Comparison of Cytoplasmic Extracts of Eight Candida Species and Saccharomyces cerevisiae

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Received 24 August 1981/Accepted 6 November 1981

Crossed-line immunoelectrophoresis of cytoplasmic extracts of eight Candida species and Saccharomyces cerevisiae demonstrated the presence of antigens reactive with a rabbit antiserum to a C. albicans extract in all species except C. glabrata. A previously defined major cytoplasmic antigen of C. albicans was also present in C. tropicalis and C. guilliermondii.

Severe infections caused by yeasts normally considered saprophytic occur frequently in compromised hosts. Candida species are the most commonly incriminated yeasts (11, 21), with C. albicans the species most commonly involved (4, 11, 21, 24). Recent reports have also emphasized the occurrence of serious infections caused by C. tropicalis (21, 33), C. glabrata (formerly Torulopsis glabrata) (1, 3, 21, 31), C. stellatoidea (23), C. parapsilosis (22, 25), C. krusei (21, 22), C. guilliermondii (30), and C. pseudotropicalis (21, 24). In addition, a related yeast, Saccharomyces cerevisiae, has been reported as a rare cause of endocarditis (25) and septicemia (6).

Because diagnosis of these yeast infections by clinical impression or culture is often difficult, numerous investigators use serological tests to facilitate early diagnosis (5). C. albicans homogenate antigens are employed in one of the most widely studied techniques to detect precipitating antibodies which reportedly recognize cytoplasmic components. However, because significant amounts of cell wall mannan are usually present in such homogenates, it is unclear whether antibody to mannan or to cytoplasmic components is being detected (15).

We have described procedures for purification of a major cytoplasmic antigen of C. albicans and the characterization of preparations enriched for this antigen (9). In the study reported here, we compared extracts from C. albicans and eight related organisms to evaluate similarities and differences in their cytoplasmic components.

Cultures of C. albicans serogroup A (strain B311) were obtained from sources previously described (15). Cultures of C. albicans serogroup B (strain 792) were obtained from John E. Bennett, National Institutes of Health, Bethesda, Md. All other organisms (C. guilliermondii [ATCC 6260], C. krusei [ATCC 6258], C. parapsilosis [ATCC 7330], C. pseudotropicalis [ATCC 4135], C. stellatoidea [ATCC 34138], C. tropicalis [ATCC 750], C. glabrata [ATCC 15545], and S. cerevisiae [ATCC 9763]) were obtained from the American Type Culture Collection, Rockville, Md. The identity of these organisms was confirmed by carbohydrate assimilation auxanography (26). Serotyping of C. albicans was performed by agar gel diffusion of cell wall extracts of the organisms against reference antiserum obtained from H. F. Hasenclever, Rocky Mountain Laboratory, Hamilton, Mont. (13).

A seed culture of each organism was prepared in Sabouraud dextrose broth (Difco Laboratories, Detroit, Mich.), inoculated into 3 liters of the same medium in a sterile Nalgene carboy, and incubated at room temperature for 36 to 72 h with constant magnetic stirring. The organisms were harvested by centrifugation at 2,000 × g and washed three times in physiological saline.

Cytoplasmic extracts of all organisms were prepared by a previously described technique (15). Their protein content was determined by the Lowry method (19). Equal amounts of cytoplasmic protein were compared in all subsequent experiments.

A partially purified preparation of a major cytoplasmic antigen of C. albicans B311 was prepared as previously described (9). Briefly, the crude cytoplasmic extract was added to a DEAE-cellulose column. After initial elution with 0.01 M NaCl in 0.067 M Tris buffer (pH 7.2), a fraction enriched for the major antigen was eluted with the Tris buffer containing 0.04 M NaCl.

Antiserum to the cytoplasmic extract of C. albicans B311 was produced in New Zealand white rabbits as previously described (15). Pooled serum from a single rabbit was used in all experiments.

Crossed-line immunoelectrophoresis was performed by modification of techniques of Kroll
(18) and Guinet and Gabriel (10), using glass plates (9.4 by 8.3 cm) and a 1% agarose gel in barbital buffer (pH 8.6; ionic strength, 0.02) (32).

Like C. albicans serogroup A, C. albicans serogroup B, C. guilliermondii, and C. tropicalis extracts contained the major cytoplasmic antigen (Fig. 1). Over 30 antigen-antibody precipitates were seen with both serogroups of C. albicans. Although the overall patterns were quite similar, several qualitative and quantitative differences were observed. The C. guilliermondii and C. tropicalis extracts formed approximately 10 precipitates with the C. albicans serogroup A antiserum. C. guilliermondii contained considerably less major cytoplasmic antigen than did the other three organisms.

Figure 2 shows the crossed-line immunoelectrophoresis patterns of four Candida species which did not contain the major cytoplasmic antigen, i.e., C. krusei, C. parapsilosis, C. pseudotropicalis, and C. stellatoidea. However, these species contained various antigens which

![Image of crossed-line immunoelectrophoresis patterns](http://iai.asm.org/)

**FIG. 1.** Crossed-line immunoelectrophoresis of 120 μg of cytoplasmic extract protein (well) and 90 μg of partially purified major cytoplasmic antigen of C. albicans serogroup A (line) against rabbit anti-cytoplasmic extract antiserum (10 μl/cm²). (A) C. albicans serogroup A; (B) C. albicans serogroup B; (C) C. tropicalis; and (D) C. guilliermondii. First-dimension electrophoresis was conducted at 15 V/cm for 1 h and second-dimension electrophoresis was conducted at 3 V/cm for 18 h with positions of the anodes (+) as shown. Gels were stained with Coomassie brilliant blue R-250.
were reactive with \textit{C. albicans} serogroup A antiserum.

The crossed-line immunoelectrophoresis patterns of \textit{C. glabrata} and \textit{S. cerevisiae} are shown in Fig. 3. The \textit{C. glabrata} cytoplasmic extract had no antigens which reacted with the \textit{C. albicans} serogroup A antiserum. \textit{S. cerevisiae} contained several cross-reactive antigens, but not the major cytoplasmic antigen.

Previous studies of the immunological relationship among yeasts have dealt primarily with surface antigens (13, 17, 28). Hasenclever and co-workers demonstrated that surface antigenic determinants of \textit{C. albicans} are expressed chiefly by cell wall mannans and divided \textit{C. albicans} into two serogroups on the basis of partial identity of their cell wall mannans. Work by these investigators (12–14, 28) and Fraser (7) demonstrated cross-reactivity of surface antigens of \textit{C. albicans} with all yeast species studied in the present report. We have confirmed this observation by demonstrating that cell wall extracts of all of these yeasts form precipitin lines when tested by double immunodiffusion against antiserum to heat-killed \textit{C. albicans} (unpublished data). Thus, antibodies to cell wall mannans of \textit{C. albicans} serogroup A, strain B311, could potentially be detected in such yeast infections.

In several studies, immunological comparisons of homogenates of \textit{Candida} species have been reported. Stickle et al. (27) used a latex
agglutination method with antisera to whole cells to demonstrate that a homogenate antigen of *C. albicans* reacts with antisera to eight species of *Candida*. However, since these reactions may have been due to mannan in the homogenates (15), a direct comparison with our findings cannot be made. Similarly, positive precipitin tests to *C. albicans* homogenate antigens in patients infected with *C. guilliermondii*, *C. parapsilosis*, *C. tropicalis*, and *C. glabrata* (8, 20, 29) may be due to antimannan antibodies or previous exposure to *C. albicans*.

Results of crossed immunoelectrophoresis of serum from a patient with *C. parapsilosis* endocarditis against *C. albicans* homogenate antigen (8) are very similar to our results with *C. albicans* antiserum and *C. parapsilosis* extract. Guinet and Gabriel (10) recently made an antigenic comparison of homogenates of *C. albicans* serogroups A and B. Although their methodology to produce homogenates differed from ours, the findings are comparable. Using a rabbit antiserum to a *C. tropicalis* homogenate, Montrocher (22) demonstrated antigens of *C. albicans* and *C. stellatoidea* homogenates that were cross-reactive with antigens in the *C. tropicalis* homogenate. Again, these findings are compatible with, although not directly comparable to, the results reported in our study.

In summary, the results of this study demonstrate antigens in the homogenates of all yeast species tested, except *C. glabrata*, which are reactive with an antiserum to the homogenate of *C. albicans* serogroup A, strain B311. This indicates that serodiagnostic tests to detect cytoplasmic antigens of *C. albicans* B311 or antibodies to such antigens could potentially be used to diagnose serious infections due to all of these yeasts except *C. glabrata*. Although we tested only one strain of each species, a previous study of eight strains of *C. albicans* revealed no qualitative interstrain variation in the number of antigens detected by crossed immunoelectrophoresis, although quantitative differences were seen (2).

These findings further indicate that detection of a previously characterized major cytoplasmic antigen of *C. albicans* serogroup A, strain B311 (9), or antibody to this antigen, could be used to diagnose infections caused by both serogroups of *C. albicans* and *C. tropicalis* and *C. guilliermondii*. This is supported by the demonstration of antibody directed primarily against the major cytoplasmic antigen in a patient with *C. tropicalis* infection (16). Enzyme-linked immunosorbent assays for detection of this antigen and antibody against it are currently being developed and tested in our laboratory.

This work was supported by the Veterans Administration and Public Health Service grant AI15682-03 from the National Institutes of Health.

We thank James Stephens for technical assistance and Cynthia Birch for editorial assistance in the preparation of this manuscript.

**LITERATURE CITED**


