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SHORT COMMUNICATION

Ultrastructural localization of specific surface antigens in the dimorphic fungus *Sporothrix schenckii*

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The ultrastructural localization of antigens recognized by an antiserum raised against formaline-killed *Sporothrix schenckii* yeast cells was investigated on yeast and mycelial phases of the fungus. Immunogold procedures revealed that these antigens were located on the cell surface of both growth phases. Labeling was heterogeneous and involved areas of Concanavalin A-binding sugar residues.

Sporotrichosis is one of the most common cutaneous and subcutaneous mycoses in Venezuela [1]. Immunocytochemical methods have been shown to be useful in the diagnosis of this infection and in the demonstration of the fungus in tissue and body fluids of infected individuals [6, 8].

In the present study, surface components of *Sporothrix schenckii* were localized using a specific anti-*S. schenkii* antiserum in an immunogold staining procedure. In addition, Concanavalin A-binding sugar residues were detected by a cytochemical technique.

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S. schenckii (strains 0073, 0260, 8770, 9977), *Paracoccidioides brasiliensis* (strain 0350), *Candida albicans* (strain 8385), *Cryptococcus neoformans* (strain B551) and *Geotrichum* species (strain 0001) were used in this investigation.

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S. schenckii strains were grown on brain-heart infusion agar slants (Difco) at 37°C in a 5% CO_2 atmosphere, to obtain yeast-like cells and in Sabouraud's glucose agar (Difco) at room temperature to obtain the mycelial phase. *P. brasiliensis* was cultured on brain heart infusion agar at 37°C and the other fungi were grown on Sabouraud's glucose agar at 25°C for 6 days to obtain the yeast phase.

Anti-*S. schenckii* SS-78-0258 antiserum was a gift from Dr W. Kaplan, CDC, Atlanta, Georgia, USA and was obtained from Dr M. C. Albornoz, Instituto de Biomedicina. This antiserum was raised in a rabbit injected intravenously with

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formalin-killed *S. schenckii* yeast-like cells grown for 6 days as described previously [5].

Pre-embedding was carried out by a modification of the procedure of De Waele *et al.* [2]. Fungal cells were washed twice with working buffer (phosphate buffered saline, PBS 0·1 M pH 7·2 containing 0·85% NaCl and 1% bovine serum albumin (BSA), prior to initiation of the immunoreaction and after each incubation step. Each washing step involved resuspension of the cell pellet in 1000 μl of working buffer and subsequent centrifugation at 300 g for 5 min at room temperature. The pellet was sequentially resuspended in 25 μl of the following solutions: anti-*S. schenckii* SS-78-0258 diluted 1:200, for 30 min at room temperature or 37°C depending on the growth phase under study; A 1:2 dilution of gold-labelled goat anti-rabbit IgG (Janssen Pharmaceutical, Belgium), for 45 min at the appropriate temperature; 2·5% glutaraldehyde in 0·1 M cacodylate buffer, pH 7·2 for 30 min at room temperature; 1% aqueous buffer, pH 7·2 for 30 min at room temperature; and 1% aqueous osmium tetroxide for 45 min at 4°C. After washing twice with double distilled water the cell pellet was embedded in 2% Noble agar and immediately dehydrated, impregnated and embedded in Epon.

A postembedding immunocytochemical procedure was also used. The cell pellet was fixed with glutaraldehyde, washed with sodium cacodylate buffer, embedded in Noble agar, dehydrated, impregnated and embedded in Epon. Ultrathin sections, 50–100 nm thick, were cut with an ultramicrotome and collected onto uncoated 300-mesh nickel grids. The grid-mounted sections were treated with 10% aqueous H_2O_2 for 10 min at room temperature followed by a 1:30 dilution of normal goat serum in Tris-buffered saline (TBS) 0·1 M pH 7·2 for 15 min. The sections were then incubated in a 1:200 dilution of anti-*S. schenckii* antibody for 45 min and then jet-washed with TBS. Gold-labelled goat anti-rabbit IgG (10–40 nm gold particles) diluted 1:2 was then added for 30 min and the sections rinsed sequentially with TBS containing 1% BSA, TBS and double distilled water.

Double detection of anti-*S. schenckii* immuno-gold and Concanavalin A (Con A) horseradish peroxidase (HRP) binding sites was performed on strain 8770 (yeast phase, 3-days-old). The cytochemical localization of Con A was carried out by incubating the cell pellet at 37°C in Con A (100 μg ml^{-1}) for 15 min followed by HRP (50 μg ml^{-1}) for 15 min. The reaction was revealed as described by Graham & Karnovsky [8] using 3,3′-diamino-benzidine as the chromogen. The cells were then treated with 1% aqueous buffer followed by aqueous osmium tetroxide as described in the pre-embedding immunocytochemical procedure.

Immunoreactivity to antiserum SS-68-0258 was observed in *S. schenckii* and was independent of the strain, the morphological phase and the age of the cultured cells (Fig. 1). The specific antiserum did not recognize antigenic components in *P. brasiliensis*, *Cr. neoformans*, *Geotrichum* species or *C. albicans*. Within any given cell population all yeast-like cells, mycelium and conidia showed variable degrees of immunoreactivity on their surface.

Antigens were localized only on the surface of the fungus and were observed on both sublayers of the cell wall (Fig. 1). These observations were confirmed by the results of post-embedding experiments.

The antigen recognized by the specific antibody did not show any specific distribution over the cell wall. It was interesting to observe, however, that gold particles were frequently associated with the microfibrillar material which apparently bridges neighbouring cells.

Con A-reactive carbohydrate components were demonstrated by the presence of an electron dense material on both sublayers of the cell wall (Fig. 2). Heterogeneity of the Con A reaction was observed, though it did not appear to be correlated to the age of the cells or to the growth phase.

Immunogold localization with antiserum SS-780258 along with cytochemical detec-

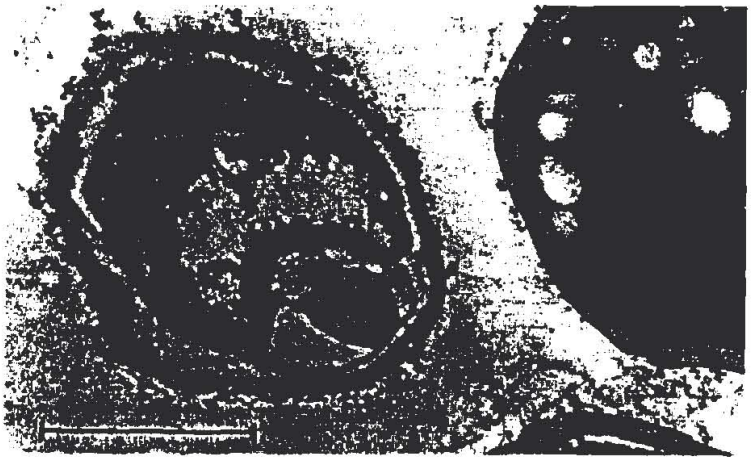

FIG. 1. Immunogold staining with antiserum SS-78-0258 on 6-day-old yeast-like cells of *S. schenckii*. Colloidal gold particles (20 nm in diameter) are localized over the cell surface. Note the heterogeneity of the immunoreaction. ×28 000, bar = 1 μm.

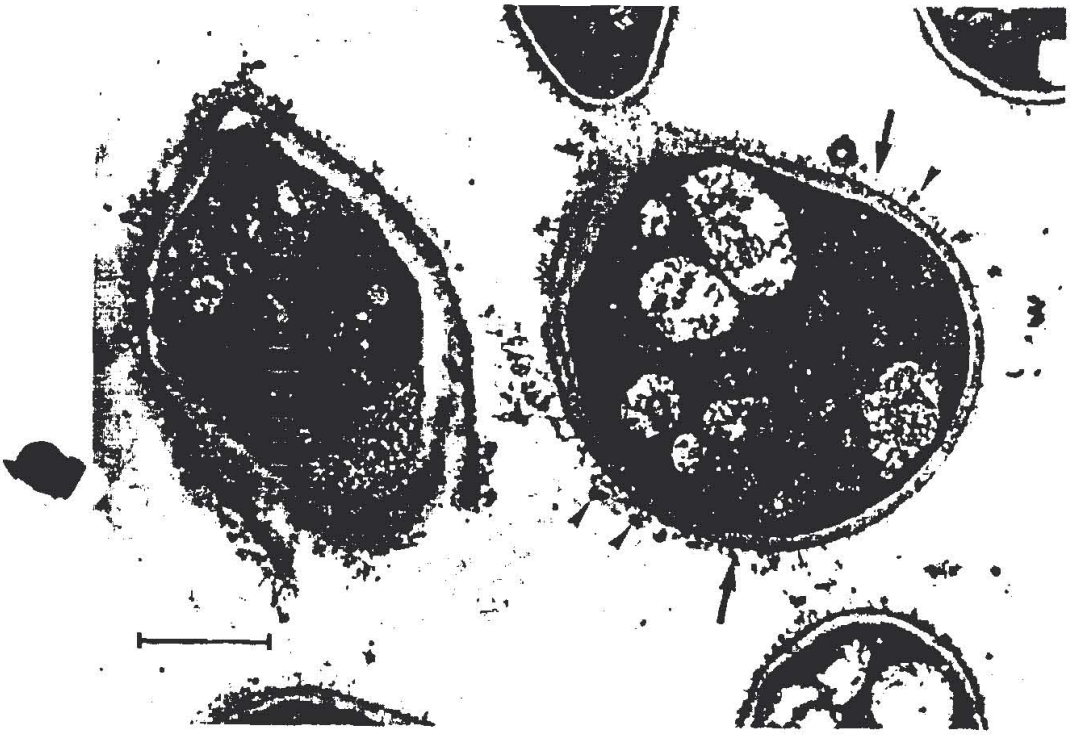

FIG. 2. Double labeling cytochemical and immunocytochemical staining of 3-day-old yeast-like cells of *S. schenckii* using the specific antiserum SS-78-0258 (arrow heads) and the lectin Con A (short arrows). Heterogeneity of the reactions can be observed as well as colocalization of antigen and Con A sugar residues. ×18 000, bar = 1 μm.

tion of sugar residues with Con A allowed a direct comparison of both cell surface constituents. Binding of Con A to the cell surface superimposed that of the specific antiserum although, as expected, Con A binding was higher (Fig. 2).

This study has demonstrated the presence of common antigens on the cell wall of both morphological phases of *S. schenckii*. The observed heterogeneity of the immunostaining of different cells might be due to differences in both the concentration and composition of the antigens exposed on the cell surface. This variability did not appear to be correlated to the age of the cells or to the growth phase although an increase in non-Con A-binding components during the morphogenetic events that take place during hyphal to yeast cell transformation has been described [9].

The observed decrease in the number of gold particles in double labelling experiments may be attributed to spontaneous loss, shedding of labelled surface components and/or to stearic hindrance between the lectin molecules and/or the markers [7].

The possibility that gold particles simply became trapped in the capsular microfibrillar mesh cannot be overlooked although this does not seem likely due to the precise localization of some of the gold particles along the extended microfibrils.

Such microfibrillar material has been postulated to be part of the capsular material [5], and may, in some way, be involved with mechanisms of cellular adhesion [3].

Future studies should be focused on the determination of the biochemical nature of the specific antigens revealed in this study and their actual roles in host-fungal interaction.

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Celulas dendriticas Epidermicas Thy-1 +
Un nuevo grupo celular asociado
con tumores cutaneos.

Martin A. Sanchez, Carlos Ritter, Felix Tapia

Año 90.

Dermatol. Venez.

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