

Current Status of Fungal Allergens

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Abstract

Several studies have been done with respect to standardization of fungal allergen extracts. The emphasis has been given to optimization of source material for quality extracts. Currently, standardization is carried out in terms of major allergen content. Recombinant methods have allowed cloning and expression of allergenic proteins in large quantities. Cloning of cross-reactive major allergenic proteins will reduce the panel of extracts required for diagnosis/therapy without compromising the efficacy. The use of recombinant mould allergens will overcome the problems associated with current form (crude extracts) of mould allergen immunotherapy.

INTRODUCTION

Fungi are among the earliest recognized allergens. Blackley (1873) reported *Penicillium* and *Chaetomium* spores as the causative agents of hay fever. Fungal spores are the normal component of outdoor airspora and also inhabit indoors such as residential buildings, offices, industrial buildings, storehouses etc in large numbers. There exists poor correlation between measured airborne allergen and related spore particles in outdoor air. This indicates potential for significant allergen exposure in absence of identifiable particles in the air¹. Valid prevalence data for large areas are difficult to secure but can be collected for small sample size¹.

Fungal spores are far more numerous in outdoor air than pollens, however indoor concentration of fungal spores varies and depends upon the human

activities. The spores are released from fruiting structures of some fungi by special spore-releasing mechanism activated by drying (*Phytophthora infestans* and *Drechslera*) while others such as *Epicoccum* and *Nigrospora* require water for sudden bursting of their turgid cells. Fungi grow on structural materials and furnishings where humidity is high or on food and stored products where there is condensation. Humidifiers, air-conditioning systems, carpeting, damp walls are the potent sources of indoor fungal allergens. Outdoor fungi such as *Alternaria*, *Aspergillus*, *Botrytis*, *Cladosporium*, *Curvularia*, *Epicoccum*, *Fusarium*, *Penicillium*, *Phoma*, *Trichoderma* etc. have been reported regularly from damp indoors. *Aspergillus*, *Eurotium* and *Penicillium* have been observed to colonize on food scraps and other organic materials².

Prevalence of fungi in a particular area depends upon various factors. The relative abundance of fungal species is influenced by plant ecology in that area. Circadian periodicity, seasons, geographical locations also affect the concentration of particular fungal spore type in the air. The fungi have been implicated in

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inflammatory reactions such as hypersensitivity pneumonitis, allergic bronchopulmonary mycoses, allergic fungal sinusitis, rhinitis, asthma, extrinsic allergic alveolitis etc. The exact prevalence of fungal allergies is not known. Studies based on skin tests suggest that at least 3-10% of adults and children worldwide are affected by fungal allergy^{1,3}. However, skin reactivity ranging from 3-91% has been reported depending upon the population studied, fungal extracts used and species tested⁴⁻⁶. The asthmatic patients (25-31%) showed more skin sensitivity to *Cladosporium*, *Alternaria* and *Aspergillus* as compared to those (10-17%) with both asthma and rhinitis⁷. In India, fungi were reported as sole offenders in 2.3% of asthmatics and contributing offenders in another 34.6% of patients³. Epidemiological studies at various occupational environment such as bakeries, poultry farms, granaries and sugar refinery revealed one or more respiratory ailment or work related respiratory dysfunction in 36-40% of workers^{8,9}.

Fungi implicated in respiratory allergy represent all major groups viz. Phycomycetes, Ascomycetes, Basidiomycetes and Deuteromycetes. The fungi such as *Alternaria*, *Aspergillus species*, *Bipolaris species*, *Curvularia lunata*, *Epicoccum nigrum* and *Fusarium solani* were isolated from air samples obtained from buildings with poor indoor air quality. They were recognized as the important causative agents of allergic fungal sinusitis in its inhabitants¹⁰. In a study to investigate the retention of inhaled fungal spores in the lungs, it was observed that the greatest number of spores was deposited in the aqueous lining layer of alveoli in close proximity to the epithelial cells. The interaction of spores with lung structures may be important for the development of respiratory allergies induced by air borne fungal allergens¹¹.

Apart from their direct allergenic effect, fungi may carry mycotoxins in their spores or produce volatile metabolites¹². Inhalation of mycotoxins such as aflatoxins, secalonic acid D, zearalenone and tricothecenes produced by *Aspergillus*, *Penicillium* and *Fusarium* spp, may affect the immunological response of the lung tissue or present other hazards

to human health¹³. The present article reviews the progress made on fungal allergens during the last two decades. It also mentions efforts being made to develop new improved allergens for use in fungal vaccines.

SOURCE MATERIAL FOR ALLERGEN PREPARATIONS

Fungal extracts contain primarily proteins and glycoproteins with molecular weights of 5-70 kD. The extract may contain polysaccharides such as mannans and galactomannans, which are essentially the cell wall components. The extracts are prepared from surface growth (spore-mycelia), mycelium, spores and culture filtrate i.e. metabolic antigens¹⁴⁻¹⁸. The number and strength of antigenic components depend on nutritional composition of growth medium and period of incubation. Different components are released at different stages of incubation. The combination of metabolic and somatic extracts from both early and late growth phase fungal cultures is recommended to obtain broad spectrum of allergenic components¹⁹. Fungi are intrinsically variable and the batches of allergenic material produced successively, even from the same strain, can differ widely. Thus standardization of growth parameters is important to obtain quality source material. The growth conditions have been worked out for cultivating certain moulds by using different nutrient media, temperature and incubation period. It has been observed that change in the metabolic states of culture influences the antigenic composition of mould extract²⁰. The use of synthetic medium has been recommended in earlier studies for cultivating moulds. However, addition of yeast extract has greatly improved the growth of some fungi in synthetic medium^{18,21,22}.

The fungus *Aspergillus fumigatus* has been extensively studied associated with several forms of allergy. Day 28 stationary culture of this fungus at 37°C released the widest range of allergenic proteins into the culture filtrate²³. Day 16 culture of *Alternaria alternata* demonstrated maximum allergen content, when extracted for 24-96 hours²⁴. On the other hand, day 11 culture (culture filtrate) of *Fusarium solani* showed maximum allergenic content as compared to earlier or late phase culture³. Recent studies with

C. lunata and *E. nigrum* showed maximum allergenic content in day 11-13 spore-mycelial culture grown in semi-synthetic Sabouraud's medium^{20,21}. Equally potent culture filtrate extract of *E. nigrum* was obtained using a modified semi-synthetic (Sabouraud's) medium that can be used for allergy diagnosis²². The essential parameters (temperature and water content) required for growing cultures of *A. fumigatus* and *A. alternata* were studied to obtain marker antigens from these fungi. The presence of marker antigens in the reference extracts would facilitate in identifying the possible risk exposure to these fungi. Antigens of 51-52 kD and 64, 56-57, 51-52 kD from water soluble extracts, while 39-41 kD and 54-55, 57-57.5, 68 kD marker antigens from culture filtrate extracts were observed in *A. fumigatus* and *A. alternata*, respectively^{25,26}.

The allergen extracts supplied by different manufacturers for allergy diagnosis are not well characterized. However, the standardized extracts show more positive skin reactions without the evidence of any non-specific reaction^{20,21,23}.

STANDARDIZED FUNGAL EXTRACTS

The efficacy of a native allergen vaccine as a therapeutic reagent is a function of the specific allergen concentration, integrity and bioavailability. Previous studies have confirmed that the allergen content of the commercially available vaccines (mould extracts) was highly variable^{16,17}. Therefore it is recommended to develop standards of each commercial allergen vaccine for comparing different lots of vaccine to maintain their quality and accurate labelling. The broad limits of comparability cause unacceptable risk to the patients (anaphylaxis), while narrow limits lead to rejection of large percentage of safe and effective lots of products supplied by the manufacturers. Hence the establishment of an acceptable range of comparability or equivalence is fundamental to the standardization process. Allergen extracts are biologically standardized on the basis of potency measurements, which depends on skin reactivity on a panel of patients. The allergenic potency of the mould extracts as measured by skin testing does not correlate with the weight/volume or protein nitrogen content of the extract. Further, in vitro methods viz. SDS-PAGE, IEF, RAST/ELISA

inhibition and immunoblot with serum pools are used and compared with in-house reference extracts. These methods measure most, if not all major/minor IgE binding components. However, change in the serum pool is unavoidable resulting differences in the measurement of allergen content^{16,17}.

Various fungal extracts have been characterized to identify their IgE binding components both by in vivo and in vitro method²⁰⁻²⁴. Many IgE binding proteins have been identified from *Aspergillus spp*²⁷. A 31 kD protein from *A. alternata* showed highest IgE binding on immunoblot and was present in both spore and mycelial extracts³. About 36 antigens of 60 identified from *Cladosporium herbarum* have been shown to react with IgE by crossed immunoelectrophoresis²⁸. A 65 kD major allergen of *F. solani* was detected in day 11 culture filtrate extract by SDS-PAGE and immunoblotting³. Studies on *E. nigrum* revealed 25 IgE binding bands in day 13 standardized spore-mycelial extract²⁰. Day 11 spore-mycelial extract of *C. lunata* showed 10-14 allergenic proteins by immunoblotting²¹. The conidial extracts of *C. cladosporoides* were prepared in different buffers and allergenic proteins were detected and quantified by passive cutaneous anaphylaxis (PCA) test on Balb/c mice and RAST. The highest allergenic potency was observed in spore extracts prepared in Tris, NaOH and NaCl buffers and the amount of allergens released was dependent on the incubation media, extraction time and spore concentration³. It was also observed that the amount and type of allergenic protein/glycoprotein from *Alternaria* extract depend on extraction time period²⁹.

Type I reactions and specific IgE antibodies to *Candida albicans* and *Pityrosporum ovale* (yeast) allergens have been reported in atopic dermatitis patients by ELISA and ELISA inhibition³. Basidiomycetes are also reported as important source of respiratory allergens and their extracts have been standardized for use in skin testing^{4,5}. Among respiratory allergy patients tested, 27% showed positive skin reactions to basidiospore-mycelial extract while 32% showed reactivity to one or more basidiospore extract⁴. The crude extracts of *Pleurotus ostreatus* contained allergens in the range of 10-25 kD and further their allergenic activity was evaluated by RAST and skin testing³. The common and specific

allergenic components of *Ganoderma lucidum* spores and whole body preparations were identified using SDS-PAGE, IEF and immunoblotting³⁰.

The potency measurement of allergen extracts by major allergen content has clear advantage over IgE based in vitro/in vivo approaches described previously. Monoclonal antibodies against major allergens can be generated now in sufficient amounts due to availability of purified allergenic proteins. The major allergens that cover >80% of specific IgE response of an allergic population can be measured and compared using these antibodies. A particular, allergen extract from all over the world can thus be compared and evaluated based on major allergen content³¹. The monoclonal antibodies against alkaline serine proteinase (allergens) of *Penicillium* and *Aspergillus* species have been generated in Balb/c mice and used for characterization by SDS-PAGE, 2-dimensional electrophoresis and immunoblotting³². A 68 kD major allergen (recognized by 56% of patient's sera) of *P. notatum* was identified by immunoblot analysis using radiolabelled monoclonal antihuman IgE³.

PURIFIED FUNGAL ALLERGENS

Fungal extracts are complex mixture of allergenic proteins that show variation in their content, even when prepared under optimized conditions^{3,14,15}. The allergy diagnosis and therapy suffers due to variations in extract. The whole mass/culture filtrate extracts contain irrelevant proteins as well, which may cause nonspecific reactions during skin testing and can lead to additional sensitivities in patients under immuno therapy. Therefore, use of purified allergenic proteins has been recommended for diagnosis and therapy. In majority of the cases, allergy is due to one or more major allergens present in the extract. The purified allergens are also required for the proper identification and characterization of the proteins by N-terminal amino acid analysis, structural epitope mapping of IgE binding sites/T cell antigenic sites and comparison of homology with other known functional proteins^{3,33,34}. The 33/34 kD allergenic proteins from *Aspergillus* species were characterized as serine protease by N-terminal sequencing^{1,35,36}. Various chromatographic techniques have been used

to purify one or more allergenic components of molecular weights (18-90 kD) from fungi (Table 1)^{1,3,32,37-47}

Purity of the proteins was checked by SDS-PAGE, HPLC, and mass spectrometry. IgE reactivity was analyzed by RAST/ELISA inhibition, immunoblot, CRIE and/or in vitro histamine release³. Recently a 3.4 kD peptide was isolated from a major allergen (*Fus s I₃₅₉₆*, MW 65 kD) of *F. solani*. The peptide was shown to bind IgE but did not release histamine. Such molecules can be explored for peptide immunotherapy⁴⁸. The information about amino acid composition, post-translational modifications and N-terminal amino acid sequences of certain allergens is available now, useful for identification and absolute quantification on molecular basis. Monospecific antibody reagents against most of purified allergens are also available. The purified natural allergens are hence better candidate for reference materials, provided that source materials can be obtained in large amounts at acceptable price using easy purification protocols.

One of the important biological functions of the purified allergens is their enzymatic activity⁴⁹. Though allergenicity is independent of such activity but it is considered to play important role in pathogenesis of the disease. The proteolytic nature of *A. fumigatus* allergens is responsible for invasion into the lung tissues and epithelial cell detachment⁵⁰. The conventional purified proteins are difficult to generate in bulk amounts and quality, hence recombinant proteins can be the better alternative.

RECOMBINANT FUNGAL ALLERGENS

The recombinant DNA techniques have a strong impact on molecular allergology since the first allergen was cloned by the end of 1980s⁵¹. The cloning of cDNA encoding allergens involves the use of mRNA isolated from allergen source. The mRNA is used to synthesize double stranded cDNA using oligo dT primer, reverse transcriptase and then DNA polymerase. The cDNA, which represents the polypeptides produced by the allergen source, is then cloned into a bacteriophage vector, which when plated out in the lawns of *E. coli* plaques represents clones containing individual cDNA molecules. The clones

of interest can be detected either by DNA hybridization using oligonucleotide probes or with specific antisera or IgE from sera of the allergic patients⁵¹. Nowadays phage display technique is also employed for this purpose^{52,53}.

Over the years, a large number of fungal allergens have been Cloned, sequenced and expressed as fusion proteins (Table I). More than 20 recombinant allergens from *A. fumigatus* have been cloned and sequenced by various workers⁵⁴⁻⁵⁸. Asp f 1 from *A. fumigatus* has been identified as a mitogillin ribotoxin whereas Asp f 2 and Asp f 3 as fibrinogen binding and peroxisomal membrane protein, respectively. Asp f 12 isolated by cDNA cloning, corresponded to heat shock protein hsp 90³. Asp f 22, an enolase has been recently cloned and sequenced which mediated both humoral and cell mediated immunity in allergic bronchopulmonary aspergillosis (ABPA)⁵⁹. The recombinant allergens from *A. alternata* (Alt a6, Alt a7 & Alt a10) and *C. herbarum* (Cla h3, Cla h4, Cla h5 & Cla h6) were reported that showed sequence homology with aldehyde dehydrogenase, enolase, yeast cell cycle protein 4 and acidic ribosomal protein P^{1,33,60} Pen c 1 to Pen c 22 from *p. citrinum* and

Pen n 13, Pen n 18 from *P. notatum*, were cloned and sequenced^{32,59,61-64}. Pen n 13 showed homology with other recombinant mold allergens such as Pen c 1, Asp f 11, Asp f 13, belonging to alkaline serine protease family⁶⁴. Pen c 2 showed high sequence similarity with vacuolar serine protease from *A. niger* and *Saccharomyces cerevisiae*, while Pen c 3 from *P. citrinum* was defined as peroxisomal membrane protein showing 82% identity with Asp f 3 from *A. fumigatus*^{32,61}. Among yeasts, *Candida spp.* is widely studied with two recombinant allergens reported till now³. Cop cl is the first recombinant allergen from a basidiomycete, *Coprinus comatus* that induced IgE binding; T cell proliferative response and skin reactivity in *C. comatus* sensitized patients⁶⁵. A 23 kD recombinant allergen from *Psilocybe cubensis* expressed as fusion protein showed homology to fungal cyclophilin. This can serve as useful model for epitope analysis of basidiospore allergens⁶⁶.

The availability of recombinant allergens has facilitated development of in vitro diagnostic techniques for quantitating major allergens in the

extract. A novel 2-site monoclonal antibody ELISA was developed for quantitating Alt al from *A. alternata* and Asp fl from *A. fumigatus* extracts⁶⁷. Some of the recombinant *Aspergillus* allergens have been analyzed for their predictive diagnostic value by direct solid phase ELISA. In a combination of IgE specific serology with rAsp f 1, rAsp f3 and rAsp f 5, 97% diagnostic sensitivity was observed in allergic asthmatics sensitized to *A. fumigatus*⁶⁸. The other recombinant allergens are yet to be worked out for their diagnostic efficiency.

Gene cloning and expression techniques have also facilitated in determining the biological activities of the allergens, demarcation of the T and B cell epitopes and an understanding of the molecular mechanism by which specific IgE antibodies are induced. Recombinant proteins have been suggested as tools for improved diagnosis and therapy of type I allergic diseases and as reference compounds for standardization of extracts. But prior to diagnostic application, it is important to know the antibody binding capacity of recombinant and natural allergens from same source. The sera from patients allergic to respective natural allergen source should show similar specific IgE binding to its recombinant counterpart^{54,55}.

CROSS-REACTIVE FUNGAL PROTEINS

Cross-reactivity is a term commonly used to express similarities of different species with respect to sensitization and reactions in the human IgE system. It denotes the presence of shared epitopes in fungal allergens and is different from parallel or independent sensitization to multiple fungal species. Cross-reactivity of IgE antibodies to allergens reflects the phylogenetic relationship between organisms. Phylogenetically related species show high degree of homology in the primary structure of the proteins. High homology in primary sequence results in homologous 3-D structures and thus potentially, in cross-reactivity. This can help in selecting the panel of cross reactive allergens to simplify diagnostic procedures and therapeutic regimens to be employed. IgE cross-reactivity is of interest for its immunological basis, particularly in relation to the regulation of allergic sensitization. It is therefore

Table I : List of important purified and recombinant mould allergens.

Fungi	Purified (Biochemical)		Recombinant Allergens (Cloned)		
	Name/Nature	MW (kD)	Name	MW (kD)	Nature
<i>Aspergillus fumigatus</i>	Protein ³	16	rAsp f1	16.9	Ribotoxin ⁵⁵
	Asp f/Ag 3 ³	18	rAsp f2	37	Cytosolic/Fibrinogen binding ²
	Ag 20, Glycoprotein ³	20	rAsp f3	18.5	Peroxisomal membrane protein ²
	Asp f2 ⁴¹	37	rAsp f4	30	NS ⁵⁵
	Metalloprotease ⁴³	40	rAsp f5	42	Metalloprotease ⁵⁵
	Protein ³	45	rAsp f6	23	Manganese superoxide dismutase ⁵⁵
	Asp f22 ⁵⁹	47	rAsp f7	12	NS ⁵⁵
	gp55 ³	50-60	rAsp f8	11	Ribosomal protein p2 ⁵⁷
	Glycoprotein ⁷⁸	58	rAsp f9	30.6	NS ⁵⁵
	Glycoprotein ⁴⁴	88	rAsp f10	34.4	Aspartic proteinase ⁵⁵
	Catalase ⁴²	90	rAsp f11	24	Peptidyl-prolyl isomerase*
	Glycoprotein ³⁹	93	rAsp f12	90	Heat shock protein ³
			rAsp f13	33/34	Alkaline serine protease ²⁷
			rAsp f15	16	Yeast membrane protein*
			rAsp f16	43	Not known, sequence homology to rAsp f9 ⁵⁸
<i>Alternaria alternata</i>			rAsp f17	-	IgE binding protein*
			rAsp f18	34	Vacuolar serine protease ⁵⁶
			rAsp f22	46	Enolase ⁵⁹
	Basic peptide, heat stable glycoprotein ³	6	rAlt a1	14.5-16	NS ³³
	Alt a29 ³	29	rAlt a2	25	Mouse RNA dependent eukaryotic initiation factor-2- α -kinase ³³
	Alt 1 ³	25-50	rAlt a3	70	Heat shock protein*
	Ag 8 ³	30	rAlt a4	57	Disulfide isomerase*
	Alt a1 ₁₅₆₃ ³	31	rAlt a5	46	Enolase ⁴⁹
	Ag 1 ³	60	rAlt a6	11	Acid Ribosomal P ₂ protein ³³
	Glycoprotein ³	70	rAlt a7	22	Yeast, YCP4 protein ³³
			rAlt a10	53	Aldehyde dehydrogenase ³³
			rAlt a11	45	Enolase*
			rAlt a12	11	Acid ribosomal protein P1*

<i>Cladosporium herbarum</i>	Cla h1/Ag 32 ²⁸	13	rCla h3	53	Aldehyde dehydrogenase ⁶⁰
	Cla h2/ Ag 54 ³	20-22	rCla h4	11.1	Ribosomal P ₂ protein ⁶⁹
			rCla h5	22	Unknown function, Yeast, YCP4
			rCla h6	48	Enolase ⁶⁰
			rCla h12	11	Acid ribosomal protein P1 *
<i>Fusarium culmorum</i>	---	---	rFus C1	11	Ribosomal protein p2 *
			rFus C2	13	Thioredoxin like protein
<i>Fusarium solani</i>	Fus s 1 ₃₅₉₆ ³⁷	65	---	---	---
<i>Fusarium equiseti</i>	Protein ³⁸	65	---	---	---
<i>Penicillium citrinum</i>	Pen c1, alkaline serine protease ⁶³	33	rPen c1	33	Alkaline serine protease ⁶⁴
	Pen c22 ⁵⁹	46	rPen c2	39	Vacuolar serine protease ⁶¹
			rPen c3	18	Peroxisomal membrane protein ³²
			rPen c18	34	Vacuolar serine protease ⁵⁶
			rPen c19	70	Heat shock protein ¹
			rPen c22	46	Enolase ⁵⁹
<i>Penicillium chrysogenum (notatum)</i>	---	---	rPen ch13	34	Alkaline serine protease ⁶²
			rPen ch18	32	Vacuolar serine protease ⁶³
			rPen ch20	68	N-acetyl glucosaminidase ³⁴
<i>Penicillium oxalicum</i>	---	---	rPen o18	34	Vacuolar serine protease ⁵⁶
<i>Candida albicans</i>	Phospholipomannan protein ⁴⁵	14-18	rCand a1	40	Alcohol dehydrogenase ³⁴
	C3d receptor binding glycoprotein ⁴⁶	50	rCand a2	29	Peroxisomal protein*
	MP65 ⁴⁰	65			
	Con A binding glycoprotein ³	70			
	Enolase ⁴⁷	46			

MW-Molecular weight, NS-No sequence similarity found, "-Submitted to NCBI database and International Allergen Nomenclature Committee.

Superscripted numbers in the table indicate corresponding cross-reference.

relevant to compare structural relationship between two allergens, its relatedness to human equivalent if any, and its influence on the immune repertoire⁶⁹.

The first report (1970) on cross-reactivity came with unexpected cross-reactions between ragweed and banana⁶⁹. Later a number of studies were carried out

to examine the cross-reactivity among the known allergens belonging to moulds⁷⁰⁻⁷⁷, pollens, mites, dander etc. Cross-reacting proteins have been demonstrated among different fungal genera, species and strains of Deuteromycetes by ELISA/RAST/Immunoblot inhibition using rabbit antiserum and/or allergic patients' sera^{3,72-74}. *Alternaria* and *Stemphylium* extracts showed multiple shared antigenic (IgG binding) determinants, while Alt a 1 was observed to be a common major allergen in *Ulocladium* and *Alternaria*³. The fungal types such as *Alternaria*, *Cladosporium*, *Curvularia*, *Epicoccum* and *Penicillium* were reported to share many IgE and IgG binding proteins among them^{70,71}. A 45 kD allergenic protein was shared by 11 different fungi as demonstrated by immunoblot with pooled sera from *Fusarium* allergic patients⁷². Extensive cross-reactivity has been observed between *Aspergillus* and *Penicillium* species^{1,73}. This may be due to close taxonomic relationship between these two genera. IgE cross-reactivity of 34 kD major allergens of *Aspergillus oryzae* and *Penicillium citrinum* was established by immunoblot inhibition and both were identified as alkaline serine protease¹. The monoclonal antibodies generated against 34 kD allergenic proteins from *Penicillium* species showed cross-reactivity with those of *Aspergillus* species³. The monoclonal antibodies prepared against 58 kD glycoprotein of *A. fumigatus* showed serological cross-reactivity with 55 kD antigen of *C. albicans*⁷⁸. Cross-reactivity is also reported within *Aspergillus* species, *Alternaria* species and *Curvularia* species^{3,74}. A number of allergenic components of molecular weight 14, 19, 35, 45, 50 and 70 kD were common among three *Fusarium* species viz. *F. solani*, *F. equiseti* and *F. moniliforme*³. The 34 and 32 kD major allergens of *P. oxalicum* and *P. notatum* showed IgE cross-reactivity with 33 kD major allergen of *P. citrinum* by immunoblot inhibition⁷⁷.

Cross-reactivity also exists among the yeast species^{3,75,76}. The 46 and 48 kD proteins of *Candida albicans* were reported to share some common epitopes with same molecular weight protein of *Saccharomyces cerevisiae* by immunoblot inhibition³. High prevalence of anti *Pityrosporum ovale* antibodies was observed that cross-react with *C. albicans* in atopic dermatitis patients sensitized to *P. ovale*³.

Various workers have reported the evidence of cross-reactivity among Basidiomycetes³. Extensive cross-reactivity was also demonstrated among *Coprinus quadrifidus*, *Psilocybe cubensis*, *Pleurotus ostreatus* of the order Agaricales and *Clavatia cyathiformes* of the order Lycoperdales using RAST, immunoblot and ELISA inhibition. The skin test reactivity to *Pleurotus ostreatus* was associated with *A. alternata*, *F. solani* and *E. purpurascens*. Despite the existence of shared allergenic determinants among select species of both the classes, cross-reactivity is minimal and its clinical significance is not clear³.

The availability of purified allergens is helpful in determining the cross-reactive IgE binding epitopes at the molecular level^{31,51,79}. The potency determination of various major allergens by in vivo/ in vitro methods will not be cumbersome since the panel of extracts required may become smaller due to knowledge of cross reactive proteins. A number of cross-reactivity studies have been done with recombinant purified plant allergens and few with mould allergens⁵¹. Among the recombinant mould allergens available, majority of them showed similarity with one or other enzymes^{1,36,47,49}. IgE cross-reactivity has been reported among 34 kD recombinant allergens from *A. oryzae*, *A. flavus*, *A. fumigatus* and *P. citrinum*^{1,36}. Quantitative information is required on cross-reactivity in the allergic population and with specific allergens rather than whole extracts. Such information is still scarce but with increasing availability of purified recombinant allergens, knowledge would accumulate in the time to come. The involvement of T cells/B cells/ mast cells in recognizing cross-reactive antigens and thereby initiating immune response is required to be studied. This information would be helpful in translating immunochemically defined cross-reactivity into clinically relevant information.

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