Cross-reactivity of Antiserum to *Absidia cylindrospora* among Some Mucorales and Other Fungi

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INTRODUCTION

Mannose-containing heteroglycans such as galactomannans and rhamnomannans are known fungal antigenic substances. Relationships between serological reactivity, chemical structure and species specificity of these glycans have been examined in some species. In the case of Mucorales, however, the serological cross-reactivity has not been examined in detail. Previously, we isolated the serologically active substance of *Absidia cylindrospora*, a member of the Mucorales, from the supernatant of disrupted mycelium and characterized it as a fucomannan-peptide (Hayashi *et al.*, 1978). The fucomannan-peptide reacted with rabbit antiserum (anti-ACE serum) against the non-dialysable fraction of the culture filtrate of *A. cylindrospora*. The present communication is concerned with the cross-reactivities of antigenic fractions from four *Mucor* species, *Rhizopus nigricans* and 10 species of fungi belonging to the Oomycetes or Fungi Imperfecti.

METHODS

Organisms. *Absidia cylindrospora* IFO 4000, *Rhizopus nigricans* IAM 6070, *Mucor hiemalis* IFO 5303, *M. javanicus* IFO 4569, *M. lamprosporus* IFO 6337, *M. mucedo* IFO 5776, *Pythium debaryanum* IFO 5919, *Alternaria solani* IFO 5924, *Aspergillus niger* IFO 4043, *A. parasiticus* IFO 4301, *Cladosporium herbarum* IFO 4459, *Fusarium solani* IFO 5232 and *Penicillium islandicum* IFO 5234 were cultured in Sabouraud's liquid medium with shaking for 2 weeks at 27 °C.

Preparation of antigens from the various organisms. Cultured mycelium suspended in cold saline was homogenized in a Waring blender (Nihon Seiki Seisakusho Co., Japan) and then treated in a French press in the cold (Hayashi *et al.*, 1978). The homogenized mycelium fluid was centrifuged at 3000 rev. min⁻¹ for 10 min, and the supernatant was dialysed against distilled water for 3 d and then lyophilized. The resulting powder was designated the I-fraction.

Each culture filtrate was concentrated to a small volume after dialysis against distilled water for 3 d, and 4 vol. ethanol was added to the concentrate. The precipitate was dried with acetone and ether, and designated the E-fraction.

Mannans and galactomannan were prepared according to methods described previously: mannans from *Candida albicans* (Duke strain) (Miyazaki, 1962) and from *Saccharomyces cerevisiae* (Peat *et al.*, 1961) and galactomannan from *Penicillium chrysogenum* (Miyazaki & Yadomae, 1968).

Preparation of ACI-B, MhI-B and RnI-B. Serologically active fractions, ACI-B, MhI-B and RnI-B, were prepared from the I-fractions of *A. cylindrospora*, *M. hiemalis* and *R. nigricans*, respectively, as described previously (Hayashi *et al.*, 1978) using affinity chromatography with concanavalin A-Sepharose, for which the B fractions had affinity.

Immunological procedures. Anti-ACE serum was prepared by immunizing a rabbit with the non-dialysable fraction of the culture filtrate of *A. cylindrospora* (Hayashi *et al.*, 1978). Precipitin tests on E- and I-fractions obtained from various fungi were performed in fine glass tubes (2 × 50 mm) using anti-ACE serum. Each of the E- and I-fractions was dissolved in saline to concentrations of 5 mg ml⁻¹ and 500 µg ml⁻¹, and was statically layered on the surface of undiluted anti-ACE serum in the fine glass tube. After incubation for 1 or 2 h at room temperature, each tube was inspected for the presence of a precipitin band.

Fig. 1. Quantitative precipitin curves of ACI-B (●), MhI-B (▲) and RnI-B (□) against anti-ACE serum.

Quantitative precipitin tests of MhI-B, RnI-B and ACI-B against anti-ACE serum were carried out as follows: 0.2 ml of undiluted rabbit antiserum was mixed with 0.1 ml of serial dilutions of antigens in saline. The mixtures were incubated at 37 °C for 1 h, stored at 4 °C for 90 h with occasional shaking and then centrifuged. The protein contents of the precipitated complexes were determined by the method of Lowry *et al.* (1951), using bovine serum albumin as a standard.

Immunodiffusion analysis and immunoelectrophoresis were carried out as described previously (Hayashi *et al.*, 1978).

RESULTS AND DISCUSSION

Anti-ACE serum reacted with E- and I-fractions from *M. hiemalis*, *M. javanicus*, *M. lamprosporus*, *M. mucedo* and *R. nigricans* as well as those from *A. cylindrospora*. Samples prepared from *Alternaria solani*, *Fusarium solani*, *Aspergillus* spp., *Cladosporium herbarum* and *Penicillium islandicum* did not react with anti-ACE serum, although these species have mannose-containing glycans (Miyazaki & Naoi, 1975; Gander, 1974). *Pythium debaryanum*, which belongs to Oomycetes in Phycomycetes, did not show any cross-reactivity. Previously, Hotchi (1967) observed cross-reactions between *Absidia lichtheimi*, *Mucor globosus* and *Rhizopus nigricans* by a fluorescent antibody technique. Antiserum against whole cells of *A. lichtheimi* gave a weak cross-reaction with a phenol extract of *Candida albicans*, but the serological cross-reactivities have not been elucidated in detail. In our experiments, neither purified mannans from *S. cerevisiae* (Peat *et al.*, 1961) and *C. albicans* (Miyazaki, 1962) nor galactomannan from *Penicillium chrysogenum* (Miyazaki & Yadomae, 1968) reacted with the antiserum to *A. cylindrospora*. These findings suggest that the anti-ACE serum has specificity only for Mucoraceae or Mucorales.

In immunodiffusion analysis of E-fractions from *Mucor* spp. and *R. nigricans* against anti-ACE serum, each of these active fractions gave a precipitin band which was identical with that of *A. cylindrospora*. The results of immunodiffusion analysis and immunoelectrophoresis of MhI-B and RnI-B, which were isolated from *M. hiemalis* and *R. nigricans*, also suggest that these fractions contained a common serologically active substance. Quantitative precipitin curves of MhI-B, RnI-B and ACI-B against anti-ACE serum are shown in Fig. 1.

From a serological examination of rhamnomannans prepared from *Sporothrix schenckii* and *Ceratomyces* species, Lloyd & Travassos (1975) concluded that the rhamnosyl side chains were important as the immunochemical determinants; but from studies of galactomannans I prepared from five species of dermatophytes, Grappel *et al.* (1968) concluded that the mannopyranoside units were the predominant groups. In Mucorales, the role of fucose units in the immunological specificities of the fucomannan-peptide is not clear. Since the purified mannans of *C. albicans* and *S. cerevisiae* did not cross-react with the anti-ACE

serum, it would appear that the chemical structure of the common immunological determinant of the serologically active substances of Mucorales may be different from those of the mannans. The chemical analysis of the common determinant is now under study.

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