

## Preliminary Description of Antigenic Components Characteristic of *Stachybotrys chartarum*

Päivi Raunio,\*<sup>1</sup> Marja Kärkkäinen,\* Tuomas Virtanen,† Jaakko Rautiainen,† and Anna-Liisa Pasanen\*

\*Department of Environmental Sciences and †Department of Clinical Microbiology, University of Kuopio, Kuopio, Finland

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### INTRODUCTION

The objective of this study was to characterize preliminarily immunogenic components characteristic of *Stachybotrys chartarum* to be used later for the development of a detection method for the fungus in environmental samples. The procedure for *S. chartarum* extract preparation was first optimized related to the age of the culture, culture type, and growth medium, and the antigenic composition of *S. chartarum* cultured in two different media was then characterized by the sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and immunoblotting method. Cross-reactivity of *S. chartarum* antigenic components with 10 other fungal species was identified by the inhibition immunoblotting method. The 10-day-old *S. chartarum* culture extract cultured in malt extract broth revealed a wider selection of proteins and antigenic components than the 30-day-old culture extract or the culture medium extracts. When cultured in cellulose broth, *S. chartarum* produced a higher number of proteins and antigenic components than in malt extract broth. The most dominant immunogenic components of *S. chartarum* cultured in cellulose broth were those of 65, 50, 37, and 27 kDa. The components of 65 and 50 kDa proved to be the most characteristic of this fungus according to the inhibition immunoblotting analyses. Several of the *S. chartarum* components were identified as glycoproteins. Carbohydrate moieties of the *S. chartarum* components also possessed an antibody binding activity. © 2001 Academic Press

**Key Words:** *Stachybotrys chartarum*; fungus; cross-reactivity; antigen; glycoprotein.

There is an increasing concern regarding contamination of toxigenic fungi, such as *Stachybotrys chartarum*, *Memnoniella echinata*, and *Aspergillus versicolor*, in moisture problem buildings. From about five *Stachybotrys* species mentioned in the literature, only *S. chartarum* (former name *S. atra*), as a saprophytic fungus growing well in wet cellulose containing materials, has been associated with mold problems in indoor environments and damp buildings. Several strains of *S. chartarum* have been observed to be capable of producing a wide variation of highly toxic mycotoxins (e.g., trichothecenes) that are known to cause adverse health effects in humans (Croft *et al.*, 1986; Jarvis *et al.*, 1998; Johanning *et al.*, 1999). Thus, *Stachybotrys* has been named one of the most harmful fungi in indoor environments and is regarded as an indicator organism for moisture problems that may cause serious health implications for occupants in affected buildings (Samson *et al.*, 1994a). Because the proliferation of *S. chartarum* contamination requires very high and somewhat constant moisture conditions (Ayerst, 1969; Grant *et al.*, 1989), this fungus is not a particularly frequent contaminant in buildings. However, it has been shown that as this fungus contaminates materials and constructions, the contamination is massive and *S. chartarum* highly dominates the fungal flora in the substrate (Croft *et al.*, 1986; Hodgson *et al.*, 1998; Nielsen *et al.*, 1998).

Recognition of fungal contamination and especially identification of harmful fungal species and their products in damaged building materials require special expertise. Methods like cultivation, microscopic counting, or complex chemical analyses are typically used for this purpose. However, there are several problems with the identification of *Stachybotrys* in buildings. Because no selective culture media for *Stachybotrys* are available and only

<sup>1</sup>To whom correspondence and reprint requests should be addressed at the University of Kuopio, Department of Environmental Sciences, Research and Development Unit of Environmental Health, Bioteeknia 2, P.O. Box 1627, FIN-70211 Kuopio, Finland. Fax: 358-17-163 750. E-mail: Paivi.Raunio@uku.fi.

less than 10% of the *Stachybotrys* spores present in the air are assumed to be viable (Miller, 1992), the cultivation method is not recommended. The present microscopic methods are not yet sufficiently advanced for identification of fungi at the genus or species level. All *Stachybotrys* strains do not produce toxins at all, or produce toxins only under certain conditions (Nielsen *et al.*, 1998; El-Maghraby *et al.*, 1991; Nikulin *et al.*, 1994; Pasanen *et al.*, 1994). There is also a wide range of different toxins that *Stachybotrys* species are capable of synthesizing, all of which have not been yet characterized precisely (Jarvis *et al.*, 1995, 1996, 1998). In addition, *M. echinata*, the most closely related fungus to *S. chartarum*, has also been observed to occur together with *S. chartarum* in the indoor environments and to produce similar or even the same toxins (Jarvis *et al.*, 1998). So, the determination of certain toxins as a detection method for *S. chartarum* is not solely complicated and expensive but may also lead to false conclusions. Finally, a delay between sampling and availability of results is a practical problem related to all the above-mentioned methods.

Immunochemical methods can provide a good alternative for the traditional detection methods and offer a fast and reliable tool for identification of fungal species. The basic requirement for an immunochemical method to be used for the detection of a certain fungus is that it should be able to identify an antigenic component characteristic of the fungus searched for and have no disturbing cross-reactivity with other fungal species that may occur and grow at the same sites with the fungus concerned. However, many fungal species contain the same or similar components, for example, enzymes or cell wall components, that may act as antigens and result in a large variety of cross-reactivity between various fungal species (Shen *et al.*, 1998; Breitenbach *et al.*, 1997; Nissen *et al.*, 1998; Verma *et al.*, 1995; Agarwal *et al.*, 1982; Brouwer, 1996; Fuhrman *et al.*, 1992; Tee *et al.*, 1987; O'Neil *et al.*, 1987). Therefore, the development of specific immunochemical methods for the detection of certain fungus is a long process that demands much background information and scientific knowledge of the composition of antigenic components of concerned and related fungi.

There are no standardized methods for preparing fungal extracts or recommendations for the source material. In some fungal species, the dominant antigenic components and relevant allergens are found in mycelium and/or spores, while in some species they are secreted in the growth medium (Nissen *et al.*, 1998; Verma *et al.*, 1995; Hoffman *et al.*, 1981;

Aukrust *et al.*, 1985; Paris *et al.*, 1990a; Fadel *et al.*, 1992; Bush and Yunginger 1997; Zhang *et al.*, 1994; Arruda *et al.*, 1992; Portnoy *et al.*, 1993a). The optimal age of source material also depends on fungal species studied (Kauffman *et al.*, 1980; Swärd-Nordmo *et al.*, 1984; van der Heide *et al.*, 1985; Kroutil *et al.*, 1984). This means that a procedure for preparing fungal extracts for the characterization of an antigenic composition should be optimized for each species individually.

In this study, we characterized the protein and antigenic composition of *S. chartarum* culture extracts and culture medium extracts prepared from cultures grown in malt extract broth for 10 and 31 days to assure the best and most versatile source material for the preparation of antigen extracts. We also compared the antigenic compositions of the *S. chartarum* extracts prepared from malt extract and cellulose broth, the latter of which resembles better the natural growth substrates of the fungus (Samson and van Reenen-Hoekstra, 1988). In addition, the role of glycoproteins as *S. chartarum* antigens and the cross-reactivity of the *S. chartarum* components with the components of 10 other fungal species that are most commonly known to grow under conditions similar to those of *S. chartarum* (moisture problem buildings; Samson *et al.*, 1994a, b; Flannigan *et al.*, 1994) were studied. Also, the strain-to-strain variability was investigated by characterizing the protein and antigenic profiles of six *S. chartarum* strains. The final goal for this study was to indicate the most suitable antigenic components characteristic of *S. chartarum* to be used later in the development of the specific detection method for the fungus.

## MATERIALS AND METHODS

### *Preparation of the Fungal Antigen Extracts*

The protocol for fungal antigen extract preparation was based on the procedures presented by Paris *et al.* (1990b), Horst *et al.* (1990), Portnoy *et al.* (1993b), and Achatz *et al.* (1995). *S. chartarum* (American Type Culture Collection (ATCC) 208877, earlier known as University of Kuopio (UKU) 10) was first cultured on 2% malt extract agar (Biokar Diagnosis, France) for 7–10 days at 25°C, after which spore suspension was prepared in phosphate-buffered saline (PBS, pH 7.4) with 0.05% (v/v) Tween 20. One milliliter of the spore suspension was inoculated in 100 mL of 2% malt extract broth in stationary Roux bottles and the culture was incubated for 10 or 31 days at 25°C. Mycelium was separated from broth by centrifugation (1000g, 20 min), dried in a vacuum dryer (VTR 5050, W.C. Heraeus

GmbH, Germany), stored at  $-70^{\circ}\text{C}$ , and resuspended at 1:20 (w/v) in PBS (pH 8.0) with 0.02% (w/v) phenylmethylsulfonyl fluoride and 0.02% (w/v) sodium azide. The mycelium suspension was homogenized mechanically (CAT homogenizer X1030D, M. Zipperer GmbH, Germany) and extracted in a shaker for 16–18 h at  $4^{\circ}\text{C}$  (200 rpm). After centrifugation (1000g, 10 min and 43,000g, 30 min at  $4^{\circ}\text{C}$ ), the supernatant (culture extract) was dialyzed (molecular weight cutoff 3.5 kDa) against three changes of PBS (pH 7.4) at  $4^{\circ}\text{C}$  for 24 h, sterile-filtered (0.2  $\mu\text{m}$ ), and lyophilized. The 10- and 31-day broth (culture medium) separated in the first centrifugation was first concentrated by lyophilization, dialyzed, filtered, and finally lyophilized again as described above. Lyophilized extract was stored at  $-70^{\circ}\text{C}$  and resuspended in MilliQ-UF water for use. The protein content of the extracts was determined by the dye-binding method of Bradford (1976; Protein Assay, Bio-Rad Laboratories, U.S.A.).

### Fungal Strains Used

To investigate the influence of the growth medium on the antigenic composition of *S. chartarum*, we cultured the fungus in cellulose broth (Dillon *et al.*, 1996) for 10 days and the culture extract was prepared as described above. For studying the cross-reactivity between *S. chartarum* and the 10 selected fungal species, *Alternaria alternata* (Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) 62006), *Aspergillus versicolor* (UKU 3), *Aureobasidium pullulans* (DSMZ 62074), *Chaetomium globosum* (UKU 4), *Cladosporium cladosporioides* (DSMZ 62121), *Memnoniella echinata* (Centraalbureau voor Schimmelcultures (CBS) 304.54), *Penicillium brevicompactum* (ATCC 58606), *Phialophora rebens* (National Public Health Institute (KTL) RE27), *Phoma* sp. (UKU 8), and *Ulocladium botrytis* (UKU 11) were cultured in cellulose broth and the culture extracts were prepared like the *S. chartarum* extract. *C. cladosporioides* and *A. pullulans* grew poorly in cellulose broth and were cultured for 30 days to obtain a sufficient amount of mycelium for preparing the culture extract. Five other *S. chartarum* strains (four Finnish strains coded KTL 296, KTL 391, KTL 530, Kuopio Regional Institute of Occupational Health (KATTI), and one strain isolated from a gypsum board sample from the United States) were studied by the sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE), immunoblotting, and inhibition immunoblotting methods to compare the strain-to-strain variability of protein and antigenic profiles.

### Rabbit Antiserum to Fungal Antigens

The *S. chartarum* 10-day culture extracts cultured either in malt extract broth or in cellulose broth were used for the immunization of three female New Zealand White rabbits. Subcutaneous immunizations were performed four times at 2-week intervals with 1 mg of extract protein per rabbit. Serum samples were collected three times: before the first immunization, before the third immunization, and 2 weeks after the last immunization (terminal serum). The sera were stored at  $-70^{\circ}\text{C}$  and the pooled terminal malt extract or cellulose extract-specific serum was used in the analyses.

### SDS-PAGE

SDS–PAGE and electrophoretic transfer were carried out for the *S. chartarum* extracts as described before (Ylönen *et al.*, 1990) with some modifications. Briefly, the proteins were separated by SDS–PAGE in a (10  $\times$  8 cm) 15% acrylamide gel using the discontinuous buffer system of Laemmli (1970). The resuspended samples were boiled with an equal amount of sample buffer for 5 min and 40  $\mu\text{L}$  of the suspensions containing 50  $\mu\text{g}$  of protein was loaded onto gel. Electrophoresis was carried out at 180 V for 1 h. The gel was stained with Coomassie blue, and scanned, and the approximate molecular weights of the protein components were analyzed with a computer program (Fragment Analysis, Molecular Dynamics, U.S.A.).

### Immunoblotting and Inhibition Immunoblotting

For the immunoblotting analysis, *S. chartarum* proteins in unstained gels were transferred to a Hybond-ECL nitrocellulose membrane (Amersham Int., UK) according to the procedure of Towbin *et al.* (1979). Electrophoretic transfer was performed at 100 V for 40 min. The nitrocellulose membrane with electrotransferred proteins of antigen extracts was blocked for 5 h with 1% (w/v) gelatin–PBS, 0.1% (v/v) Tween 20 (Fluka Chemie, Switzerland). The membrane was incubated overnight with the pooled terminal *S. chartarum*-specific rabbit antisera (malt extract culture or cellulose culture-specific, depending on the extract used), at a dilution of 1:5000 and thereafter with peroxidase-conjugated swine anti-rabbit immunoglobulins (P0399, Dako, Denmark) at a dilution of 1:5000 for 1 h. All the incubations were at room temperature. The membrane was washed with PBS–Tween several times. The immunologically reactive protein components were detected by

the ECL chemiluminescence method (Amersham). In the inhibition immunoblotting analyses, SDS-PAGE was performed using preparative combs; 500 µg of the cellulose extract protein was loaded onto gels. The membranes were cut into strips prior to the blocking. To perform the inhibition, the *S. chartarum* cellulose extract-specific antisera were first incubated at 37°C for 1 h with several 10-fold dilutions (2–2000 µg/mL) of different fungal extracts. Thereafter the analyses were completed as described above.

#### Lectin Blotting Assay and Periodate Treatment

The specific binding of lectins to the components of *S. chartarum* cellulose extract was evaluated by the DIG Glycan Differentiation Kit (Boehringer-Mannheim, Germany) according to the manufacturer's procedure. The significance of the carbohydrate moieties for the antibody binding activity was analyzed by the periodate treatment followed by immunoblotting with the specific rabbit antisera. The periodate treatment was performed as described by Heelan *et al.* (1991). In brief, after transferring the *S. chartarum* extract onto nitrocellulose membrane, all strips were rinsed with 50 mM sodium acetate buffer (pH 4.5). The control strips were incubated in the same buffer for 1 h at 23°C. The test strips were exposed to 50 mM sodium *m*-periodate in sodium acetate buffer (pH 4.5) for 1 h in the dark at 23°C. Both the control and test strips were rinsed with

50 mM sodium acetate buffer followed by incubation with 50 mM sodiumborohydride in PBS (pH 7.4) for 30 min at 23°C. The strips were washed in TBS for 5 min and the immunoblotting was performed as described above.

## RESULTS

### *Protein and Antigenic Profiles of the S. chartarum Extracts*

The total numbers of protein components in the 10- and 31-day culture extracts and the corresponding culture medium extracts of *S. chartarum* cultured in malt extract broth were 12, 10, 9, and 11, respectively. The specific rabbit antisera produced against the 10-day culture extract recognized 12, 7, 4, and 7 of the protein components in the extracts (Table 1). The extracts prepared from malt extract broth contained nonimmunogenic components with a small molecular weight (<14 kDa) that caused intense smearing of the Coomassie-stained SDS-PAGE gel (Fig. 1). The 10-day culture extract prepared from *S. chartarum* cultured in cellulose broth contained 13 protein components in the SDS-PAGE analysis. The specific rabbit antisera recognized 18 immunogenic components in the *S. chartarum* cellulose extract in the immunoblotting analysis. The protein and antigen profiles of the different *S. chartarum* strains were similar (data not shown).

TABLE 1

Schematic Presentation of the Protein and Antigen Profiles of 10- and 31-Day Culture and Culture Medium Extracts of *S. chartarum* Cultured in Malt Extract Broth

| SDS-PAGE protein profile |                 |        |                        |        | Antigen profile, immunoblotting with the rabbit antiserum |                 |        |                        |        |
|--------------------------|-----------------|--------|------------------------|--------|-----------------------------------------------------------|-----------------|--------|------------------------|--------|
| Molecular mass (kDa)     | Culture extract |        | Culture medium extract |        | Molecular mass (kDa)                                      | Culture extract |        | Culture medium extract |        |
|                          | 10-day          | 31-day | 10-day                 | 31-day |                                                           | 10-day          | 31-day | 10-day                 | 31-day |
| 92                       | XX              | X      | X                      | X      | 92                                                        | ( )             |        | ( )                    |        |
| 77                       | (X)             | (X)    | (X)                    | (X)    | 77                                                        | ( )             | ( )    | ( )                    |        |
| 67                       | (X)             | (X)    | (X)                    | (X)    | 64                                                        | ( )             | ( )    | ( )                    |        |
| 64                       | (X)             | (X)    | (X)                    | (X)    | 57                                                        | XX              | XX     | XX                     | XX     |
| 57                       | XXX             | ( )    | (X)                    | ( )    | 54                                                        |                 |        |                        | XX     |
| 45                       | X               | X      | ( )                    | (X)    | 48                                                        | (X)             |        |                        | X      |
| 43                       | X               | (X)    | ( )                    | ( )    | 45                                                        | (X)             | ( )    |                        | X      |
| 37                       | X               | X      | (X)                    | X      | 37                                                        | XX              |        |                        |        |
| 31                       | ( )             | X      |                        | X      | 33                                                        |                 |        |                        | XX     |
| 25                       | XXX             | ( )    |                        |        | 31                                                        | (X)             | ( )    |                        | X      |
| 20                       | ( )             |        |                        | X      | 30                                                        | (X)             |        |                        |        |
| 17                       | X               |        | ( )                    | (X)    | 25                                                        | XXX             | ( )    |                        |        |
|                          |                 |        |                        |        | 20                                                        | ( )             | ( )    |                        |        |
|                          |                 |        |                        |        | 14.4                                                      | (X)             |        |                        | ( )    |

Note. ( ), very weak; (X), weak; X, distinct; XX, very distinct; XXX, strong band.

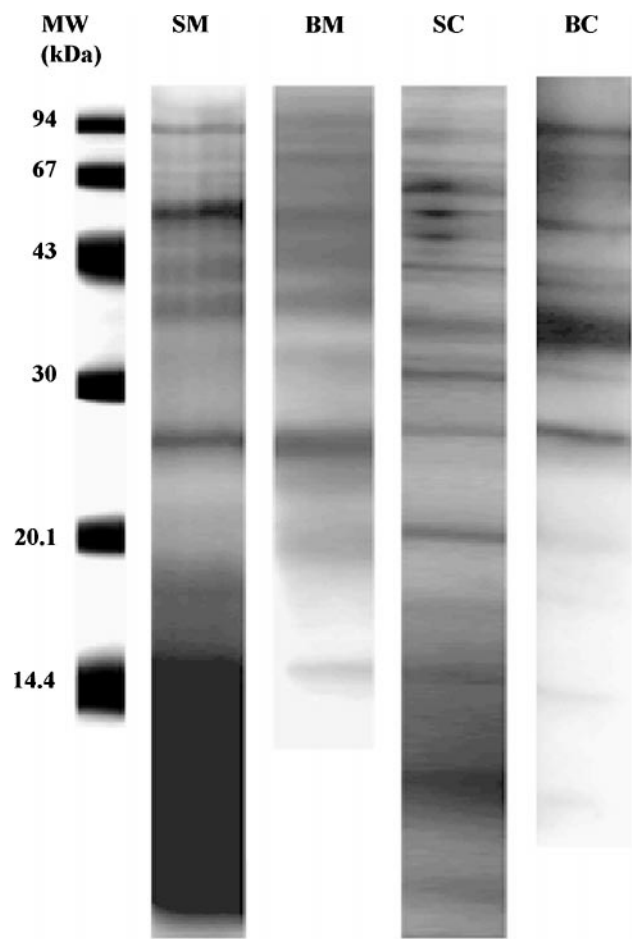


**FIG. 1.** SDS-PAGE (S) and immunoblotting (B) profiles of the *S. chartarum* culture extracts cultured in malt extract (M) and cellulose extract (C) broth for 10 days. MW, molecular weight markers.

*Glycoproteins and Their Antigenicity*

The most abundant carbohydrate moiety in the *S. chartarum* cellulose extract identified by the DIG Glycan Differentiation Kit was mannose (Fig. 2). Galactose- $\beta$ (1-4)-*N*-acetylglucosamine and sialic acid were also detected in some components of 100–24 kDa. The periodate treatment generally decreased the antibody binding capacity of the most *S. chartarum* components (Fig. 2). Also components of 37 and 17 kDa, which were not detected by the lectin blotting analysis indicating that they contained sugars other than could be detected by the kit, partly lost their antibody binding capacity during the periodate treatment. The component of 31 kDa totally lost its antigenicity during the peri-

odate treatment. The treatment did not have any effect on the antigenicity of the glycoprotein component of 50 kDa, which contained only weakly detectable galactose- $\beta$ (1-4)-*N*-acetylglucosamine residues.

*Cross-Reactivity*

The inhibition immunoblotting analyses were performed using the *S. chartarum* antiserum obtained by immunizing the rabbits with the *S. chartarum* extract cultured in cellulose broth. Fungal extracts were prepared from growth cultured in cellulose broth. As an example, the results of the analysis in which *U. botrytis* was used as the inhibiting preparation are presented in Fig. 3. In the analyses, the level of inhibition gradually increased in parallel with the increasing concentrations of inhibitors (Table 2). All the components of *S. chartarum* were

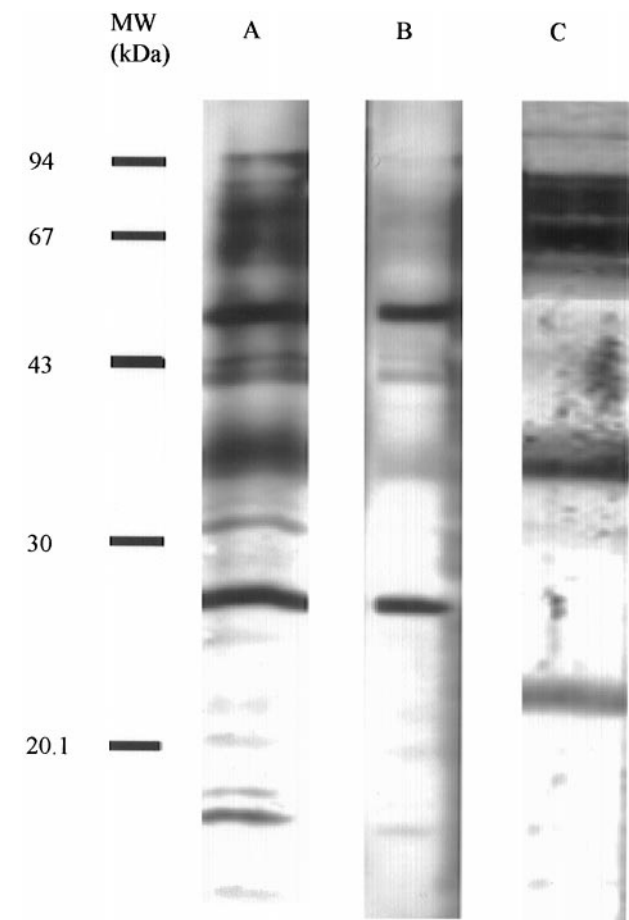


**FIG. 2.** The effect of periodate treatment on the antibody-binding capacity of the *S. chartarum* cellulose extract components. Immunoblotting before (A) and after the periodate treatment (B) and the glycoprotein components of the extract identified by the DIG Glycan Differentiation kit (C). MW, molecular weight markers.

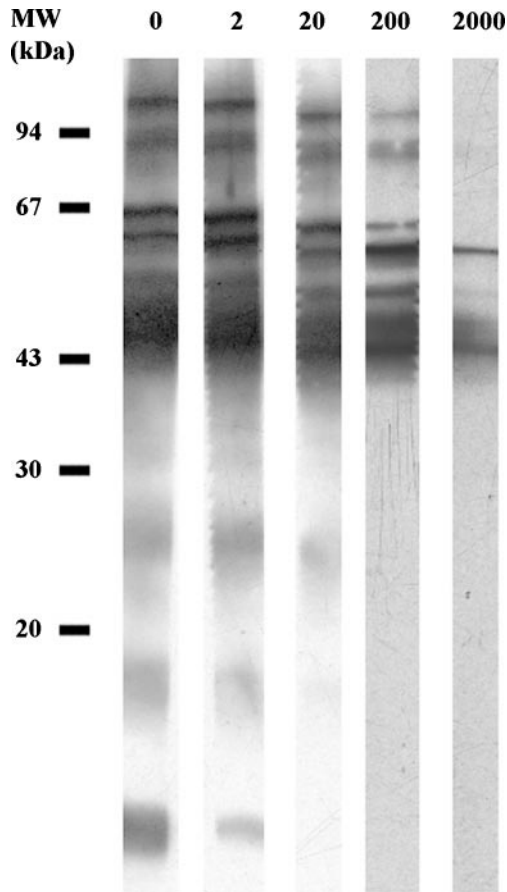


**FIG 3.** Inhibition-immunoblotting analysis using *U. botrytis* as inhibitory preparation at concentrations of 0–2000  $\mu\text{g/mL}$ . MW, molecular weight markers.

observed to be at least slightly cross-relative with those of the other fungal extracts when high protein concentrations (200–2000  $\mu\text{g/mL}$ ) of the inhibiting fungal preparations were used. At low concentrations (2–20  $\mu\text{g/mL}$ ), slight if any cross-reactivity could be observed in most cases. At the concentration of 20  $\mu\text{g/mL}$ , *U. botrytis* completely inhibited the visualization of 36% of the *S. chartarum* components in the immunoblotting analysis. The figure with *M. echinata* was 19% and with the other extracts 0–12%. *S. chartarum* completely inhibited the binding of the *S. chartarum*-specific antiserum to 50% of its own components at a concentration of 2  $\mu\text{g/mL}$  and at a concentration of 20  $\mu\text{g/mL}$ , the inhibition was complete. All *S. chartarum* strains tested yielded similar inhibition profiles (data not shown). The most cross-reactive *S. chartarum* components were those below 50 kDa (Fig. 3). The components of 65 and 50 kDa proved to be the most characteristic of this fungus according to the inhibition immunoblotting analyses (Fig. 3).

## DISCUSSION

Culture (cellular) extracts have usually been favored in the production of fungal antigen extracts (Shen *et al.*, 1998; Brouwer *et al.*, 1996; Horst *et al.*, 1990; Achatz *et al.*, 1995; Aden *et al.*, 1999; Deards *et al.*, 1991; Paris *et al.*, 1991; Vijay *et al.*, 1991; Portnoy *et al.*, 1990). There are only a few studies comparing the antigen yields of culture extracts and culture medium extracts. Zhang *et al.* (1994) reported that the culture extracts of *Cladosporium herbarum* contained considerably more IgE binding components than the culture medium extract. In the study of Nissen *et al.* (1998), the culture extract and the culture medium extract of *Fusarium moniliforme* contained equal amounts of IgE binding components (partly shared, partly different), while the culture medium extract of *Penicillium notatum* contained more IgE binding components than the culture extract. Also, the culture medium extract of *Fusarium solani* contained more IgE binding components than the culture extract (Verma *et al.*, 1994). An elevated number of IgE binding components in the culture medium extract is possibly due to a fact that some fungal species may secrete important antigenic components into the culture medium during fungal growth (Portnoy *et al.*, 1993c; Arruda *et al.*, 1992). In our studies, the 10-day culture extract of *S. chartarum* cultured in malt extract broth proved to contain more protein components than the 31-day culture extract or the culture medium extracts of different ages (Table 1). This was the

**TABLE 2**  
Cross-reactivity between *S. chartarum* and 10 Other Fungal Strains Expressed as a Percentage of Vanished Components

| Fungal species                      | Inhibitory concentration ( $\mu\text{g/mL}$ ) |     |     |      |
|-------------------------------------|-----------------------------------------------|-----|-----|------|
|                                     | 2                                             | 20  | 200 | 2000 |
| <i>Stachybotrys chartarum</i>       | 50                                            | 100 | 100 | 100  |
| <i>Ulocladium botrytis</i>          | 21                                            | 36  | 57  | 79   |
| <i>Memnoniella echinata</i>         | 0                                             | 19  | 75  | 79   |
| <i>Alternaria alternata</i>         | 0                                             | 0   | 15  | 64   |
| <i>Cladosporium cladosporioides</i> | 0                                             | 12  | 25  | 87   |
| <i>Penicillium brevicompactum</i>   | 0                                             | 0   | 21  | 93   |
| <i>Phoma</i> sp.                    | 0                                             | 0   | 0   | 100  |
| <i>Phialophora rebens</i>           | 0                                             | 0   | 7   | 57   |
| <i>Chaetomium globosum</i>          | 8                                             | 8   | 15  | 46   |
| <i>Aspergillus versicolor</i>       | 0                                             | 0   | 23  | 77   |
| <i>Aureobasidium pullulans</i>      | 0                                             | 0   | 0   | 0    |

*Note.* *S. chartarum*-specific rabbit antiserum was incubated with different concentrations of the fungal extracts before blotting.

reason the 10-day culture extract was chosen for the production of *S. chartarum*-specific antiserum for the immunoblotting analyses. The 10-day culture extract also contained the widest spectrum of rabbit IgG binding components. Anyhow, because the rabbits were immunized with the 10-day culture extract, it is possible that the other extracts contained other components that were not detected by the immune serum used.

The composition of growth media affects the growth pattern of fungi, and changes in the metabolic state influence the resulting antigenic composition. In general, fungal extracts cultured in high-nutrient media seem to contain a higher number of antigenic components than extracts cultured in low-nutrient media (van der Heide *et al.*, 1985; Portnoy *et al.*, 1993c). The *S. chartarum* extract prepared from the culture grown in cellulose broth that simulates the natural growing media (straw, gypsum board) of this fungus contained a higher number of protein components as well as immunogenic components than the extract prepared from the culture grown in malt extract broth (Fig. 1). This was not surprising, because cellulose broth contains more complex carbohydrate components that must be split by fungal enzymes before ingestion. The induced enzymes may act as antigens, resulting in a broader spectrum of immunogenic components detected in the immunoblotting analyses. Also, because the detection method under development is aimed at detecting *S. chartarum* contamination in moisture problem buildings where the substrate most probably contains cellulose, cellulose broth was chosen as a growth medium in this study.

The inhibitory potency of *S. chartarum* extract in the immunoblotting analyses was generally 100-fold higher than the potency of the other extracts, indicating that *S. chartarum* contains antigenic components distinct from those of the other fungal species studied, even though cross-reactivity was detected between the species (Table 2). Some species (*U. botrytis*, *M. echinata*, *C. cladosporioides*, and *Ch. globosum*) showed cross-reactivity with some *S. chartarum* components at low inhibitory concentrations (2–20 µg/mL), while intensive cross-reactivity (total disappearance of >50% of the components in the inhibition immunoblotting analysis) was usually observed first at the highest concentration tested (2 mg/mL; to reach a concentration of 2 mg/mL, intense concentration of the extracts was needed). Molds are commonly known to contain sugar residues due to their cell wall structure. About 80–90% of the fungal cell wall dry weight consists of polysac-

charide components (Williams and Kirk, 1988). There are several reports suggesting that carbohydrates participate in antibody binding, and they are also found to have a role in cross-reactivity (Heelan *et al.*, 1991; Aalberse *et al.*, 1981; Batanero *et al.*, 1996; Faye *et al.*, 1988; Hansen *et al.*, 1994; Afferini *et al.*, 1999; Petersen *et al.*, 1996; Hijikata *et al.*, 1994; Tretter *et al.*, 1993; Mucci *et al.*, 1992). The periodate treatment generally decreased the antigenicity of the *S. chartarum* glycoprotein components, showing that sugar epitopes had a marked role in antigen binding (Fig. 2). Thus, the broad cross-reactivity between *S. chartarum* and several other fungal species at the high concentrations of the preparations used as inhibitors may be non-specific and due considerably to the common carbohydrate moieties rather than shared identical protein components between the fungi. The fact that the periodate treatment did not have any effect on the antigenicity of the component of 50 kDa, the component that also showed poor cross-reactivity, points to this assumption.

The fact that the visualization of some *S. chartarum* components was prevented at the low concentrations of the inhibitors in the immunoblotting analysis by some fungal species (Table 2) may be due also to shared components, for example, enzymes, between the fungal species. On the other hand, to prevent the visualization of some *S. chartarum* components (those of about 50 and 65 kDa, Fig. 3), high concentrations of the inhibitors were needed. We regard the components of about 50 and 65 kDa as the most characteristic components of *S. chartarum*. According to some pilot tests (data not shown), the 50-kDa component also seems to be prevalent in *S. chartarum* growth; the extracts prepared from 20-, 35-, or 70-day-old *S. chartarum* cultures in cellulose broth contained this component. In addition, the 50-kDa component could be extracted by only gently mixing the mycelium with PBS. This indicates that the 50-kDa component can be detected in rather diluted solution. Even though the molecular weight analysis did not yield any component of 50 kDa in the *S. chartarum* extract cultured in malt extract broth, the cellulose broth culture-specific rabbit antisera detected this component from the extract in the immunoblotting analysis. In conclusion, this study showed that the 50-kDa component is the most promising candidate for a specific component of *S. chartarum*, and a closer characterization of the molecular structure and specificity of the 50-kDa component establishes a good basis for the development of the *Stachybotrys*-specific detection method.

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