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Allergenic components of *Ganoderma applanatum*

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Spores of *Ganoderma applanatum* were collected by gravity. The spore extract was prepared and its biochemical and immunological properties were examined. In isoelectric focusing, the extract had 10–12 bands with pI values between 3.5 to 6.0, the strongest being at 3.5, 3.75, 4.55 and 5.2. In crossedradioimmunoelectrophoresis using human atopic sera, the extract had two dominant and two minor allergens. In IgE immunoblots of sodium dodecylsulphate polyacrylamide gel electrophoresis, the extract showed reactivity at 82, 45, 33, 26, 16, 11 kDa MW, the strongest being at 45 kDa MW. These results indicate that *G. applanatum* contains a complex mixture of allergens.

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Respiratory allergic reactions have been demonstrated to the airborne mold spores (Salvaggio & Aukrust 1981). The available data indicate that basidiospores are prevalent in aerospora worldwide (Hasnain et al. 1985 and Lehrer et al. 1986). Thus clearly, basidiospores are potentially a major source of aeroallergen (Herxheimer et al. 1969). Lopez et al. (1976) and Sprenger et al. (1986) demonstrated that extracts prepared from mycelia or culture fluid of selected Basidiomycetes grown *in vitro* were allergenic in humans and antigenic in rabbits. Tarlo et al. (1979) found *Ganoderma* spores to be the most numerous fungal aerospore in an Ontario, Canada study. These authors reported human sensitization to *Ganoderma* extract. Hasnain et al. (1985) in their studies with *Ganoderma* extracts proposed that persistent exposure to this organism stimulates a base level of allergic responsiveness in susceptible atopic patients. Buller (1922) reported that *Ganoderma applanatum* can release spores from a particular fruiting body for up to six months per year. Additionally, *G. applanatum* has a perennial fruiting body that may persist three years, or occasionally longer. Other Basidiomycetes may release spores for several days, or only several hours, as is the case with *Coprinus quadrifidus*. Therefore, the present study was carried out to investigate the antigenic and allergenic

components of *Ganoderma applanatum* spores so that the patients can be skin tested adequately and desensitized efficiently with the extract of this mold.

MATERIALS AND METHODS

Collection of *Ganoderma applanatum* spores

Twenty trees of *Acer saccharum* Marsh., which provided support for *G. applanatum* caps, were selected in a sugar Maple forest located at Lantier, Quebec (46° 10'N; 74° 15'W), at 450 m a.s.l. in the Laurentides mountains.

A glass plate (30 × 15 cm) was suspended 10 cm under each *Ganoderma* cap and spores were collected by gravity and removed from the plate with a razor blade at regular intervals in July and August 1989. Spores (~20 g) were dried at room temperature in small cups and then filtered through a 63 µm sieve.

Preparation of *G. applanatum* spore extract

Before extraction, spores of pulverized caps were defatted twice with diethyl ether (1:20 w/v) for 3 h at room temperature. The defatted material was extracted with 1:20 w/v ammonium bicarbonate buffer (0.125 mol/L, pH 8.1) and stirred magnetically for 18 h at 4°C. The suspension was centrifuged at 1,000 g at 4°C for 20 min, then at 30,000 g at 4°C for 30 min to remove traces of suspended particles. The resulting supernatant was concentrated and washed with water using an Amicon YM-5 membrane. The retentate was filtered through a 0.45 µm filter and lyophilized. The lyophilized product was termed as *Ganoderma applanatum* (GA) spore extract.

Table 1. Potency of *G. applanatum* spore extract in direct RAST assays

*Total radioactivity in 50 µl (8 ng) of anti-human IgE-I125 was 28,173 cpm

Defatted mold spores (g)	Spore extract		Direct RAST* % Counts bound
	Yield (mg)	Protein (%)	
4.68	82.9	8	13

Biochemical assays

Protein content of the spore extract was determined by a commercial dye-binding assay (Bio-Rad Laboratories), using bovine serum albumin as a standard (Bradford 1976). The extract was screened for the determination of 19 enzymes with Api-Zym system: alkaline and acid phosphatases, three lipases, five peptidases, phosphodiesterase and eight glycosidases (Vijay et al. 1985). Analytical isoelectric focusing (IEF) was performed in thin layer polyacrylamide gel plates with a pH range of 3.5–9.5 (Vijay et al. 1984). The gel plates were stained with Coomassie brilliant blue R-250 (Bio-Rad Laboratories).

Immunological assays

Previously reported procedures (Vijay et al. 1981, 1984) were followed for the production of antisera to the spore extract in New Zealand white rabbits and for direct RAST assays. The IgE antibody preparation was made by mixing equal volumes of sera from 3 patients allergic to *Ganoderma* (kindly provided by Dr. S. B. Lehrer, New Orleans) and the solid phase disc was coupled with 200 µg of the extract.

Crossed-immunoelectrophoresis (CIE) and CRIE were performed as described by Axelsen et al. (1973) and Aukrust (1979) respectively, with slight modifications (Vijay et al. 1989).

Protein components of the spore extract (1200 µg containing 96 µg of protein) were separated on SDS-PAGE by the method of Laemmli (1970) and were electrotransferred on to nitrocellulose (NC) membrane (Bio-Rad; 0.45 µm pore size) in a Bio-Rad Transblot cell as described by Towbin et al. (1979). Proteins reactive with specific IgE to *G. applanatum* were detected by incubating the NC membrane with the same serum pool used in CRIE and RAST assays, followed by incubation with anti-human IgE-I125 and autoradiography as reported earlier (Vijay et al. 1989).

RESULTS

Extraction and biochemical analysis

Extraction of 4.68 g of dry defatted *G. applanatum* spores with ammonium bicarbonate yielded 82.9 mg of water soluble material MW > 5,000 having 8%

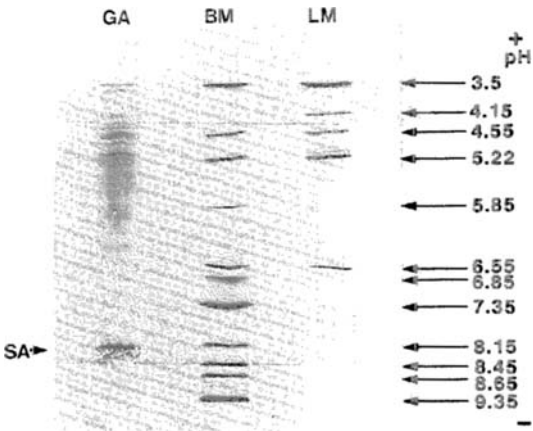


Fig. 1. Analytical isoelectric focusing of *G. applanatum* (GA) spore extract in polyacrylamide gel containing pH 3.5–9.5 ampholines. The standard pI markers were run in the lanes marked BM (broad pI) and LM (low pI) and their values are given at the side. SA: Sample application point.

protein content (Table I). The extract contained large amounts of acid phosphatase, α -galactosidase, β -glucosidase and β -glucosaminidase. Moderate amounts of alkaline phosphatase and leucine aminopeptidase were also present. Isoelectric focusing demonstrated 18 bands with pI values between 3.75–6.35; 8 strong bands with pI values between 3.75–5.15 (Fig. 1).

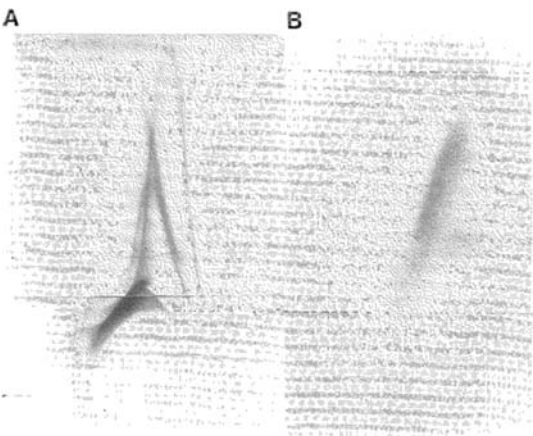


Fig. 2. A–B. The antigenic components of *Ganoderma*. (A) CIE patterns of *G. applanatum* extract. (B) Autoradiograph showing the CRIE pattern of duplicate unstained CIE plate.

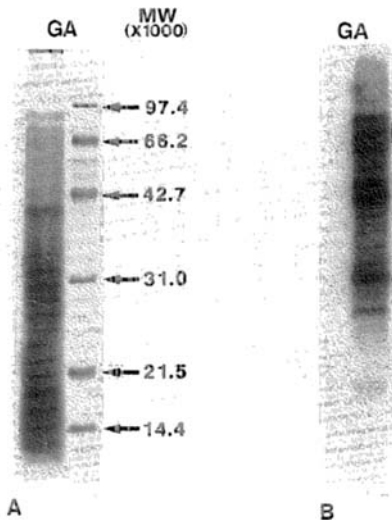


Fig. 3 A–B. SDS-PAGE of the extract. (A) Coomassie brilliant blue R-250 staining of GA extract and molecular weight standards. (B) An autoradiograph of an immunoblot of unstained gel after incubation with the atopic serum pool followed by anti-human IgE-I125.

Crossed-immunoelectrophoresis

The antigenic components of *G. applanatum* spore extract were detected by CIE. The extract had 12 to 14 precipitin arcs (Fig. 2A). No cathodically migrating antigens were observed. In similar experiments with heterologous antisera, no antigenic cross-reactivity was observed among *G. applanatum*, *Cladosporium herbarum* and *Alternaria alternata* extracts.

To detect the allergenic components, the CIE plates were incubated with a serum pool from three atopic patients followed by anti-human IgE-I125. The autoradiographs showed (Fig. 2B) that the extract had two dominant and two minor allergens.

SDS-PAGE and immunoblotting analysis

The allergenic components of the extract were further characterized by SDS-PAGE followed by immunoblotting with the human atopic serum pool and anti-human IgE-I125. The autoradiographs of the immunoblots showed that the extract had strong binding of specific IgE antibodies at 26, 33, 45, 66, 70 and 82 kDa MW along with moderate reactivity at 18 and 24 kDa MW (Fig. 3A,B).

Immuno-assay

The potency of the extract was determined by *in vitro* direct RAST assay. Table I shows that the extract had 13% RAST activity.

DISCUSSION

The spore extract of *G. applanatum* contained at least 12 to 14 antigens by CIE suggesting that the spores of this organism are immunogenic in rabbits. Similar results were obtained by Cutten et al. (1988) in their studies with *Ganoderma*. These authors reported that there are common antigens in both spore and fruiting body extracts of *Ganoderma* by CIE. Some patients were skin positive to antigens in spore extract alone, some to fruiting body extract alone, while others reacted to both. O'Neil et al. (1988) studied a number of Basidiomycete extracts including *G. lucidum* for antigenic and allergenic relationship by immunodiffusion and RAST inhibition tests. These authors demonstrated a high degree of cross-reactivity among spore extracts of different *Basidiomycetes*. Cross-reactive allergens among various Basidiomycete species including *G. meredithae* have been reported by immunoprint-inhibition studies (de Zubiria et al. 1990). In our studies, no antigenic cross-reactivity was observed among *G. applanatum*, *C. herbarum* and *A. alternata* by CIE. This could be explained due to quantitative or qualitative differences in allergenic determinants. In order to have better understanding of the molecular structure of allergens of this organism, more work is required to purify and characterize individual allergen(s) of *Ganoderma* species. Potency of the spore extract by RAST assays in the present study confirms IgE dependent sensitization in the patients. These results appear to be in agreement with Tarlo et al. (1979), O'Neil et al. (1988) and Butcher et al. (1987) in their studies with basidiospores by RAST assays. In the present study, it is interesting to note that the extract contained several allergen species in the MW range between 82 to 18 kDa.

From the battery of immunological tests applied to the extract in the present study, it appears that the spore extract contains a potent but complex mixture of allergens of *G. applanatum*. Following purification and characterization of allergens, test extracts could be prepared for adequate diagnosis and ultimately, for immunotherapy. It would be desirable to

investigate the antigenic and allergenic components from mycelial extracts of this organism. Also, more data are required on the Aerobiology pathway of *Ganoderma* in order to understand its role in allergic diseases.

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