

Evaluation of Basidiomycete and Deuteromycete (Fungi Imperfecti) extracts for shared allergenic determinants

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Summary

Aqueous extracts of select members of the Basidiomycetes and Deuteromycetes (Fungi Imperfecti) were evaluated for the presence of shared allergenic determinants using skin prick and radio-allergosorbent test (RAST) inhibition. Twenty adults with perennial symptoms of rhinitis, with or without asthma, were skin-prick tested with six species of Deuteromycetes and seven species of Basidiomycetes. Positive weal-and-flare reactivity to *Pleurotus ostreatus* was associated with *Alternaria alternata*, *Fusarium solani* and *Epicoecum purpurescens*. Positive skin reactivity to *Calvatia cyathiformis* was also associated with *A. alternata* and *F. solani*. *Coprinus quadrifidus* was associated only with *F. solani*, and *Psilocybe cubensis* was only associated with *Aspergillus fumigatus*. No other skin test associations were demonstrated. For every allergen tested by RAST inhibition, significant dose-dependent homologous inhibition was demonstrated. Although the ability of an individual heterologous extract to inhibit the direct RAST varied, inhibition was generally minimal. In the most extreme example, no heterologous allergen inhibited the *A. alternata* RAST. However, the *Armillaria tabescens* RAST was inhibited 52.6%, 38.1% and 25.1% by *A. fumigatus*, *E. purpurescens*, and *Penicillium notatum*, respectively, suggesting significant cross-reactivity. These results suggest that, although shared allergenic determinants exist between select species of Basidiomycetes and Deuteromycetes, crossreactivity is minimal and its clinical significance is not clear. These data confirm that for reliable diagnosis of fungal allergy, representatives of both major groups must be used.

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Introduction

Four major groups of true fungi—Zygomycetes, Ascomycetes, Basidiomycetes, and Deuteromycetes (Fungi Imperfecti) contribute most of the airborne spore load. Together, these groups contain at least 60 000 separate species. Although all of these groups include clinically important species, which are associated with symptoms of respiratory allergy, the two latter groups contain most of the important allergens. Basidiomycetes, which include mushrooms, puffballs, rusts, and shelf or bracket fungi [1], have only recently been demonstrated to be important

aero-allergens [2–6]. Deuteromycetes is an artificial group which contains the asexual stages of primarily Ascomycetes, but also some Basidiomycetes as well as other fungi without known perfect stages [1]. The Deuteromycetes include most of the 'traditional' fungal allergens used for diagnosis and treatment of mould-induced allergy; important genera are: *Alternaria*, *Aspergillus*, *Fusarium*, *Cladosporium* and *Penicillium*. Over 60 fungal genera have been reported to be allergenic [7]. As characterized extracts of other genera or species become available, this number will continue to grow.

Many mould-sensitive patients exhibit broad patterns of positive skin reactivity to both Basidiomycetes and Deuteromycetes. For example, we have shown previously that when atopic individuals were skin-prick tested with

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15 basidiospore antigens, 11 of 24 (45.8%) reacted to five or more extracts and two of 24 (8%) reacted with 12 extracts [2]. The prevalence of skin reactivity to basidiospores was similar to or exceeded the prevalence of reactivity to commercial extracts of several Deuteromycetes. Moreover, a significant linear correlation between the number of positive skin tests to Basidiomycete (basidiospore) extracts and Deuteromycetes has also been demonstrated [2]. Although patients may have multiple independent sensitivities to several fungal classes, the possibility also exists that some fungal species contain shared allergenic determinants. As it is clearly impossible to test patients with extracts of all species of clinically relevant fungi, it is important to determine the degree of crossreactivity between these two major fungal groups. The present study was undertaken to determine whether select species of Basidiomycetes and Deuteromycetes contain shared allergenic determinants.

Materials and methods

Extract preparation

Collection of basidiospores and preparation of extracts has been described previously [3]. Briefly, field-collected caps from *Armillaria tabescens*, *Pleurotus ostreatus* or *Coprinus quadrididus* were placed gills down on Whatman No. 1 filter paper and covered for 24–48 hr to collect spore prints; spores were then scraped from the paper. Spores from *Psilocybe cubensis* were purchased (Florida Mycology Institute, Pensacola, U.S.A.). Spores from *Calvatia cyathiformis*, *Scleroderma* sp. and *Pisolithus tinctorius* were obtained by harvesting the puffball at maturity, drying at room temperature, cutting the ball in half, and removing the internal spore mass. Purity of samples was ascertained by light microscopy. For radio-allergosorbent (RAST) inhibition testing only, powdered defatted *Fusarium solani*, *Aspergillus* species (*A. glaucus* group), *A. fumigatus*, *Alternaria alternata*, *Penicillium notatum*, *Epicoccum purpureescens* and *Cladosporium herbarum*, containing both mycelia and spores, were purchased from Greer Laboratories (Lenoir, NC, U.S.A.).

All fungi were extracted separately by grinding 1 g of material in 40 ml ammonium bicarbonate buffer (0.125 M, pH 8.1) with glass beads using a Braun homogenizer (Braun Melsungen AG, Melsungen, FRG). Seventy millilitres of additional buffer were used to wash the homogenate from the beads. The material was centrifuged (48 000 g); the supernatant was lyophilized and stored desiccated at room temperature [3].

Skin-prick testing

Skin-prick test results from 20 adults who presented to the Charity Hospital Allergy Clinic with perennial symptoms

of rhinitis, with or without asthma, and who had at least one positive skin-test reaction to any fungal allergen were evaluated statistically. This represents approximately 27% of patients screened at this clinic. Subjects signed an informed consent and were tested with commercially available Deuteromycete extracts: *A. alternata*, *F. solani*, *A. fumigatus*, *C. herbarum*, *Epicoccum* sp. and *P. notatum* (Hollister Stier, Spokane, WA, U.S.A., 1:20 (w/v) in 50% glycerol) and basidiospore extracts, prepared as above, and diluted to 10 mg/ml with phosphate-buffered saline (PBS) (diluted 1:1 with glycerol). Histamine diphosphate (1 mg/ml) and PBS:glycerol (1:1) were the positive and negative controls, respectively. A skin-test reaction was considered positive if the average weal diameter was at least 2 mm larger than the PBS control. Statistical associations between reactions to basidiospore and Deuteromycete extracts were determined using Fischer's exact test [8]. A *P*-value ≤ 0.01 was considered statistically significant.

RAST inhibition

Appropriate serum pools were prepared from equal volumes of sera obtained from skin- and RAST-positive individuals. Serum used for *Aspergillus* sp. RAST inhibition studies was from one individual only. Pools for the *F. solani* RAST and for all Basidiomycete RASTs were used undiluted; all other serum pools were diluted 1:1 with physiological saline.

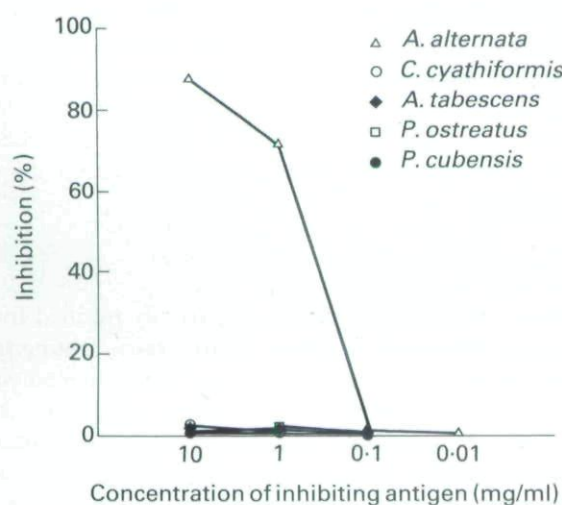
RAST inhibition was performed using the method currently in use in our laboratory [9]. Briefly, *A. tabescens*, *A. alternata*, *C. cyathiformis*, *F. solani* or *Aspergillus* sp. (100 µg/disc) in borate buffer (pH 8.0) were coupled with cyanogen bromide-activated filter paper discs [10]. For the test, 50 µl of the serum pool and 50 µl of increasing concentrations (unless noted otherwise, these were 0.01, 0.1, 1.0 and 10.0 mg/ml in PBS) of the inhibiting antigen, or PBS, were added simultaneously to an allergen-coupled disc. Assays were performed in duplicate. Percentage inhibition was calculated as follows:

$$\left[1 - \frac{\text{c.p.m. test antigen}}{\text{c.p.m. PBS control}} \right] \times 100\%.$$

Significant inhibition was considered to be greater than 20%. To evaluate the results further percentage inhibition of the ascending linear portion of the dose-response curve was fitted to the logarithm of the concentration using a least-squares regression. Slopes and intercepts were calculated and compared using multiple regression techniques [11]; parallel slopes were indicative of shared allergenic determinants [12].

Table 1. Associations between skin-prick test results with selected Basidiomycete and Fungi Imperfecti extracts

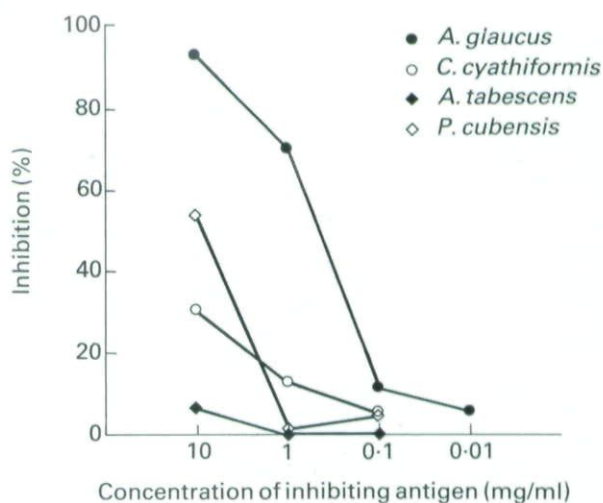
	Fungi Imperfecti						Basidiomycete total
	<i>A. alternata</i>	<i>F. solani</i>	<i>A. fumigatus</i>	<i>C. herbarum</i>	<i>Epicoccum</i> sp.	<i>P. notatum</i>	
Basidiomycetes							
<i>A. tabescens</i>	3	2	2	3	3	2	7
<i>C. quadrifidus</i>	6	5*	3	4	5	2	10
<i>P. ostreatus</i>	6*	5*	4	4	5*	3	6
<i>P. cubensis</i>	7	4	5*	5	4	3	9
<i>C. cyathiformis</i>	7*	5*	4	4	5	3	8
<i>P. tinctorius</i>	2	0	1	0	0	0	6
<i>Scleroderma</i> sp	8	5	4	5	4	2	13
Fungi Imperfecti total	9	5	5	7	6	3	

* $P \leq 0.01$.**Fig. 1.** Inhibition curves of *Alternaria alternata* RAST with basidiospore extracts.

Results

Skin-test associations

Several statistically significant associations were observed between skin-test reactions to some Deuteromycetes and basidiospores (Table 1). Reactivity to *P. ostreatus* showed highly significant associations ($P=0.002$, $P=0.0004$ and $P=0.007$) with reactivity to *A. alternata*, *F. solani* and *E. purpurescens*, respectively. Weal-and-flare reactivity to *C. cyathiformis* was also associated with *A. alternata* ($P=0.003$) and *F. solani* ($P=0.004$). *Coprinus quadrifidus* was only associated with *F. solani* ($P=0.01$) and *P. cubensis* only with *A. fumigatus* ($P=0.008$). No other statistically significant skin-test associations were noted.

**Fig. 2.** Inhibition curves of *Aspergillus glaucus* RAST with basidiospore extracts.

RAST inhibition analysis

RAST inhibition dose-response curves are presented in Figs 1–5. For every allergen system tested significant homologous inhibition was observed. Although the ability of an individual extract to inhibit the direct RAST varied with the extract tested, in general, inhibition with heterologous materials was minimal. In the most extreme example, none of the heterologous allergens tested inhibited the *A. alternata* RAST; whereas, homologous allergen inhibited the system by 87.4%. Inhibition of other Fungi Imperfecti RASTs was marginal. In the *F. solani* RAST, for example, only *A. tabescens* (22.9%) and *P. cubensis* (25.3%) demonstrated significant inhibi-

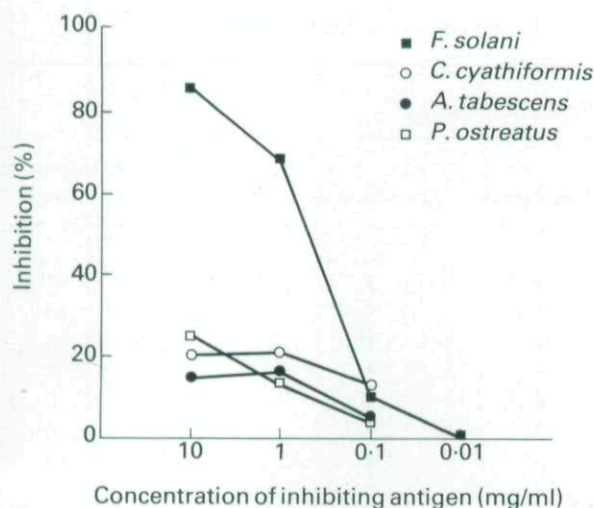


Fig. 3. Inhibition curves of *Fusarium solani* RAST with basidiospore extracts.

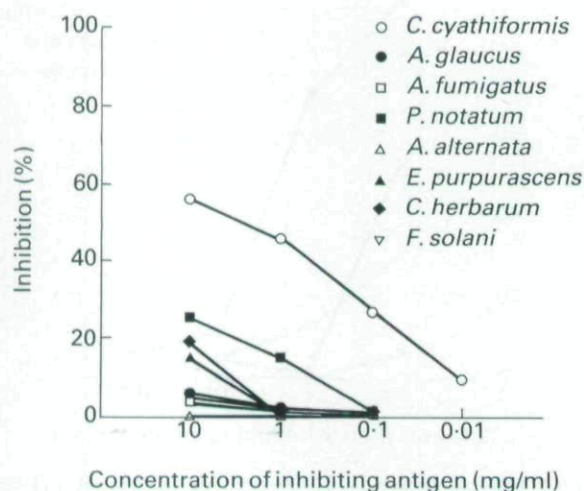


Fig. 4. Inhibition curves of *Calvatia cyathiformis* RAST with Fungi Imperfecti extracts.

tion which was seen only at the highest test concentration. Inhibition of basidiospore RASTs by Deuteromycete species was also minimal; however, some heterologous inhibition was seen. As shown in Fig. 5, the *A. tabescens* RAST was inhibited 52.6%, 38.1%, and 25.1% by *A. fumigatus*, *E. purpureus* and *P. notatum*, respectively.

Evaluation of slopes and intercepts of the dose-response curves (regression lines not shown) demonstrated that some regression lines had parallel slopes (Table 2). No lines were coincident. These results suggest that some Basidiomycetes and Deuteromycetes may

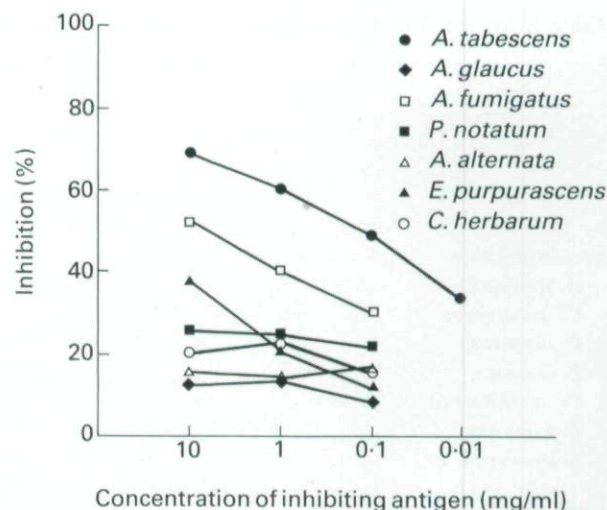


Fig. 5. Inhibition curves of *Armillaria tabescens* RAST with Fungi Imperfecti extracts.

share some allergenic determinants. The regression lines of *C. cyathiformis* demonstrated parallel slopes with three species of Deuteromycetes: *C. herbarum*, *E. purpureus* and *P. notatum*. *Armillaria tabescens* showed cross-reactivity with *A. fumigatus* and *E. purpureus*. Lastly, *Aspergillus* sp. demonstrated some crossreactivity with *P. cubensis*.

Discussion

Previous studies using crude or partially purified fungal allergens have demonstrated crossreactivity among select basidiospore extracts [13] or among the Deuteromycetes [7,9,14,15]. Crossreactivity is most apparent within genera, as is exemplified by the form genus *Aspergillus* [9]; however, shared allergenic determinants have also been demonstrated among genera. For example, within the Deuteromycetes, *Alternaria*, *Stemphylium*, *Curvularia* and *Stachybotrys* crossreact extensively [reviewed in 7]. Furthermore, a high level of crossreactivity among genera of Basidiomycetes has been shown [13]. Previous limited studies have failed to demonstrate the presence of shared allergenic determinants between major fungal groups [16-18]. The one description of crossreactivity between airborne fungi and dermatophytic fungi was based solely on skin-test reactivity [18]; thus the presence of concomitant allergies in the test subjects could not be eliminated. The present study demonstrated that, although positive skin tests to some Deuteromycete and Basidiomycete extracts were statistically associated, there was only limited *in-vitro* crossreactivity among selected members of these groups.

Lehrer *et al.* [2] demonstrated a significant correlation between the number of positive skin tests to basidiospore

Table 2. Statistical evaluation of dose response curves for inhibition of Basidiomycete and Fungi Imperfecti RAST

Inhibiting antigen	Discs coupled to:				
	<i>F. solani</i>	<i>C. cyathiformis</i>	<i>A. glaucus</i>	<i>A. alternata</i>	<i>A. tabescens</i>
<i>F. solani</i>	—	⋈	n.d.	n.d.	⋈
<i>C. cyathiformis</i>	⋈	—	⋈	⋈	n.d.
<i>A. glaucus</i>	n.d.	⋈	—	n.d.	⋈
<i>A. tabescens</i>	⋈	n.d.	⋈	⋈	—
<i>A. fumigatus</i>	n.d.	⋈	n.d.	n.d.	
<i>C. herbarum</i>	n.d.		n.d.	n.d.	⋈
<i>A. alternata</i>	n.d.	⋈	n.d.	—	⋈
<i>E. purpureus</i>	n.d.		n.d.	n.d.	
<i>P. ostreatus</i>	⋈	n.d.	n.d.	⋈	n.d.
<i>P. cubensis</i>	⋈	n.d.		⋈	n.d.
<i>P. notatum</i>	n.d.		n.d.	n.d.	⋈

|| = parallel lines, slopes equal $P > 0.05$.⋈ = not parallel lines, slopes different $P < 0.05$.

and Deuteromycete extracts; however, comparisons of individual species were not made. Results from the present study extend our initial observation by the use of a direct test of association between specific allergens. It is noteworthy that positive skin-prick test results to *P. ostreatus* showed strong statistical associations with three of the Deuteromycetes. Our previous RAST inhibition study [17], however, failed to demonstrate the presence of shared allergens in *P. ostreatus* and *A. alternata* or *F. solani*. *Aspergillus fumigatus* was, however, not evaluated in that study. Thus, although statistical associations between paired skin-test reactions suggest that the allergens may contain shared determinants, they do not rule out multiple, parallel sensitivities.

RAST inhibition, in the present study, demonstrated that crossreactivity among selected species of Basidiomycetes and Deuteromycetes varied considerably. Some fungal extracts used in this study showed no crossreactivity. *Alternaria alternata*, although demonstrating dose-dependent homologous inhibition, could not be inhibited by any basidiospore extract tested. The Basidiomycete extracts used in those experiments had been shown previously to be highly reactive by skin test and RAST [3]. This suggests that these basidiospore extracts are active in our populations. On the other hand, inhibition of *Aspergillus* sp. RASTs by basidiospore extracts did consistently demonstrate the presence of shared allergenic activity; for example, *P. cubensis* inhibited the RAST by more than 50%. Inhibition of Basidiomycete RASTs by Deuteromycete extracts also produced variable results.

These findings differ from previous ones where no crossreactivity between select species of Basidiomycetes and Deuteromycetes could be demonstrated by either RAST inhibition [17] or immunoelectrophoretic techniques [16]. Dissimilarities between data obtained in those studies and the present one may be the result of species tested, use of different serum pools, the wider range of allergens used in this study, or the varying ability of proteins present in basidiospores and Deuteromycetes to bind with cyanogen bromide-activated discs.

Because of potential differences in binding capabilities of these two groups of fungi to RAST discs and, subsequently binding of serum IgE, comparison of RAST results in general, and RAST inhibition in particular, is complex. In the *C. cyathiformis* and *A. tabescens* RAST inhibition experiments, only 3.6% and 5.2% of total ^{125} I-labelled anti-IgE added bound to the respective discs. However, binding observed in Deuteromycete RAST systems was 21.5%, 13.5% and 13.7% for *Aspergillus* sp., *A. alternata* and *F. solani*, respectively. These values are routinely observed for these extracts, regardless of the serum tested. Low activity of some basidiospore extracts in the RAST may produce spurious interpretations; furthermore, even minimal intra-assay variability could profoundly affect the results. On the other hand, low binding should increase the sensitivity of the RAST inhibition. Reasons for low binding of specific IgE to basidiospore extract-coupled discs are not clear. However, this has been a consistent observation, not only with Basidiomycetes, but with fungi in general. It is possible

that basidiospores have fewer free amino groups to bind to the disc or that allergens of these fungi may be composed of non-proteinaceous material. Sufficient information about the biochemical composition of fungal allergens is not yet available, so these remain hypotheses rather than fact.

Care must be taken when evaluating RAST inhibition data by statistical evaluation of slopes. An example of questionable conclusions from this type of assessment is seen in the inhibition of the *C. cyathiformis* RAST. Statistically, slopes of three heterologous Deuteromycete extracts were parallel with that of the homologous material, suggesting the presence of shared allergens. Visual examination of the raw data, however, showed that for *C. herbarum* and *E. purpurescens* inhibition was virtually zero, except at 10 mg/ml where it approached 20%. Furthermore, the limited number of data points available from these experiments could result in a loss of statistical power. Therefore, maximal percentage inhibition was also considered when assessing these data. For example, in the *A. glaucus* RAST inhibition, *P. cubensis* demonstrated a maximal inhibition of nearly 60%; coupled with the parallel slope this strongly suggests the presence of shared allergens. The patterns observed in this system, as well as in the other systems presented here, were consistent over at least three trials.

Collectively, these studies confirm that relationships among fungal extracts cannot be evaluated by skin testing alone. They also suggest that, although shared allergenic determinants could be demonstrated in select species of Basidiomycetes and Deuteromycetes, crossreactivity is minimal and its clinical significance is unclear. However, the irregular pattern and minimal degree of cross-reactivity between Basidiomycetes and Deuteromycetes does indicate that testing with extracts of one group will not reliably predict reactivity to the other group.

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