

Short communication

Isolation and immunobiochemical characterization of a major allergen (65 kDa) from *Fusarium equiseti*

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Fusarium equiseti is one of the most important species in the class Deuteromycetes (Fungi Imperfecti). For proper diagnosis and immunotherapy, isolation and characterization of allergens of *F. equiseti* are necessary. In the present study, culture filtrate (CF) extract of *F. equiseti* was resolved into 35–37 bands on isoelectric focusing pI (3–9) and SDS–PAGE (mol. wt. 10–100 kDa). Most of them were glycoproteins, as identified by PAS staining. *F. equiseti* CF revealed 15 allergenic proteins on immunoblot with an allergic serum pool. It was fractionated into nine fractions (I–IX) on a Superose-12 column by FPLC. Fraction IV (65 kDa) and fraction VI (25 kDa) were found to be highly allergenic by IgE ELISA. A 65-kDa protein was observed as a major allergen because it was recognized by most of the patient sera on immunoblot. After elution from SDS–PAGE gel, it gave two bands of pI 7.4 and 6.0. Inhibition in IgE-binding components of *F. equiseti* CF with CF extracts of *F. solani* and *F. moniliforme* by immunoprint inhibition assay indicated the allergenicity shared between the extracts of *Fusarium* species. Data suggested that the 65-kDa is the major allergen in the *Fusarium* species and can be used for the treatment of allergic patients.

Key words: allergens; characterization;
Fusarium equiseti; immunoprint inhibition.

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Among the Fungi Imperfecti, *Fusarium solani* is considered one of the most prevalent species responsible for allergic diseases (1, 2). Antigenic and allergenic differences have been demonstrated between various sources of fungal extracts (3, 4). It has been observed that culture filtrate, mycelium, and spore extracts of *F. solani* contain a number of allergens. They may be unique to one species or may show shared allergenicity with related species or genera (5, 6). A 65-kDa major glycoprotein allergen has been purified from *F. solani* culture filtrate (CF) and characterized by immunochemical techniques (7). But other species of *Fusarium* have not been characterized.

Of the genus *Fusarium*, *F. equiseti* is common in temperate and tropical areas of the world. It has also been isolated from avocado fruit, bean, cabbage, hemp, soybean, etc. It produces toxins such as zearelenone and zereulenol (8), but its role in antigenic/allergenic activity is unclear.

In the present study, CF extract of *F. equiseti* was prepared under optimum conditions to identify and characterize the major allergenic proteins that could be used for diagnosis and immunotherapy of allergic patients.

Material and methods

Culture of F. equiseti and preparation of extract

F. equiseti, strain 3896, obtained from the Indian Agriculture Research Institute, Delhi, was grown at 30°C in 250-ml flasks containing 100 ml of synthetic medium under constant agitation (125 rpm) to prevent sporulation. *F. equiseti* CF was prepared by the same procedure as described for the preparation of *F. solani* CF extract (1). On the basis of maximum protein content and number of bands on isoelectric focusing (IEF) (pI 3–9), an 11-day-old culture of *F. equiseti* was selected for the

preparation of antigenic extract. The extract was stored at -70°C until analysis.

Protein and carbohydrate

The protein content was estimated by the method of Lowry et al. (9), and carbohydrate concentration in CF extract of *F. equiseti* was estimated by the phenol-sulfuric acid method (10).

IEF

Of each extract (CF extract of *F. equiseti* and 65-kDa protein of *F. equiseti* which was eluted from SDS-PAGE gel), 3 μg protein was resolved on ready-made gel (Pharmacia, pI 3–9) by the Phast system. The separated protein components were silver-stained with a kit (Pharmacia).

Patient sera

Sera were collected from 10 allergic patients attending the Clinical Research Centre, V. P. Chest Institute, Delhi, with a clinical history of bronchial asthma, in whom allergy to *Fusarium* antigen had been confirmed by intradermal tests. All patient sera showed specific IgE binding to *F. equiseti* CF when screened by ELISA.

Fractionation of *F. equiseti* extract

F. equiseti CF was fractionated on a Superose-12 column by FPLC (Pharmacia). An amount of 18 mg of protein was eluted with 0.1 M phosphate-buffered saline (PBS) (pH 7.4). All the fractions of each resolved peak were pooled, dialyzed, and lyophilized. The molecular weight of each fraction was calculated by eluting the standard protein markers on the same column. The allergenic activity of each fraction was checked by IgE-specific ELISA with pooled sera of patients allergic to *Fusarium* (1).

SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

F. equiseti CF (250 μg) was resolved on (10% acrylamide) stacking gel by the discontinuous buffer system of Laemmli on a midget apparatus (11). Resolved proteins were stained with Coomassie brilliant blue R-250.

Periodic acid-Schiff (PAS) staining for glycoproteins

Proteins of *F. equiseti* separated on SDS-PAGE gel were incubated at room temperature with 1%

periodate, prepared in acetic acid for 1 h (12). The gel was washed with 7% acetic acid and incubated at 4°C with PAS for 2 h. The gel was finally washed with water.

Elution of a 65-kDa protein from SDS-PAGE gel

A 65-kDa protein band was excised from the gel. To remove the dye, extraction was done with distilled water by vigorous shaking for 2–3 h. The gels were then kept in 0.05 mol/l acetate buffer, pH 8.5, for 18 h at room temperature under shaking. The gel was discarded and solution containing the protein lyophilized (13).

Immunoblot analysis

An amount of 250 μg protein of *F. equiseti* CF, resolved on SDS-PAGE (10% gel), was electrophoretically transferred to a nitrocellulose membrane in tris-glycine buffer with 20% methanol, as described by Towbin et al. (14), with a dry-blot apparatus (LKB, Pharmacia). After saturation with 1% bovine serum albumin in PBS, the membrane was incubated with (1:10 v/v) diluted patient serum. The blot was developed as described earlier by Verma & Gangal (5).

Immunoblot inhibition

Immunoblotting was performed to study the inhibition of binding of protein components of *F. equiseti* with patient serum IgE by CF proteins of the other two species of *Fusarium*. Proteins of *F. equiseti*, resolved on SDS-PAGE in four different lanes, were blotted on nitrocellulose membrane. Individual lanes were cut out, and each *F. equiseti* CF blotted strip was incubated at 4°C with 500 μl (1:5 v/v) diluted patient serum mixed with 500 μl (1 mg/ml) CF antigens of *F. equiseti*, *F. solani*, and *F. moniliforme*. For positive control, an *F. equiseti* CF blotted strip was incubated with (1:10 v/v) diluted pooled patient sera only. Other steps were as described for immunoblotting by Verma & Gangal (5).

Results

It can be seen from the growth curve of *F. equiseti* (Fig. 1) that the maximum protein and dry weight of mycelium were observed in 11-day-old culture (stationary phase). On IEF pI (3–9), the number of protein components in CF was also found to be greatest on the same day. Hence, 11-day-old cultures were used for the preparation of *F. equiseti* CF.

F. equiseti CF was fractionated into nine fractions (I–IX) on a Superose-12 column. Fractions IV

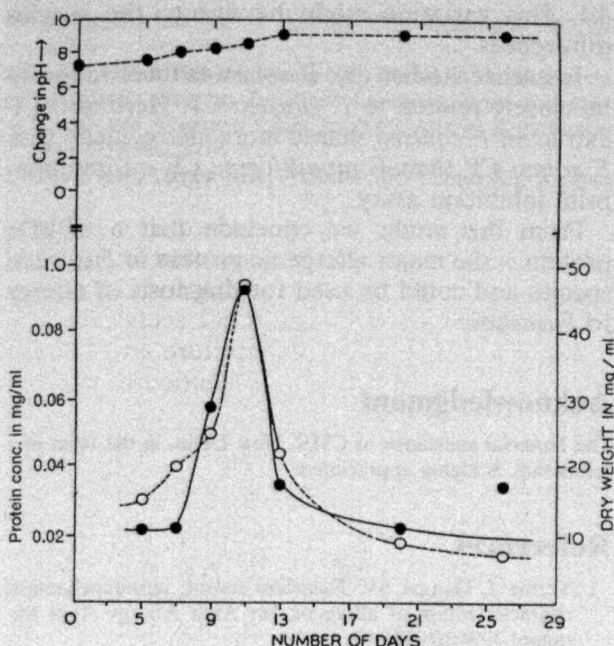


Fig. 1. Metabolic changes during growth of *F. equiseti*. (●—●): pH of culture medium, (●—●): protein content in CF, (○—○): dry weight of mycelium after different days of cultivation.

(65 kDa) and VI (25 kDa) were highly allergenic in comparison to other fractions, as checked by IgE ELISA with pooled sera of patients (Fig. 2).

The carbohydrate/protein ratio was found to be 1:0.8. On SDS-PAGE, *F. equiseti* resolved into 35 protein bands (Fig. 3, lane 1). Of these, 15 were allergenic, as visualized on immunoblot with pooled patient sera (Fig. 3, lane 3). On PAS staining, glycoproteins appeared as pink bands at 14–92 kDa (Fig. 3, lane 2). Although a number of allergenic proteins in the range 14–92 kDa were detected on immunoblot of *F. equiseti* CF with individual patient serum, a 65-kDa protein component was found to be a major allergen because it was strongly visible in nine out of 10 patient sera tested, showing 90% frequency of IgE binding (data not shown).

F. equiseti CF exhibited 37 silver-stained bands in the pI range 3–9, while a 65-kDa protein eluted from SDS-PAGE gel showed two bands at pI 7.4 and 6.0, suggesting the presence of two subunits of a 65-kDa protein of *F. equiseti* (Fig. 4).

A high degree of inhibition in IgE-binding components of *F. equiseti* with homologous antigen was observed on immunoblot as compared to heterologous antigens (not shown). At 500 µg protein, the CF extract of each of the three species of *Fusarium* showed complete inhibition of binding of protein bands of 28, 29, 30, 40, 41, 43, and 44 kDa. *F. solani* CF inhibited the binding of 65-, 90-, and 92-kDa

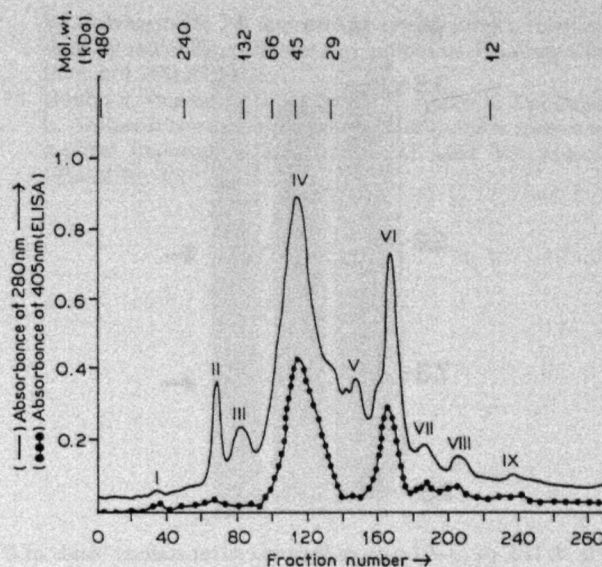


Fig. 2. Elution profile (—) of *F. equiseti* CF on Superose-12 column. Eluent: 0.1 mol/l PBS (pH 7.3); flow rate: 0.5 ml/min; sensitivity: 0.2 arbitrary units; chart speed: 0.2 mm/min; fraction size: 0.5 ml. (●—●): IgE-binding capacity of each eluted fraction, as determined by ELISA.

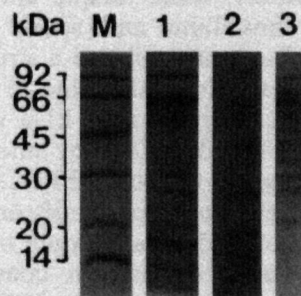


Fig. 3. SDS-PAGE pattern. Lane 1) Coomassie brilliant blue-stained bands of *F. equiseti* CF, 2) PAS-stained glycoprotein bands in *F. equiseti* CF, 3) immunoblot analysis of IgE-binding components in *F. equiseti* CF with allergic serum pool. M: mol. wt. markers.

components also, while *F. moniliforme* did not inhibit the same. This indicates that *F. equiseti* CF shared more allergenicity with *F. solani* than *F. moniliforme*.

Discussion

Fusarium has been described as one of the most important aeroallergens in India (2, 5). Mold-allergic subjects often display parallel and independent multiple sensitivity to different species.

In the previous studies (15), crude extract of *F. solani* has been used in combination with other mold extracts for immunotherapy. Verma & Gangal (1994) have characterized CF, mycelium,

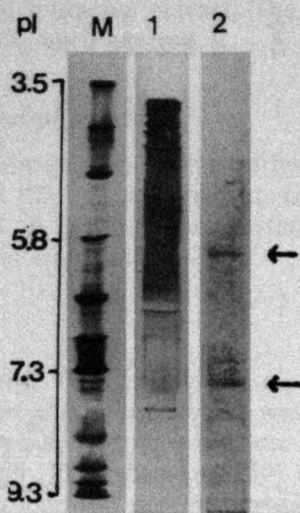


Fig. 4. IEF pI (3–9) pattern showing silver-stained bands of *F. equiseti* CF (lane 1) and 65-kDa protein eluted from SDS-PAGE gel (lane 2). M: mol. wt. markers.

and spore extracts of *F. solani* by IEF, SDS-PAGE, and immunoblot. It has been observed that CF contains the greatest number of allergenic proteins. However, some unique allergens were identified in mycelium and spore extracts of *F. solani* (1). *F. solani* exhibited a strong band of 65 kDa on immunoblot with all the 15 patient sera tested. A major allergen of 65 kDa was isolated from *F. solani* CF and purified by anion-exchange chromatography, gel filtration, FPLC, and HPLC (7). In the present study, to identify and study the characteristics of a 65-kDa allergen in other species of *Fusarium*, the protein components of *F. equiseti* CF were resolved on IEF and SDS-PAGE. Out of nine fractions obtained from a Superose-12 column by FPLC, a 65-kDa fraction was found to be highly allergenic on ELISA with pooled patient sera. On immunoblot, a 65-kDa component of *F. equiseti* was also recognized by most of the patient sera. Therefore, it was identified as a major allergen.

Among the species of the same genus, antigenic/allergenic similarities may exist. Both shared and unique antigenic/allergenic components among *Alternaria tenuis* and *A. solani* have been reported by various investigators (4, 16). Similarly, Karr et al. (17) have examined the antigenic relationships among *Aspergillus fumigatus*, *A. glaucus*, and *A. flavus*, using RAST inhibition. Antigenic relationships among *Cladosporium* species have been demonstrated by Honbo et al. (18). In the present study, a 65-kDa glycoprotein allergen of *F. equiseti* CF exhibited two bands of pI 6.0 and 7.4 on IEF, while a 65-kDa protein of *F. solani* CF showed a homogeneous band at pI

4.1. This variation might be due to the species differences.

In earlier studies (5), *F. solani* CF was found to be closely related to *F. equiseti* CF. Here also CF extract of *F. equiseti* shared more allergenicity with *F. solani* CF than *F. moniliforme* CF on immunoprint inhibition assay.

From this study, we conclude that a 65-kDa protein is the major allergenic protein in *Fusarium* species and could be used for diagnosis of allergy to *Fusarium*.

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