Detection of Two *Blastomyces dermatitidis* Serotypes by Exoantigen Analysis

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We evaluated the diagnostic value of antibody to *Blastomyces dermatitidis* A precipitin arcs for identifying *B. dermatitidis* cultures by the exoantigen technique with 88 isolates from the United States and Canada, 12 from Africa, and 1 each from India and Israel. In addition, we studied 190 mycelial-form isolates of other fungi which could be confused antigenically or morphologically with *B. dermatitidis*. Antigen extracts from all of the North American isolates, the Indian and Israeli isolates, and one of the African isolates reacted with the *B. dermatitidis* A antibody. The anti-A serum did not react with antigens from the other 11 African isolates or with antigens from any of the 190 heterologous fungi. Studies with unadsorbed and adsorbed antisera to selected North American and African isolates of *B. dermatitidis* showed that the North American isolates not only produced the distinct A antigen, but also formed another antigen that was common to all 11 of the antigenically less complex African isolates. The results of this study indicate that at least two serotypes of *B. dermatitidis* exist. All of the North American, Indian, and Israeli isolates and one of the African isolates contained the A antigen and were designated serotype 1. Most of the African isolates (11 of 12) constituted a second serotype that was deficient in A but showed an antigen designated K.

The immunoidentification by exoantigens of such mycelial-form fungal pathogens as *Coccidioides immitis*, *Exophiala jeaneselmei*, *Histoplasma capsulatum*, *Paracoccidioides brasiliensis*, and *Wangiella dermatitidis* (15; L. Kaufman, P. Standard, and A. A. Padhye, Mycopathology, in press) is proving to be a valuable diagnostic procedure. Reports to date, although preliminary, indicate that *Blastomyces dermatitidis* mycelial-form cultures may also be rapidly and accurately identified by the exoantigen technique.

Manych and Sourek (12) were the first to use agar gel precipitation to identify *B. dermatitidis* cultures. Identification was based on the formation of a tube precipitin band after incubating a culture with *B. dermatitidis* rabbit antiserum for 4 weeks. The means of determining the specificity of the precipitate were not described. In 1977, Rippon et al. (13), using washings from a slant culture and sera from a patient with blastomycosis and a rabbit with *B. dermatitidis* antibodies, reported that reverse immunodiffusion may be used to identify fungus cultures. In 1973 (5), a specific precipitin, designated A, was found in patients with blastomycosis. Later, Kaufman and Standard (7) used sera containing antibody to A antigen to show the apparent specific production of exoantigen A in some *B. dermatitidis* isolates. The present study evaluated more extensively the specificity and sensitivity of the *B. dermatitidis* exoantigen test with a large number of *B. dermatitidis* isolates obtained from various parts of the world, as well as with many morphologically related hyphomycetous fungi.

**MATERIALS AND METHODS**

**Antisera and antigens.** The antisera used to produce antisera were prepared from three yeast-form cultures of *B. dermatitidis* (strains X-79, B-832, and B-3015) that had been grown in brain heart infusion broth (Difco Laboratories). The broth cultures were shaken for 1 week at 150 to 160 rpm during incubation at 37°C. The cultures were killed by adding merthiolate to a concentration of 1:5,000. After appropriate sterility checks, the yeast cells were removed by centrifugation, and the supernatants were concentrated 10 times and retained. The yeast cells were washed twice in merthiolated saline and adjusted to a turbidity equivalent to a no. 3 McFarland nephelometric standard for immunization.

The standardized yeast suspensions, in doses of 1, 2, and 2 ml, respectively, were injected intravenously into rabbits on the first, second, and third days of the week for 2 consecutive weeks. Each rabbit was bled 1 week after the final injection, and the serum titers were determined against the homologous 10-times-concentrated supernatant antigens (5). Rabbits whose sera failed to give well-defined precipitin bands re-
The arcs were technique in vitro been received an additional weekly series of injections until well-defined precipitates developed. Control antisera against the A antigen were also prepared in rabbits with newly isolated immunoelectrophoretic precipitin arcs as a vaccine by the method of Green et al. (3, 4). The arcs were produced from the concentrated B. dermatitidis culture supernatants and A arc antiserum.

**Adsorption procedure.** Antigens for adsorption consisted of whole cells grown and harvested in the same manner as for vaccine production, but the cells were washed and suspended in phosphate-buffered saline (pH 7.2) that contained merthiolate (1:10,000). Antisera were adsorbed with half volumes of packed cells at 37°C in a water bath for 2 h. Adsorptions were repeated until precipitins to the adsorbing strains had been eliminated.

**Cultures studied.** B. dermatitidis isolates, including 87 from the United States, 12 from Africa, and 1 each from Canada, India, and Israel (total, 102), were examined. Data for the African isolates are given in Table 1. All 12 African isolates were obtained from patients with proven cases of blastomycosis. The colonial morphology of all the B. dermatitidis isolates was studied on Sabouraud dextrose agar at 25°C. Their microscopic features were studied by slide culture technique on potato dextrose agar and corn meal agar. The in vitro conversion of each isolate from mycelial to yeast form was studied on cotton seed agar (21) at 37°C. All of the African and Asian isolates were paired with the + and − mating type tester strains of Ajellomyces dermatitidis on yeast extract agar (10).

A total of 190 isolates of heterologous fungi, including species of Coccidioides immitis, H. capsulatum, and a variety of saprophytic fungi were selected for study. The species and number of isolates of saprophytic fungi included were: Arachniotus reticulatus, 1; Arthroderma tuberculatum, 2; Aspergillus fumigatus (atypical), 1; Auxarthron umbrinum, 1; Chrysosporium asperatum, 1; Chrysosporium keratinophilum, 2; Chrysosporium lucknowense, 1; Chrysosporium mer- darium, 1; Chrysosporium parvum, 5; Chrysosporium tropicum, 1; Chrysosporium sp., 1; Corynascus sepedonum, 3; Geomyces pannorum, 1; Renispora flavisima, 5; Sepedonium spp., 4; and Sepedonium zylo- genum, 1.

**Exoantigen tests.** Culture extracts were prepared by the slant extraction method described by Kaufman and Standard (6). Extracts from 2- to 3-week-old cultures believed to be B. dermatitidis were concentrated 50 times with an Amicon Minicon B-15 Macrosolute concentrator. Using the microimmunodiffusion procedure and rabbit anti-B. dermatitidis serum, we tested the concentrated extracts in parallel with control B. dermatitidis A antigen. The antisera was placed in the central well and incubated for 1 h at 25°C. Reference antigens were then placed in the upper and lower wells of each pattern, and the unknown antigens were placed in duplicate in the lateral wells. Each plate was incubated in a moist chamber at 25°C for 24 h. Studies in our laboratory showed that the results obtained with these incubation conditions were similar to those obtained for plates incubated at 37°C for 48 h, as reported earlier (7).

**RESULTS**

Isolates of B. dermatitidis obtained from the United States and other countries were tested with antiserum to B. dermatitidis A arcs for their ability to produce the A exoantigen (Table 2). All 88 North American isolates, as well as the Indian (8) and Israeli (9) isolates, produced the A antigen. Of the 12 African isolates, only one, strain B-3016 from Algeria (11), produced the A antigen; six produced an antigen unrelated to A. The colonies of the Algerian (strain B-3016),

<p>| TABLE 1. African isolates of B. dermatitidis |</p>
<table>
<thead>
<tr>
<th>Isolate no.</th>
<th>Received from (original strain no.) and reference</th>
<th>Country of origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>B-781</td>
<td>F. Gatti, Republic of Congo (human case) (16)</td>
<td>Zaïre</td>
</tr>
<tr>
<td>B-832</td>
<td>H. F. Hasenclaver (NIH 6071; King* (16)</td>
<td>Uganda</td>
</tr>
<tr>
<td>B-833</td>
<td>H. F. Hasenclaver (NIH 6066; H. I. Lurie* (16)</td>
<td>South Africa</td>
</tr>
<tr>
<td>B-838</td>
<td>C. Magalhanes (16)</td>
<td>Mozambique</td>
</tr>
<tr>
<td>B-2490</td>
<td>E. S. McDonough; R. B. Scott* (1419) (16)</td>
<td>South Africa</td>
</tr>
<tr>
<td>B-3007</td>
<td>E. S. McDonough (no. 24); Vandepitte*</td>
<td>Rwanda</td>
</tr>
<tr>
<td>B-3013</td>
<td>C. N. Young (A-449) (14)</td>
<td>South Africa</td>
</tr>
<tr>
<td>B-3015</td>
<td>C. N. Young (A-2402) (22)</td>
<td>South Africa</td>
</tr>
<tr>
<td>B-3016</td>
<td>F. Mariat (no. 61) (11)</td>
<td>Algeria</td>
</tr>
<tr>
<td>B-3017</td>
<td>F. Mariat (M-268) (18)</td>
<td>Tunisia</td>
</tr>
<tr>
<td>B-3018</td>
<td>F. Mariat (M-973)</td>
<td>Morocco</td>
</tr>
<tr>
<td>B-3064</td>
<td>G. A. de Vries (CBS 514.14) (1)</td>
<td>Angola</td>
</tr>
</tbody>
</table>

* *, Previous provider of isolate.

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**TABLE 2. Reactions of rabbit anti-B. dermatitidis A arc serum and anti-B. dermatitidis B-832 and B-3015 seraa with extracts from isolates of B. dermatitidis and heterologous fungi**

<table>
<thead>
<tr>
<th>Species and origin (no. of isolates tested)</th>
<th>A arc antigen</th>
<th>Unrelated antigensb</th>
<th>Yeast cells of isolates B-832 and B-3015</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. dermatitidis, Africa (12)</td>
<td>1</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>B. dermatitidis, North America (88)</td>
<td>88</td>
<td>41</td>
<td>88</td>
</tr>
<tr>
<td>B. dermatitidis, India (1)</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>B. dermatitidis, Israel (1)</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Coccidioides immitis (76)</td>
<td>0</td>
<td>68</td>
<td>0</td>
</tr>
<tr>
<td>H. capsulatum (83)</td>
<td>0</td>
<td>27</td>
<td>0</td>
</tr>
<tr>
<td>Saprophytic fungi (31)</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

*a These two antisera lacked antibody to antigen A.

*b One to two antigens were noted.
Israeli (strain B-2280), and Indian (strain B-3463) isolates on Sabouraud dextrose agar were velvety, flat, white to creamy white, becoming tan, and measured 28 to 32 mm in diameter after 2 weeks at 25°C. Microscopic examination of the slide cultures showed that the three isolates produced moderate to large numbers of conidia, 3 to 6 by 2 to 4.5 μm, that were hyaline, pyriform, subspherical to globose, smooth, and holoblastic. They were borne either directly on the sides of vegetative hyphae or on short or long pedicels. On cotton seed agar at 37°C, the three isolates became creamy, yeast-like, and white to creamy white, with smooth or heaped irregular surfaces. Under microscopic examination, the yeast cells were thick-walled, globose to subglobose, and 8 to 12 μm in diameter, with persistent buds attached to the parent cells by 3- to 5-μm-wide bases.

When paired with the tester strains of *Ajjelomyces dermatitidis*, isolate B-3016 showed strong stimulation of growth and mycothelial initials with the + tester strain. At the junction of the two colonies (strains B-3016 and B-784*), pseudogymnothecia were produced, indicating that the Algerian isolate was of the + mating type. The isolates from Israel and India reacted similarly when paired with the B-788* tester strain and were thus of the + mating type.

The remaining 11 African isolates differed from isolates B-2280, B-3916, and B-3463 mentioned above and from the North American isolates in colonial and microscopic morphology. After 2 weeks at 25°C on Sabouraud dextrose agar, colonies of the 11 African isolates were glabrous, moist, and folded or radially furrowed, with centrally located coremia. They were white at first, becoming greyish white to light tan after aging. The African isolates generally grew more slowly than the North American isolates did. Of the 11 colonies, 3 developed small sectors which were white with felt-like aerial mycelium after 5 weeks of incubation. When examined microscopically, these sectors were devoid of conidia. Microscopically, the 11 isolates showed hyaline, septate, and branching mycelium. Conidia were present in large to moderate numbers, globose to subglobose to pyriform in shape, and either sessile or borne on short or long conidiophores. The conidia were hyaline, one-celled, and smooth to finely echinulate and measured 3 to 7 by 2 to 3 μm. Echinulations on the outer walls were seen in matured conidia in 5 of the 11 isolates.

All 11 isolates eventually converted to yeast form at 37°C after being subcultured on cotton seed agar (21). Colonies on cotton seed agar were white to light tan, smooth or heaped with irregular surfaces, and slow growing. Microscopic examination of the yeast-like colonies showed globose to subglobose, thick-walled cells 9 to 18 μm in diameter, with persistent budding cells attached to the parent cells with broad, 3- to 5-μm-wide bases. Many chains of three to five budding cells were also observed. In 4 of the 11 isolates, complete conversion into yeast form was not observed. Even though numerous characteristic budding cells were seen, some hyphal elements, consisting of swollen, bizarrely shaped cells, always persisted.

When paired with the tester strains of *Ajelloomyces dermatitidis*, none of the 11 African isolates that were A antigen negative showed any growth stimulation or formation of pseudogymnothecia with either the + or − tester strain.

None of the 76 *Coccidioides immitis*, 83 *H. capsulatum*, or 31 saprophytic fungi isolates produced the A antigen; many, however, particularly the *Coccidioides immitis* isolates, produced at least one unrelated antigen.

Although the anti-*B. dermatitidis* A serum was produced in rabbits against the immunoelectrophoretic A arcs, it was not specific and contained precipitins other than A. Reactions to at least one of these precipitins occurred with the antigen extracts from many of the fungi studied (Table 2).

Antisera to the intact yeast-form cells of one North American (strain X-79) and two African (strains B-832 and B-3015) isolates of *B. dermatitidis* were produced in rabbits. These antisera were tested for precipitin reactivity before and after adsorption with yeast cells of the homologous and heterologous *B. dermatitidis* isolates. The results of these studies were compared with those obtained with the unadsorbed and similarly adsorbed rabbit anti-*B. dermatitidis* A arc serum (Table 3). The reactions noted with the antigens of and the antiserum to the African isolate B-832 were identical to those noted with the antigens of and the antiserum to the African isolate B-3015.

The non-African isolates reacted with the unadsorbed *B. dermatitidis* A arc antiserum to form an A precipitate. Similar studies with the unadsorbed *B. dermatitidis* A arc antiserum indicated that, except for isolate B-3016, the African isolates lacked the A antigen (Fig. 1, Table 2). However, studies with the unadsorbed antisera to the North American and African isolates showed that they reacted with extracts of all the African and North American isolates used in this study, suggesting that these isolates share at least one antigen unrelated to the A antigen (Fig. 1, Table 2 and 3).

The extracts of all of the North American isolates produced an A precipitate and another unique precipitate with the unadsorbed antiserum to North American *B. dermatitidis* X-79. The extracts from all of the African isolates
showed a single common band that was identical to the second band that noted with the extract from the North American isolate (Fig. 1). The extracts of both the African and North American isolates reacted with unadsorbed antisera to the African isolates, producing a single band that was apparently identical to the second band described above. This band was not apparent with extracts from any of the heterologous fungi studied (Table 2).

Adsorbing the B. dermatitidis A arc antiserum with the antigens of African isolates B-832 and B-3015 had no effect on the A precipitin. Adsorbing the antiserum to North American isolate X-79 with cells of either of the two African isolates yielded an antiserum with the A pattern of reactivity. However, this adsorption eliminated the second precipitin common to the African isolates. Adsorbing antisera to the B. dermatitidis A arcs and to the African and North American B. dermatitidis isolates with the antigens of North American isolate X-79 rendered all of the antisera negative for precipitins.

**DISCUSSION**

Studies with antigen extracts of B. dermatitidis isolates from North America, India, and Israel showed that the isolates were homogeneous for production of the specific A antigen. These isolates were readily and accurately identified by the exoantigen test. In contrast, our studies with the African isolates indicated that they were antigenically heterologous and devoid of the A antigen, with the exception of isolate B-3016. The B. dermatitidis A arc antiserum could not be used to identify such isolates because it gave false-negative results. We discovered a second antigen, designated K, that was apparently common to all of the B. dermatitidis isolates studied but, like the A antigen, was absent from the heterologous fungi. Our studies suggested that the K antigen is unrelated to the previously described B. dermatitidis antigens (4, 5). The K antigen is currently under extensive study and will be described in detail in a later publication.

Cross-adsorption studies with antisera to the North American isolate X-79 and two African isolates (B-832 and B-3015) showed the existence of serotypes among the B. dermatitidis isolates (Table 3). These findings confirmed the occurrence of two B. dermatitidis serotypes. These were first suggested by the immunofluorescence studies done by Sudman and Kaplan (16). The North American isolates were for the most part antigenically more complex than the African isolates that we studied. In addition to the specific A antigen, they shared at least one other unique antigen with the African isolates. Whether the use of antibody to the latter antigen will permit the immunoidentification of all B. dermatitidis isolates has yet to be determined.
The African isolate B-3016 and the isolates from Israel and India also produced the A antigen. They had cultural and microscopic characteristics similar to those of the North American isolates of *B. dermatitidis*. The other African isolates differed from the North American *B. dermatitidis* isolates in colonial morphology and microscopic features, especially in the echinulate conidia produced by 5 of the 11 African isolates. The presence of echinulations on the conidia of some African isolates of *B. dermatitidis* was previously pointed out by Vermeil et al. (18, 19, 20) and by Van Oorschot (17). Vermeil et al. (19) proposed a subgenus (*Echinulatum*) to include the anamorphs of *Ajellomyces dermatitidis* and *Ajellomyces capsulatus* without providing a Latin description of their subgenus. The subgenus *Echinulatum* was not validly proposed because the proposal violated Article 36 of the International Code of Botanical Nomenclature. Article 36 clearly specifies that a Latin description of the new taxon must be provided to publish the name of a new taxon on or after 1 January 1935. Van Oorschot (17) preferred the genus *Zymonema* de Beurn and Gougerot to classify *B. dermatitidis*. The fact that some of the African isolates produced echinulate conidia clearly supports Carmichael’s classification of *B. dermatitidis* in the genus *Chrysosporium*.

When the African isolates were paired with the tester strains of *Ajellomyces dermatitidis*, none was stimulated. Similar observations on the incompatibility of African isolates with *Ajellomyces dermatitidis* were reported by Kwon-Chung (10). Our 11 incompatible African isolates with atypical morphological and genetic features all lacked antigen A.

Just as two varieties of *H. capsulatum* (*H. capsulatum* var. *capsulatum*, *H. capsulatum* var. *duboisii*) exist in Africa, it appears that Africa also harbors at least two varieties of *B. dermatitidis*.

Our findings indicate that at least two serotypes of *B. dermatitidis* occur. Serotype 1 has specific antigens A and K. Of the two specific antigens, serotype 2 has only K. Currently, *B. dermatitidis* serotype 2 is only known to occur in Africa. In contrast, serotype 1 isolates have been found in Canada, the United States, Israel, India and Algeria. Studies continue in our laboratory to compare and determine the value of the two apparently specific *B. dermatitidis* antigens for the immunoidentification of isolates of *B. dermatitidis* and for the serodiagnosis of blastomycosis.

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**LITERATURE CITED**