Detection of Fungal Spore Antigens by Serologic Methods

It has become increasingly evident that carbohydrate-containing cell surface components are intimately involved in the antigenicities and immunoresponses associated with blood-typing[1], gamete recognition in animal[2], and bacterial cells[3], tissue-typing and histo-compatibility[4], tumor-specific antigens[5], differentiating neural retina tissue[6], reaggregation of dissociated tissue[7], and cell recognition[8]. Plant host-parasite specificity, which must be considered cellular recognition and compatibility, may also depend on carbohydrate-containing surface antigens as the participative, complementary molecules. This suggestion is supported by the fact that complementary molecules of glycoprotein composition have been isolated from male and female strains of *Chlamydymonas*[9], and also from a sexually agglutinative yeast, *Hansenula wingei*[10]. However, information regarding the chemistry, architecture and antigenic structure of the outermost layer of pathogenic fungal spores and hyphae which would be intimately involved in host recognition and invasion has been hitherto largely lacking or limited to comparisons of the histocompatibility antigens between parasites and hosts, both susceptible and resistant strains.

Preliminary studies by KLEINSCHUSTER and BAKER[11] of the macroconidium of *Fusarium roseum* 'Avenaceum' and later *Fusarium solani*[12] using enzymatic and chemical surface alteration and a technique employing several plant lectins, which agglutinate cells in a fashion resembling immunological reactions by binding to specific carbohydrate-containing receptor sites on cell surfaces, revealed in part the relative qualitative and quantitative carbohydrate composition of the outermost layer of these spores. Additional studies by KLEINSCHUSTER and BAKER investigated the lectin-detectable carbohydrate composition of normal and altered macroconidial surfaces of *Fusarium roseum* 'Avenaceum' and *Fusarium solani* and clearly demonstrated for the first time chemical-structural and apparent antigenic differences in the outer cell surfaces of two host-distinct, though closely related pathogens. We report here confirmation of these chemical-structural differences by demonstrating different antigenic properties of the spore surfaces of *F. roseum* and *F. solani* by the use of serologic methodology unique to plant sciences.

Methods and materials. Macroconidida of *F. solani* f. sp. phaseoli and *F. roseum* 'Avenaceum' were obtained from 4-week-old slant cultures by introduction of 5 ml of phosphate buffered saline (PBS) into the culture tubes, gentle shaking and filtering through several layers of cheesecloth to remove mycelial fragments, followed by a single wash in PBS. The concentration of conidia used in all experiments was adjusted to approximately 1.5×10^6/ml of PBS.

Rabbits were used for immunization with the spores of *F. solani*. Before immunization, 15 ml of blood was withdrawn via the lateral ear vein for agglutination tests with normal sera. The immunization schedule consisted of an initial single innoculation of a 1 ml PBS spore suspension injected i.m. into the hind extensors of the

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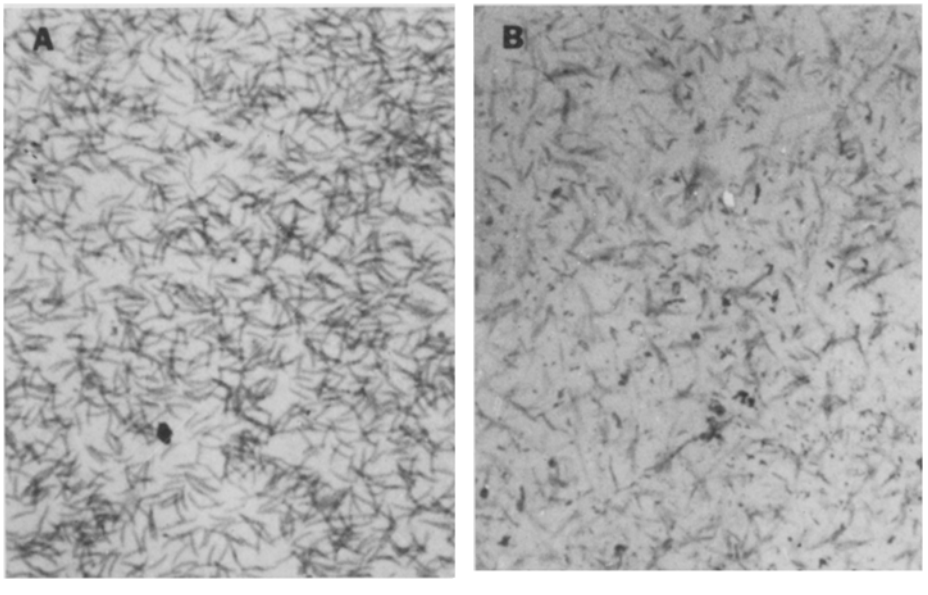

Fig. 1. Representative photographs of spores challenged with control (normal rabbit) sera. A) *Fusarium solani*; B) *Fusarium roseum*. ×630.

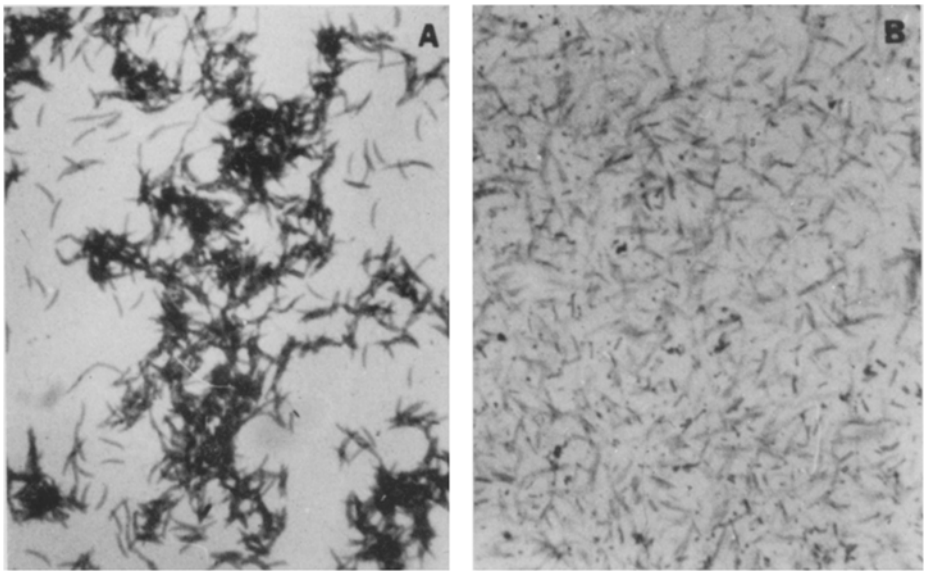

Fig. 2. Representative photographs of spores challenged with *Fusarium solani* antisera. A) *Fusarium solani;* B) *Fusarium roseum.* ×630.

animals. After 1 week, a 2nd inoculation, similar to the 1st, was administered i.p. and a 3rd inoculation, also i.p., was given 2 weeks after initial inoculation. One week following the 3rd inoculation, 15 ml of blood was obtained from the lateral ear vein.

Agglutination reactions were performed on ordinary glass slides and at room temperatures. To each slide was added a 0.05 ml of spore suspension and 0.05 ml of either normal rabbit or *F. solani* antiserum. The slides were gently swirled for 2 min to assure maximum cell contact. At the end of this period, the agglutination reactions were photographed.

Results and discussion. As seen in Figure 1, control (normal rabbit) sera did not agglutinate to any degree condida of either *F. solani* or *F. roseum*. However, as seen in Figure 2, *F. solani* antisera massively agglutinated the conidida of *F. solani*, while *F. roseum* spores showed no agglutination. It is of interest to note that the agglutination reactions of the spores did not decrease following repeated washings with PBS. This evidence indicates distinct antigenic differences between the spores of these 2 taxonomically related, though host-distinct, plant pathogens and is probably reflective of their host specificities.

It would appear that the above described method of assay of the antigenic properties of fungal spores may be an important investigative technique and should be exploited in future studies as the agglutination reaction is one of the most sensitive assays known. Thus, when this methodology can be applied to such large cells as fungal spores (4 to 5 times more volume than erythrocytes) with such apparent specificity, the intimacies of the host-parasite recognition and invasion mechanisms can be more easily investigated and elucidated.

Zusammenfassung. Immunologische Agglutinations-Reaktionen eignen sich als Methoden zur Untersuchung von spezies-spezifischen Antigenen von Pilzsporen.

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A Convenient Colourimetric Method for Routine Assay of Brain Adenylate Cyclase

Brain is a tissue rich in adenylate cyclase and enzyme activity has been measured with unlabelled substrate by ion exchange and UV-spectroscopy[1]. To provide a simpler method suitable for undergraduate teaching a direct nonchromatographic method of similar sensitivity and precision was devised.

Materials and methods. Brain adenylate cyclase: Whole, freshly obtained cow brains were immediately immersed in icecold buffer consisting of glycylglycine (glygly), 2×10^{-3} *M*, pH 7.5; $MgSO_4$, 1×10^{-3} *M*; and NaCl, 2×10^{-3} *M*. The cortex was minced, and homogenized in a Teflon glass Potter-Elvehjem apparatus, according to Bitensky et al.[2] and the homogenate was processed according to Sutherland et al.[3] and stored at −20 °C.

The standard incubation medium (4.0 ml) consists of ATP, 1.0×10^{-3} *M*; $MgSO_4$, 3.6×10^{-3} *M*; caffeine, $5.0 \times$

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