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Delayed Hypersensitivity Cross-Reactions Between *Sporothrix schenckii* and *Ceratocystis* Species in Sporotrichotic Patients

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Cutaneous delayed hypersensitivity to antigens prepared from *Sporothrix schenckii* and several *Ceratocystis* species, including *C. stenoceras*, *C. ulmi*, *C. ips*, and *C. minor*, was tested in 14 patients with known cutaneous sporotrichosis. Extensive cross-reactions were observed. Nonsporotrichotic people (controls) did not react to these antigens. The correlation coefficient between antigens of *S. schenckii* and each *Ceratocystis* species was calculated from the areas of the cutaneous reactions. Among the *Ceratocystis* species tested, the correlation coefficient between *S. schenckii* and *C. stenoceras* was 0.91.

Comparative morphological, chemical, and serological studies of *Sporothrix schenckii* and various *Ceratocystis* species have been reported (2, 3, 5-11, 17-22; H. Ishizaki, R. Wheat, and N. F. Conant, Abstr. Annu. Meet. Am. Soc. Microbiol. 1974, Mm 27, p. 140). The close relationships indicated in these reports between *S. schenckii* and several *Ceratocystis* species raise some doubt about the specificity of the sporotrichin skin test. This is of some importance since the sensitive sporotrichin skin test has been widely used both in the diagnosis of sporotrichosis and in epidemiological studies of the disease (14).

This paper reports the results of cutaneous tests in sporotrichotic patients and normal controls to antigens prepared from *S. schenckii* and various *Ceratocystis* species and the significant relatedness correlations of reactions between the *S. schenckii* and cross-reactive *Ceratocystis* antigens.

MATERIALS AND METHODS

Preparation of antigens. *S. schenckii* (ATCC 10268), *Ceratocystis stenoceras* (Colorado State University 922), *C. minor* (Colorado State University 811), *C. ips* (Colorado State University 445-A), and *C. ulmi* (Department of Forestry, Duke University) were grown on a dialysate medium (prepared by dialyzing 0.5% peptone and 0.5% yeast extract at 60 C for 6 h) to which 2% glucose was added before autoclaving. After inoculation, cultures were incubated for 1 week at 35 C with constant shaking. Formalin was added to a final concentration of 1%, and the culture was then held overnight at room temperature.

Antigens. Culture filtrate was obtained by centrifugation and was dialyzed against running tap water for 5 days and then concentrated to 1/20 volume by pervaporation. To the concentrated retentate, 5 volumes of 99% ethanol were added, and the precipitate was obtained by centrifugation and then dried with acetone. The acetone-dried precipitate was dissolved in 0.25% phenol-physiological saline (100 μg/ml) as skin test antigen.

Skin test. Each antigen (0.1 ml) was injected intradermally on the volar surface of arms of 14 patients with known cutaneous sporotrichosis and 7 normal controls. After 48 h, the diameters of induration measured in two directions at 90° were recorded, and the areas of reactions were calculated. The correlation coefficient (r) between *S. schenckii* and each *Ceratocystis* species was calculated from the formula $r = \Sigma xy/(\sqrt{\Sigma x^2}\ \sqrt{\Sigma y^2})$ (15).

RESULTS

The area of cutaneous reactions to each antigen and the correlation coefficient between *S. schenckii* and each of the *Ceratocystis* species antigens tested are shown in Tables 1 and 2, respectively.

DISCUSSION

Recently, several laboratories, both in France and in the United States, have recognized that aberrant isolates of *S. schenckii* from human cases of sporotrichosis have been identified as *C. stenoceras* (7; L. Ajello and W. Kaplan, Abstr. Annu. Meet. Am. Soc. Microbiol. 1969, Mm10, p. 112; S. McMillen, Abstr. Annu. Meet. Am. Soc. Microbiol. 1973, Mm21, p. 133), which is usually considered to be a

TABLE 1. *Area of cutaneous reactions (in square millimeters)*

Case	*S. schenckii*	*C. stenoceras*	*C. ips*	*C. ulmi*	*C. minor*
1	777	200	7	14	188
2	141	200	24	5	3
3	491	94	160	3	24
4	154	254	0	86	0
5	796	509	38	86	380
6	314	201	0.8	3	188
7	311	201	5	121	9
8	2,748	1,236	141	589	177
9	633	570	7	528	361
10	231	0	0	0	0
11	1,943	653	7	0	212
12	176	13	0	0	94
13	510	653	16	113	214
14	2,826	1,256	2,826	20	1,256
Normal controls					
15	0	0	0	0	0
16	0	0	0	0	3
17-21	0	0	0	0	0

TABLE 2. *Correlation coefficients between S. schenckii and Ceratocystis spp. antigens*

Organism	Correlation coefficient
S. schenckii versus *C. stenoceras*	0.91 ($P < 1\%$)
S. schenckii versus *C. ips*	0.63 ($P < 2\%$)
S. schenckii versus *C. ulmi*	0.36
S. schenckii versus *C. minor*	0.67 ($P < 1\%$)

white pine pathogen (i.e., a phytopathogen) transmitted or vectored by various species of bark beetles. It is therefore of interest to note that *C. stenoceras* is closely related to the well-known *C. ulmi*, which is the cause of Dutch elm disease in Europe and which is presently sweeping across the United States. *S. schenckii* has been suggested to be the imperfect stage of one (or more) *Ceratocystis* species, including *C. stenoceras* (7-9), but a variety of lines of evidence from several laboratories, reviewed below, do not substantiate this hypothesis.

A comparison of several *Ceratocystis* species with *S. schenckii*, from the aspects of some cultural, morphological, serological, and mouse virulence characteristics, showed that several *Ceratocystis* species were not significantly different from typical strains of *S. schenckii* (17). Furthermore, most *Ceratocystis* and *Graphium* species, which were reported by Spencer and Gorin (16) to produce rhamnose (and possibly mannose), were found to cross-react serologically with *S. schenckii* (Ishizaki et. al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1974, Mm27, p. 140). These include Spencer and Gorin groups 1 through 8, none in group 9 (mannans only), three rhamnose producers in group 10 (predominantly galactomannan producers), and none in group 11 (no Cu-precipitable [i.e., Fehling solution] mannans). It has been reported that polysaccharides of *S. schenckii* are mainly composed of rhamnose and mannose (1, 4-6, 12), and rhamnose also can be found in many *Ceratocystis* species (16), although, in general, rhamnose is restricted to a very few genera of fungi.

The polysaccharides of *S. schenckii, C. stenoceras, C. ulmi, C. minor,* and *C. pilifera* have α-L-rhamnopyranosyl-(1 → 3) and α-L-rhamnopyranosyl-(1 → 2)-L-rhamnopyranosyl side chain units (21). The α-L-rhamnosyl or α-L-rhamnopyranosyl-(1 → 3)-D-Man structure was reported to be the immunodominant structure in yeast-form *S. schenckii* grown at 37 C and in several *Ceratocystis* species grown at 25 C (6). The structures of the rhamnomannans from *S. schenckii* and a few selected *Ceratocystis* species have been compared by methylation analysis (21), nuclear magnetic resonance spectroscopy (22), and serological analyses (6). Also studied were deoxyribonucleic acid base compositions in 17 strains of *S. schenckii* and *Ceratocystis* species and the percentage of deoxyribonucleic acid hybridization between *S. schenckii* and *C. stenoceras, C. minor,* and *C. pilifera* (12). The conclusion derived from these investigations was that some *Ceratocystis* species studied are closely related to *S. schenckii* but are not identical.

The correlation coefficient between *S. schenckii* and *C. stenoceras* was 0.91, indicating significant correlation (Table 2). However, the meaning of this correlation is not to be interpreted, because the antigens used in this study

are crude and probably differ in composition both qualitatively and quantitatively.

In view of the cross-reactions observed between *Ceratocystis* species and *S. schenckii*, the specificity of the sporotrichin skin test deserves a few comments. The primary epidemiological work on sporotrichosis has been done with the sporotrichin test, and a high percentage of positive reactions was found in horticulture workers (14). The possibility that positive skin tests to sporotrichin were due to cross-reactions in persons sensitized to *Ceratocystis* species was not excluded, and, since *Ceratocystis* and *S. schenckii* can be isolated from animals, soils, and plants at the same time (10), the meaning of such studies needs to be reevaluated. Our work indicates the need for the development of specific sporotrichin (and possibly ceratocystin) antigens, especially for use in epidemiological research.

On the other hand, *Ceratocystis* species that have not been studied so far should be studied for cross-reactivities in skin testing. Information developed from these approaches would be helpful in answering such questions as whether sporotrichosis is caused by a single organism or is a disease complex caused by several organisms and in aiding in attempts to identify a possible perfect form of *S. schenckii*.

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