Comparative Antigenic Studies of Species of Basidiobolus and Other Medically Important Fungi

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An immunodiffusion technique was used to evaluate the antigenic relationship of various pathogenic and saprobic Basidiobolus spp., Conidiobolus spp., isolates of the order Mucorales, and several other medically important nonzygomycetous fungi. The antiserum to Basidiobolus haptosporus shared two lines of identity, designated inner (Y) and outer (X), when tested against exoantigens of known strains of B. haptosporus and Basidiobolus ranarum as well as exoantigens of a human Nigerian isolate and several wild isolates tentatively identified in B. ranarum. Both bands were heat stable at 56°C for 30 min. Exoantigens of strains of Basidiobolus meristosporus, Basidiobolus microsporus, Conidiobolus incongruus, Conidiobolus coronatus, and other wild isolates of Basidiobolus spp. tested formed only the N immunoprecipitin band. Exoantigens of 10 isolates from other taxa did not produce any cross-reactive precipitin line. B. meristosporus antiserum that was tested against exoantigens of Basidiobolus spp. and of Conidiobolus spp. developed only an N band without a Y band. These data suggest that B. haptosporus and B. ranarum are antigenically similar to each other and distinct from B. meristosporus. Basidiobolus spp. and Conidiobolus spp. share a common N immunoprecipitin band, which implies a taxonomic relationship between these two genera. The absence of lines of identity between Basidiobolus spp. and other fungi tested suggests that, antigenically, Basidiobolus is a distinct genus.

Species of Basidiobolus and Conidiobolus are etiologic agents of the subcutaneous mycotic infections entomophthoromycosis basidiobolae and entomophthoromycosis conidiobolae, respectively. Although seldom life-threatening, these infections cause disfigurement and morbidity and are clinically significant where endemic, as in tropical Africa and Asia (2, 6, 11, 14, 16).

There has been controversy regarding the taxonomy of Basidiobolus spp. Zygospor wall undulation, optimum temperature of growth, production of meristospores, microspore formation, and Streptomyces-like odor production are criteria that have been used for differentiation of species within this genus but that have not been totally accepted (1, 3-5, 9, 10, 12, 13, 18). The only species unequivocally recognized is B. microsporus, which produces exogenous microspores. McGinnis (13) proposes that until a taxonomic revision is conducted, the remaining species should be considered as B. ranarum.

In this study, an immunodiffusion technique (17, 19) was adapted to determine if some of the putative species of Basidiobolus can be distinguished from each other and to compare with the two pathogenic species of Conidiobolus and with several other nonrelated medically important fungi.

MATERIALS AND METHODS

Fungal isolates. Human isolates of B. haptosporus ATCC 34122, ATCC 16108, and ATCC 16109; B. ranarum ATCC 24670, ATCC 24671, and ATCC 14052; B. meristosporus ATCC 36599, and ATCC 36600; C. incongruus ATCC 24293; C. coronatus ATCC 42063; Mucor ramosissimus ATCC 28933; Cunninghamella bertholletiae ATCC 42115; Saksenaea vasiiformis ATCC 28740; the wild isolate B. microsporus ATCC 14708; and Rhizopus microsporus ATCC 14050 were obtained from the American Type Culture Collection, Rockville, Md. Other fungi studied included a human Nigerian isolate (B. ranarum) and other wild-type isolates of Basidiobolus (5112, L20, L21, L24, L25, and L26; all tentatively identified as B. ranarum), which were gathered from lizard guts in Nigeria except B. ranarum GA5, which was obtained from a lizard gut from Florida. An unknown species of Rhizopus was obtained from the University of South Florida Department of Medical Microbiology, Tampa, Fla. Isolates of Absidia corymbifera and Mucor sp., and antiserum and exoantigens from Blastomyces dermatitidis, Coccioides immittis, and Histoplasma capsulatum were generously supplied by A. A. Padhye and Paul Standard from the Centers for Disease Control (CDC), Atlanta, Ga.

Inocula. All Basidiobolus isolates were grown on Sabouraud dextrose agar to a 50-mm diameter at 25°C for approximately 5 to 7 days. Each colony was scraped off, suspended in 5 ml of sterile phosphate-buffered saline (pH 7.2), and homogenized in a tissue homogenizer (Tall Boy Instrument Corp., Emerson, N.J.) at 700 to 1,000 for 5 min or until a smooth mycelial slurry was formed.

Exoantigen production. A sample (2 ml) of the homogenized inoculum was transferred to a flask (125 ml) containing 50 ml of yeast nitrogen base plus glucose and asparagine (V. Baker, M. S. thesis, University of South Florida, Tampa, 1984; manuscript in preparation). The suspensions were grown at 25 to 27°C for 6 weeks in a Dubnoff metabolic shaking incubator. The mycelium was killed by adding a 1% thimerosal solution to yield a final concentration of 1.5,000.

The thimerosal-treated cultures were shaken for 24 h. Nonviableity of cultures was confirmed by plating samples of the mycelium on brain heart infusion agar or Sabouraud dextrose agar. The cells were separated from the media aseptically with a coarse sintered-glass filter. The exoantigen was then filtered through a 0.20-μm-pore filter (Nalgene

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Fusion plate against the Basidiobolus exoantigen complete adjuvant were ATCC 36600. ATCC 34122 concentrated the center to ascertain antibody adjuvant. any antibody reached weekly initially bled, and their antibody was formed between B. haptosporus and B. ranarum.

Labware Div., Malge/Sybron Corp., Rochester, N.Y.) and concentrated 10 times in a B15 Minicon cell concentrator (Amicon Corp., Lexington, Mass.) (19). Exoantigens were also elicited for the immunodiffusion test by adding 5 ml of 1:5,000 thimerosal to 7-day-old slant cultures for 24 h or until visible sporulation or hyphae were noted in the supernatant fluid; the exoantigens were then concentrated 50 times in a B15 cell concentrator.

Antiserum production. New Zealand white rabbits were initially bled, and their serum was run in a macroimmunodiffusion plate against the Basidiobolus exoantigen to reveal any antibody production before immunization. Four rabbits were injected subcutaneously on day 1 with 0.5 ml of Freund complete adjuvant and 0.5 ml of exoantigen from B. haptosporus ATCC 34122 concentrated 10 times. Two rabbits were also injected on day 1 with 0.5 ml of Freund complete adjuvant and 0.5 ml of exoantigen from B. meristosporus ATCC 36600. They were bled 1 week later to ascertain antibody production against these antigens, and each serum was run in an immunodiffusion plate by placing the serum in the outer well and the reference antigen in the center well (17, 19). During week 3 and every other week the rabbits were again injected with exoantigen, but with Freund incomplete adjuvant. Bleedings and immunizations were alternated weekly until a significant line of identity was detected on the immunodiffusion plate and the antibody titer reached a peak. The antibody titer was measured with the tanned-cell hemagglutination test (7, 8). Peak titers (1:256 to 1:1,024) were reached by week 6.

Immunodiffusion test. Macroimmunodiffusion plates were prepared with phenolized agar medium, and individual 4-mm wells were punched in a pattern (Fig. 1) (19). Antisera to B. haptosporus ATCC 34122 and B. meristosporus ATCC 36600 were placed in each of the center wells of a macroimmunodiffusion plate and allowed to diffuse for 1 to 2 h at room temperature. The reference antigens were placed in the upper and lower wells of each pattern, and the unknown antigens were placed in duplicate in the lateral wells. Plates were incubated at 25°C in a humic chamber. At 24 to 48 h, they were checked for the production of the inner (N) and outer (Y) bands specific for B. haptosporus.

Controls. The yeast nitrogen base plus glucose and asparagine was concentrated 10, 25, and 50 times. B. haptosporus antiserum and the reference antigen were used to check for antibody titer against the medium in an immunodiffusion plate. Heat stability of the bands was determined by incubating the antiserum at 56°C for 30 min and performing the immunodiffusion test.

RESULTS

Various isolates were tested with rabbit antisera raised against B. haptosporus ATCC 34122 and B. meristosporus ATCC 36600 (Table 1). Two common lines of identity (the N and Y bands, as in Fig. 1) were evident when the antiserum to B. haptosporus was tested against exoantigens of B. haptosporus ATCC 34122, ATCC 16108, and ATCC 16109, B. ranarum ATCC 14052, ATCC 24670, and ATCC 24671, and various isolates (human Nigerian and wild GA5 and 5112) tentatively identified as B. ranarum. Both bands were heat stable at 56°C for 30 min. Exoantigens of B. meristosporus ATCC 36600 and ATCC 35699, B. microsporus ATCC 14708, C. incongruus ATCC 24293, and wild isolates L-20, L-21, L-24, L-25, and L26 formed only an N band (Table 1 and Fig. 2). Exoantigens A. corymbifera CDC, C. bertholletiae ATCC 42115, Mucor sp. (CDC), M.

![Image](http://jcm.asm.org/)

**FIG. 1.** Relationship of antiserum against B. haptosporus to exoantigens of B. haptosporus and B. ranarum. The center well contains the control antiserum against B. haptosporus ATCC 34122. Wells 1 and 4 contain the reference antigen. Wells 2 and 3 contain the antigen from B. haptosporus ATCC 16108. Wells 5 and 6 contain the antigen from B. ranarum ATCC 14052. Common N and Y bands are formed between B. haptosporus and B. ranarum.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Presence (+) or absence (−) of precipitin bands* with antiserum against:</th>
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<tbody>
<tr>
<td></td>
<td>B. haptosporus ATCC 34122</td>
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<tr>
<td>B. haptosporus ATCC 34122</td>
<td>+</td>
</tr>
<tr>
<td>B. haptosporus ATCC 16108</td>
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</tr>
<tr>
<td>B. haptosporus ATCC 16109</td>
<td>+</td>
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<tr>
<td>B. ranarum ATCC 14052</td>
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<tr>
<td>B. ranarum ATCC 24670</td>
<td>+</td>
</tr>
<tr>
<td>B. ranarum ATCC 24671</td>
<td>+</td>
</tr>
<tr>
<td>B. meristosporus ATCC 36600</td>
<td>−</td>
</tr>
<tr>
<td>B. meristosporus ATCC 35699</td>
<td>−</td>
</tr>
<tr>
<td>B. microsporus ATCC 14708</td>
<td>−</td>
</tr>
<tr>
<td>B. ranarum GA5</td>
<td>+</td>
</tr>
<tr>
<td>B. ranarum (human Nigerian isolate)</td>
<td>+</td>
</tr>
<tr>
<td>B. ranarum 5112</td>
<td>+</td>
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<tr>
<td>B. ranarum L-20</td>
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<tr>
<td>B. ranarum L-21</td>
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<td>B. ranarum L-24</td>
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<td>B. ranarum L-25</td>
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<tr>
<td>B. ranarum L-26</td>
<td>+</td>
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<tr>
<td>C. coronatus ATCC 42063</td>
<td>+−</td>
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<tr>
<td>C. incongruus ATCC 24293</td>
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* Determined by immunodiffusion test.
+ +/− Equivocal presence.
* USF, University of South Florida.

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**TABLE 1.** Antigenic relationships among species of Basidiobolus, Conidiobolus, related mucorales, and various nonrelated pathogenic fungi

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*USF, University of South Florida.*
formed among fraction as an an-tics. Greer and Basidiobolus exoantigens. Lines. and C. ATCC ramosissimus elicited the reference antigen. contain reciprocal hemagglutination meristosporus exoantigens of ATCC incongruus. VOL. 23, only showed and 6 contain contain reference antigen ATCC 3i600. Wells and 3 contain antigen from B. meristosporus, ATCC 34122. A common N band is produced between B. haptosporus and B. meristosporus.

**FIG. 2.** Relationship of antiserum against B. haptosporus to exoantigens of B. meristosporus and C. incongruus. The center well contains the B. haptosporus control antiserum. Wells 1 and 4 contain the reference antigen. Wells 2 and 3 contain the antigen elicited from B. meristosporus ATCC 36600. Wells 5 and 6 contain the antigen from C. incongruus ATCC 24293. A common N band is formed among B. haptosporus, B. meristosporus, and C. incongruus.

**FIG. 3.** Relationship of antiserum against B. meristosporus to Basidiobolus exoantigens. The center well contains the control antiserum against B. meristosporus ATCC 36600. Wells 1 and 4 contain reference antigen to B. meristosporus ATCC 36600. Wells 2 and 3 contain antigen from B. meristosporus ATCC 36599. Wells 5 and 6 contain antigen from B. haptosporus ATCC 34122. A common N band is produced between B. haptosporus and B. meristosporus.

**DISCUSSION**

There have been few attempts at resolving the taxonomic controversy about B. haptosporus, B. ranarum, and B. meristosporus by comparison of their antigenic characteristics. Greer and Friedman (8, 9) studied the antigenic relationship of several species of Basidiobolus. In these investigations, they used a polysaccharide moiety of a whole cell fraction as an antigen and detected the specific antibody by reciprocal hemagglutination inhibition and agglutin absorption technique. They concluded that B. meristosporus is antigenically different from B. ranarum. They found that there were common antigenic determinants among most human isolates of this fungus and among some of the saprophytic isolates. They suggested that these isolates were most closely aligned with B. meristosporus.

Our study shows that there is a close antigenic relationship among some pathogenic and wild isolates of Basidiobolus and a closely related Conidiobolus species when tested with B. meristosporus and B. haptosporus antisera. The isolates all possess the N band. In recent antigenic investigations, Polonelli and Morace (15) used a polysaccharide antigen, a concentrate-soluble filtrate antigen, and a mycelium homogenate antigen to also find several common bands by immunodiffusion among isolates of B. haptosporus, B. ranarum, B. meristosporus, and B. microsporus.

Although some antigens are shared among species of Basidiobolus, it is apparent in this study and those by Greer and Friedman (8, 9) and Polonelli and Morace (15) that there is enough antigenic diversity among species for developing serodiagnostic antibodies. Furthermore, our exoantigen immunodiffusion test supported morphological and chemical studies which indicate that the genus Basidiobolus is related to the genus Conidiobolus and distinct from other fungi. Still, the taxonomic delineation among species and human isolates is not yet clear. Confirmation of the technique and taxonomic relationships may depend upon the future opportunity to evaluate sera from human or animal cases of entomophthoromycosis basidiobolae.

**ACKNOWLEDGMENT**

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**LITERATURE CITED**