number of strains comprising this enormous form-genus, but such comparisons as have been made clearly suggest that the organisms are closely related to *S. schenckii.*

Kaplan has studied five of our isolates (HO 1001, HO 1003, HO 1004, HO 1006, HO 1010) by fluorescent-antibody techniques (Kaplan and Ivens, 1960). He reported that: "Spores and filaments of all five strains were stained by our labeled *S. schenckii* antibodies. The intensity and pattern of the staining reactions did not differ in any significant respect from those shown by our mycelial-phase cultures of pathogenic strains of *S. schenckii*" (Kaplan, *personal communication*). Thus, the saprophytic isolates are also related antigenically to *S. schenckii.*

The saprophytic isolates differed from *S. schenckii* in several physiological aspects: only two of nine strains initiated growth at 37 C; and some of the isolates did not require thiamine, which is known to be essential to the growth of *S. schenckii.* These differences hardly preclude consideration of the saprophytes as *S. schenckii.* Pathogenic strains of this fungus demonstrate considerable variation in pigmentation (Davis, 1915), and in ability to convert to a yeast-cell phase of growth (Campbell, 1945; Salvin, 1947). It is, therefore, conceivable that saprophytic *S. schenckii* might show variations in ability to initiate growth at different temperatures of incubation and in requirements for certain growth factors.

Under the conditions reported, some of the organisms were feebly pathogenic for mice. De Beurmann and Gougerot (1908) reported that strains of this fungus recovered from human patients and maintained in the laboratory gradually lost virulence for experimental animals. Such a loss in virulence could usually be restored by animal passage. We also observed these facts

with strains of *S. schenckii* used in the present study. De Beurmann and Gougerot (1908) and Sartory (1915) further noted that there saprophytic isolates of *S. schenckii* possessed a low order of virulence, and that augmentation of virulence accompanied animal passage. Thus, variation in virulence of strains of *S. schenckii* has been repeatedly documented.

In conclusion, we suggest that, inasmuch as the saprophytes are morphologically indistinguishable from and antigenically related to *S. schenckii,* the variations displayed by these strains hardly exclude them from consideration as variants of *S. schenckii.*

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