

Prevalence of IgE reactivities in mold-allergic subjects to commercially available fungal enzymes

W. Elliott Horner, Ph.D.,*§ Maricelis Armstrong, M.D.,*# Jane El-Dahr, M.D.,*#
Marjorie McCants, B.A.,* Gerald Reese, Ph.D.,* Aaron K. Kobernick, M.D.,*# and
Samuel B. Lehrer, Ph.D.*#

ABSTRACT

*Fungi are important aeroallergens. However, fungal allergen sources of consistent quality for clinical testing are not readily available. Because some allergens have been identified as enzymes, we assessed the prevalence of IgE reactivity to commercially available fungal enzymes. The purpose of this study was to determine IgE antibody reactivity by radioallergosorbent assay (RAST) to commercially available fungal enzymes in mold-allergic individuals. Sera from 20 subjects with symptoms of respiratory allergies and skin test reactivity to 2 or more fungal allergens (4 conidial [imperfecti] fungi and/or 8 basidiomycetes) were selected. Controls were six atopic individuals with neither history of fungal allergy nor skin test reactivity to fungi. Seventeen commercial fungal enzymes were used as antigens to evaluate the subjects' IgE antibody reactivity by RAST. Sera from most fungus-allergic individuals showed substantial IgE antibody reactivity to enzymes; control sera showed little or no reactivity. The mean reactivity to all commercial enzymes of all subjects tested was RAST $\geq 3\%$ with only one exception. The most reactive fungal enzymes were invertase (bakers' yeast, *Saccharomyces cerevisiae*), cellulase (*Trichoderma viride*), and glucosidase (brewers yeast, *S. cerevisiae*) with mean binding of 14.6, 9.5, and 8.8%, respectively. Using RAST results with a combination of four enzymes from *S. cerevisiae* (brewers yeast glucosidase, bakers' yeast maltase, invertase, and invertase V), a sensitivity of 100% was shown for detecting mold-allergic patients. The studies suggest that fungal enzymes may be useful source materials for the identification of fungal allergens and may also provide readily available source materials to produce improved diagnostic and therapeutic reagents.*

(Allergy Asthma Proc 29:629–635, 2008; doi: 10.2500/aap.2008.29.3174)

Key words: Allergens, allergy, enzyme sources, fungus, IgE antibody, mold

Fungi are a diverse group of organisms; some are infectious agents, some are facultative parasites, whereas others are strictly saprophytic.¹ They produce allergens² and toxins³ and can grow almost everywhere. Airborne spores are present in outdoor as well as indoor air throughout the year, and spore numbers outdoors frequently exceed pollen concentrations by 100- to 1000-fold, depending on environmental factors such as water and nutrient availability, temperature, and wind.^{2,4} Allergen databases identify 189 fungal species that may be allergenic, although the real number is probably much higher.⁵ In addition, >200 toxins have been identified from fungal growth on plant materials including those used for food and as indoor construction materials.

Allergic, IgE antibody-mediated type I reactivities to ingested fungi or fungal components have been de-

scribed.^{6–8} Furthermore, allergies to inhaled fungal components can also manifest either as rhinitis, asthma, or skin reactions. The prevalence of allergenic sensitization to fungi is not exactly known but has been estimated in some studies to be as high as 6% of the general population.^{2,4,9} Based on the analysis of Mudarri and Fisk, an estimated 21% of current asthma in the United States is attributable to dampness and indoor mold exposure.¹⁰ Diagnosis of allergies to fungi has historically been difficult, caused by, in part, the generally poor quality of commercial fungal extracts as well as significant cross-reactivity among different mold species that is not clearly delineated.¹¹

Recent advances in allergen preparation have increased the availability of specific fungal allergens, in particular, use of cDNA cloning and the production of recombinant allergens.^{12,13} These molecular techniques have isolated a number of allergenic fungal proteins. The isolation and serum testing of fungal allergens may be improved by new source material; this may aid in diagnosis and treatment of patients with fungal allergy. Because of current cost of preparation and narrow spectrum of availability, however, use of specific fungal allergens remains of limited clinical utility. Furthermore, significant cross-reactivity among fungal

From the *Section of Clinical Immunology, Allergy and Rheumatology, Department of Medicine, and #Section of Allergy and Rheumatology, Department of Pediatrics, Tulane University School of Medicine, New Orleans, Louisiana, and §Air Quality Sciences, Inc., Atlanta, Georgia

Address correspondence and reprint requests to Samuel B. Lehrer, Ph.D., Tulane University Medical Center, 1700 Perdido Street, SL-57, New Orleans, LA 70112

E-mail address: sblehrer@gmail.com

Copyright © 2008, OceanSide Publications, Inc., U.S.A.

genera has been established.¹¹ The relevance of this cross-reactivity has not been discerned but may be caused by similar posttranslational modifications among fungal genera.

As with any other allergic disease, diagnosis is based on the clinical symptoms of the patient, skin test reactivity, and/or the identification of allergen-specific IgE antibody reactivity by radioallergosorbent test (RAST).¹⁴ Skin test and RAST are most reliable with standardized allergen extracts, but such extracts are not available for many species of allergenically important fungi.

Fungal enzymes have long been used widely in the food, chemical, and pharmaceutical industries. Hence, a broad array of these enzymes is available in commercial quantities. Some fungal enzymes are known to be allergenic to exposed workers.^{6,8,15-17} If these commercial fungal enzymes were allergenic for other subjects, they would be a relatively inexpensive source of material for fungal allergen research as suggested^{18,19} and could substitute as source material for extracts used in *in vitro* diagnostic studies. Hence, we assessed the prevalence of IgE antibody reactivity to commercially available fungal enzymes using sera from mold-allergic subjects who had no known occupational exposure to fungal enzymes.

MATERIALS AND METHODS

Subjects' Sera

Sera were obtained from 20 subjects with symptoms of respiratory allergies and skin test reactive to 2 or more fungal allergens. These included commercial extracts of four conidial fungi: *Alternaria alternata*, *Aspergillus niger*, *Cladosporium herbarum*, and *Fusarium solani*, (Greer Laboratories, Lenoir, NC) and eight basidiomycete spore extracts²⁰ prepared from *Psilocybe cubensis*, *Pleurotus ostreatus*, *Calvatia cyathiformis*, *Coprinus commatus*, *Scleroderma geaster*, *Pisolithus tinctorius*, and *Ganoderma lucidum* at Tulane University Medical Center, New Orleans, LA, from spores (95% purity) collected locally. Sera from six atopic subjects with no reactivity to fungi by skin test or RAST were used as controls.

Fungal Enzymes

Seventeen fungal enzyme preparations were purchased from Sigma-Aldrich, St. Louis, MO, and Worthington Biochemical Corp., Freehold, NJ. These included an α -amylase (type X-A, *Aspergillus oryzae*), cellulase (*Trichoderma viride*), enolase (*Saccharomyces cerevisiae*, bakers' yeast) α -glucosidase (*S. cerevisiae*, brewers' yeast), invertase grade V (*S. cerevisiae*, bakers' yeast), maltase (*S. cerevisiae*, bakers' yeast), protease XIX (*A. sojae*), protease XIII (*A. saitoi*), protease II (*A. oryzae*), and proteinase K (*Tritirachium album*; Sigma). Purified maltase (*S. cerevisiae*, bakers' yeast), cellulase

(*Trichoderma reesei*), carboxypeptidase (*S. cerevisiae*, bakers' yeast), lipase (*Candida cylindracea*), pectinase (*A. niger*) and basidiomycete tyrosinases (Worthington Biochemical Corp.) were also included.

Radioallergosorbent Assay

The fungal extracts were coupled to paper discs (100 μ g/disc) by the method of Ceska.¹⁴ One hundred microliters of sera were tested in duplicate and incubated overnight with the antigen-coated disk on a rotator at room temperature. Discs were then washed 3 $\times$ with PBS to remove unbound antibody and then again incubated overnight with I¹²⁵-labeled anti-human IgE (15,000 cpm/disc; Kallestad Diagnostics, Chaska, MN). The discs were washed again and bound radioactivity was determined in a γ -counter. The average counts per minute were obtained for each set of sera and the percent of RAST binding was calculated.

RESULTS

The ability of the fungal enzyme extracts to bind IgE from fungal allergic as well as atopic individuals who are not allergic to molds is summarized in Figs. 1, 2, and 3. RAST reactivity of 3% binding is considered to be positive based on previous studies because this is consistently at least 2 SD greater than the mean reactivity of nonatopic individuals.²¹ Generally, sera from nonallergic subjects showed little or no RAST reactivity (usually <4% binding) to all fungal enzymes tested. The only major exception was control sera 1, which showed significant reactivity (>4%) to tyrosinases from mushrooms, cellulase from *T. reesei* and pectinase from *A. niger*. All other values of the fungal nonallergic sera were <3% binding. In contrast, significant IgE binding was shown by a number of the sera from fungus-allergic individuals. Although sera varied in their reactivity, percent binding was rather substantial such as shown for serum 2 to *Aspergillus* enzymes (Fig. 1), yeast enzymes (Fig. 2), and other fungal enzymes (Fig. 3). Significant reactivity was shown by a number of other sera. The most potent fungal allergens appeared to be invertase (bakers' yeast), two cellulases (*T. viride*, *T. reesei*), and glucosidase (brewers yeast) with mean RAST binding of 17.7, 9.5, 19.3, and 13.1%, respectively.

Of the 20 sera from fungal-allergic individuals tested, 16 showed significant ($\geq 3\%$ binding) RAST reactivity to at least two fungal extracts. However, reactivity to individual enzymes varied: of the 16 sera tested, 5 reacted to *T. album* proteinase, 7 reacted to *C. cylindracea* lipase, 9 reacted to *A. oryzae* protease II, 8 reacted to *A. niger* pectinase; 7 reacted to carboxypeptidase from bakers' yeast, 7 reacted to *T. viride* cellulase, 11 reacted to glucosidase from brewers yeast, 9 reacted to enolase

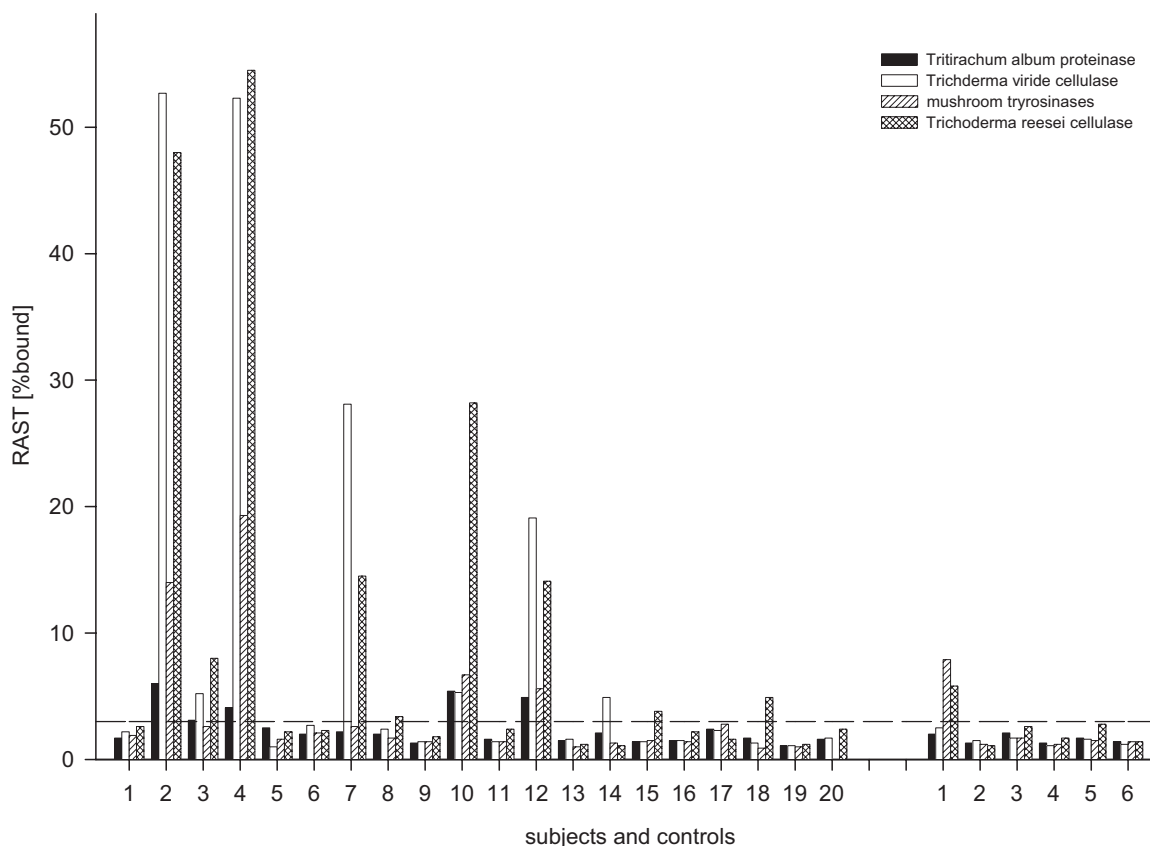


Figure 1. IgE reactivities to *Aspergillus* enzymes: *A. sojae* protease XIX, *A. oryzae* protease II, *A. oryzae* amylase, and *A. niger* pectinase.

from bakers' yeast, and all 16 reacted to invertase from bakers' yeast.

The individual IgE reactivities were compared by Friedman repeated measures analysis of variance of ranks for significant differences among all reactivities and were compared pair-wise by Tukey test. Among the IgE reactivities to *Aspergillus* enzymes, no significant differences were detected. Furthermore, IgE reactivities to *Aspergillus* enzymes and *T. album* proteinase, *T. viride* cellulase, *T. reesei* cellulase, and basidiomycete tyrosinases were statistically similar. The only IgE reactivities that were different were those to *S. cerevisiae* (bakers' yeast) invertase; IgE reactivities to this enzyme were significantly different from those to *A. sojae* protease XIX, *A. oryzae* protease II, *A. oryzae* amylase, *A. niger* pectinase, *S. cerevisiae* invertase V, *S. cerevisiae* carboxypeptidase, *S. cerevisiae* maltase, *S. cerevisiae* invertase VII, *C. cylindracea* lipase, *T. album* proteinase, *T. viride* cellulase, and *T. reesei* cellulase.

Maximal RAST reactivities to fungal enzymes were exceptionally high for sera from mold-allergic subjects; 31% binding for *A. sojae* protease II, 40.9% for *A. saitoi* protease XIII, 51% for invertase from bakers' yeast, and 52.7% for *T. reesei* cellulase. To determine the most potent fungal enzyme allergens, mean RAST percent bound for the allergic sera compared with the control

sera were determined (Table 1). By far, the cellulases of *T. viride*, *T. reesei*, and yeast enzyme invertase were the most potent allergen, with mean ($n = 20$) RAST of 23.94, 19.93, and 17.71%, respectively. However, the high SD indicates a large variability of individual allergic sera reactivity. The second most potent enzyme allergen was observed with two different cellulase preparations. All enzymes showed substantial SD indicating variability of reactivity, but, nonetheless, significant reactivity was detected to a number of enzymes.

Table 2 shows sensitivities and specificities of positive RAST to correctly identify mold-allergic subjects with each fungal enzyme tested. The different enzymes vary in their sensitivity between 20 and 80% whereas the specificity is at least 83.33% for all enzymes. The highest sensitivity (80%) is achieved with *S. cerevisiae* invertase and combined with a high specificity of 100%. The accuracy of a positive RAST reactivity (positive predictive value) range from 85 to 100%, indicating that an IgE binding of at least 3% to almost any fungal enzyme, is a good measure to show a sensitization to molds. Four enzymes from *S. cerevisiae* varieties (brewers yeast glucosidase, bakers' yeast maltase, invertase, and invertase V) have a combined sensitivity of at least 50% with high specificity of at least 80%.

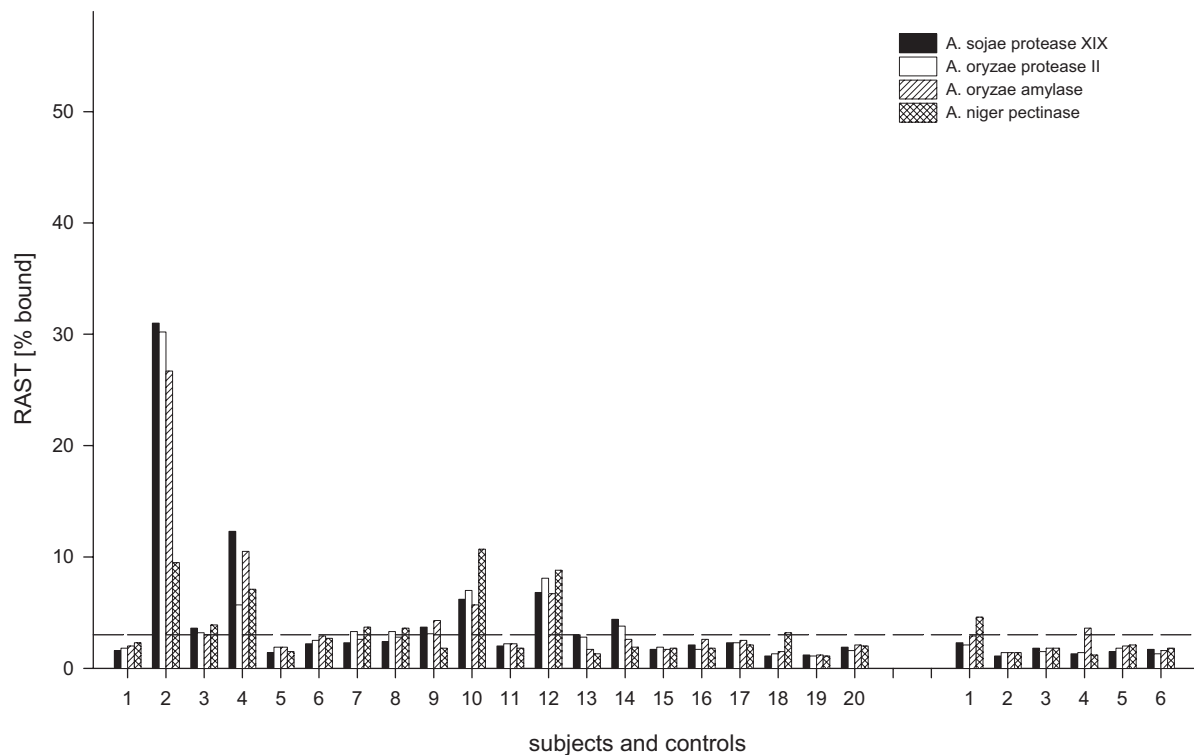


Figure 2. IgE reactivities to yeast enzymes: *S. cerevisiae* (brewers' yeast) glucosidase, *S. cerevisiae* (bakers' yeast) enolase, *S. cerevisiae* (bakers' yeast) maltase, *S. cerevisiae* (bakers' yeast) invertase, *S. cerevisiae* invertase V, *S. cerevisiae* carboxypeptidase, *S. cerevisiae* maltase, *S. cerevisiae* invertase VII, and *C. cylindracea* lipase.

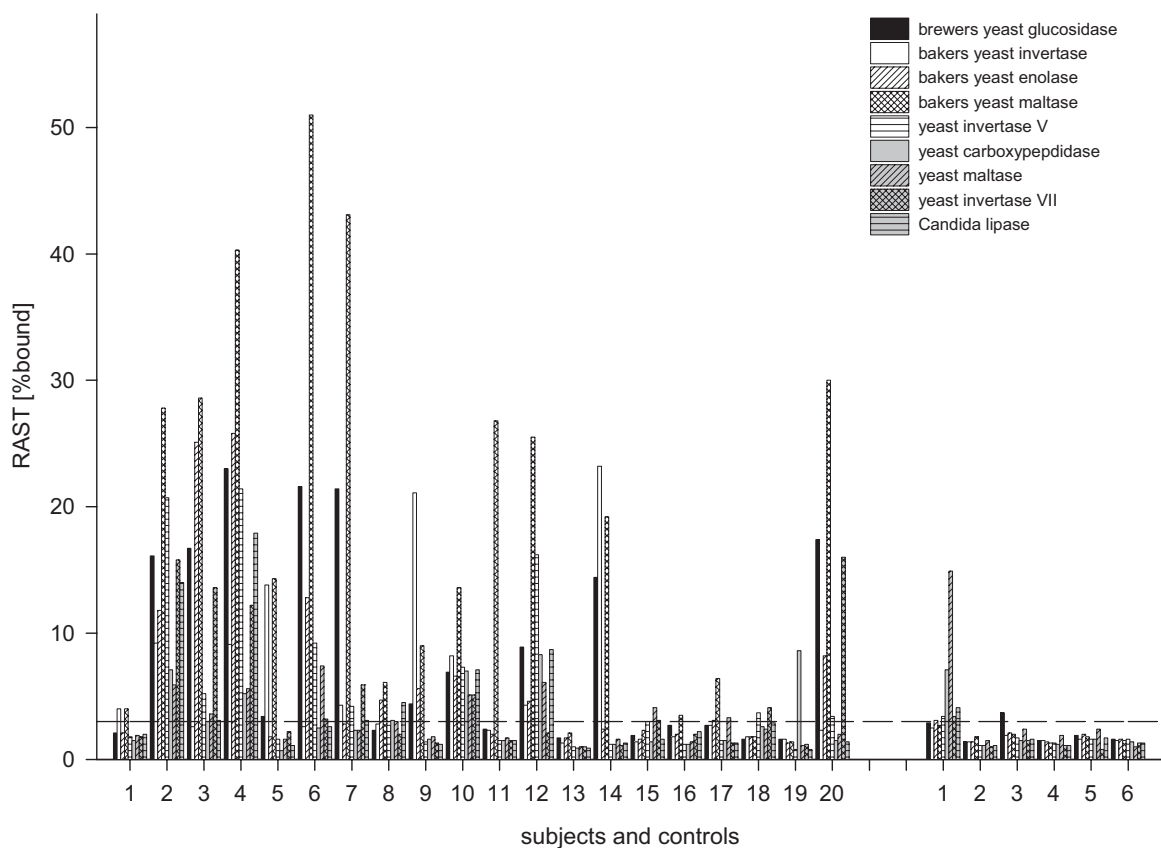


Figure 3. IgE reactivities to *T. album* proteinase, *T. viride* cellulase, *T. reesei* cellulase, and basidiomycete trypsinases.

Table 1 RAST reactivities of mold-allergic and control subjects to fungal enzymes

Enzyme	Mold-Allergic Subjects			Control Subjects		
	n/20	%B Mean	SDEV	n/6	%B Mean	SDEV
<i>A. sojae</i> (protease XIX)	8	8.88	9.43	0	—	—
<i>A. oryzae</i> (protease II)	9	7.52	8.7	0	—	—
<i>A. oryzae</i> (amylase)	6	9.48	8.81	1	3.6	—
<i>A. niger</i> (pectinase)	8	6.31	3.07	1	4.6	—
<i>S. cerevisiae</i> brewers yeast (glucosidase)	10	13.12	6.74	1	3.7	—
<i>S. cerevisiae</i> bakers' yeast (enolase)	9	10.8	7.17	0	—	—
<i>S. cerevisiae</i> bakers' yeast (maltase)	10	10.83	8.31	1	3.1	—
<i>S. cerevisiae</i> bakers' yeast (invertase)	16	17.71	14.46	0	—	—
<i>S. cerevisiae</i> (invertase V)	10	9.43	7.28	1	3.4	—
<i>S. cerevisiae</i> (carboxypeptidase)	7	6.04	2.32	1	7.1	—
<i>S. cerevisiae</i> (maltase)	9	4.9	1.49	1	14.9	—
<i>S. cerevisiae</i> (invertase VII)	9	8.78	5.52	1	3.4	—
<i>C. cylindracea</i> (lipase)	7	8.34	5.7	1	4.1	—
<i>T. album</i> (proteinase)	5	4.7	1.13	0	—	—
<i>T. viride</i> (cellulase)	7	23.94	21.34	0	—	—
<i>T. reesei</i> (cellulase)	9	19.93	19.42	1	5.8	—
basidiomycetes (tyrosinases)	4	11.4	6.45	1	7.9	—

RAST = radioallergosorbent assay; n = number of subjects RAST reactivity (% binding, %B) $\geq 3\%$; $B\%_{mean}$ = mean RAST reactivity; SDEV = standard deviation. *Aspergillus* species: *A. oryzae*, *A. niger*, *A. sojae*. Yeasts: *Saccharomyces cerevisiae* (bakers' yeast, brewers' yeast), *Candida cylindracea*; Others: *Tritirachium album*, *Trichoderma reesei*, basidiomycetes.

Table 2 Sensitivity, specificity, positive predictive value, and negative predictive value in percent of RAST reactivities to 17 different fungal enzymes

Enzyme	Sensitivity (%)	Specificity (%)	Positive Predictive Value (%)	Negative Predictive Value (%)
<i>A. sojae</i> (protease XIX)	40.0	100.0	100.0	33.3
<i>A. oryzae</i> (protease II)	45.0	100.0	100.0	35.3
<i>A. oryzae</i> (amylase)	30.0	83.3	85.7	26.3
<i>A. niger</i> (pectinase)	40.0	83.3	88.9	29.4
<i>S. cerevisiae</i> brewers yeast (glucosidase)	50.0	83.3	90.9	33.3
<i>S. cerevisiae</i> bakers' yeast (enolase)	45.0	100.0	100.0	35.3
<i>S. cerevisiae</i> bakers' yeast (maltase)	50.0	83.3	90.9	33.3
<i>S. cerevisiae</i> bakers' yeast (invertase)	80.0	100.0	100.0	60.0
<i>S. cerevisiae</i> (invertase V)	50.0	83.3	90.9	33.3
<i>S. cerevisiae</i> (carboxypeptidase)	35.0	83.3	87.5	27.8
<i>S. cerevisiae</i> (maltase)	45.0	83.3	90.0	31.3
<i>S. cerevisiae</i> (invertase VII)	45.0	83.3	90.0	31.3
<i>C. cylindracea</i> (lipase)	35.0	83.3	87.5	27.8
<i>T. album</i> (proteinase)	25.0	100.0	100.0	28.6
<i>T. viride</i> (cellulase)	35.0	100.0	100.0	31.6
<i>T. reesei</i> (cellulase)	45.0	83.3	90.0	31.3
basidiomycetes (tyrosinases)	20.0	83.3	80.0	23.8

RAST = radioallergosorbent assay.

Analyzing the results of these four enzymes gives an overall sensitivity of 100%, specificity of 50%, a positive predictive value of 86.96%, and a negative predictive value of 100%.

DISCUSSION

Although fungal allergens in the form of mycelia or spores are one of the most common inhaled particles that can cause respiratory allergies, fungal allergen extracts have not been as well studied as other aeroallergens because of a number of difficulties in characterizing them.^{2,7} Additionally, standardization of fungal allergen products has been inconsistent because of inherent manufacturing difficulties.¹⁴ Despite this and the fact that there are literally thousands of species of fungi that contribute potential allergens to the atmosphere, it is essential for us to identify and characterize fungal allergens not only to develop better diagnostic reagents but also to improve therapeutic regimens. Indeed, in occupational studies it has been shown that proteases can induce profound IgE sensitization in enzyme detergent workers.^{22,23} It is well known that enzymes from fungi can be potent occupational aeroallergens.^{8,16,24–26} Likewise, several very important allergens (*e.g.*, dust-mite major allergens Der p 1 and Der f 1) are enzymes.^{27–32} Thus, it was proposed that enzymes from fungi might be important sources of fungal allergens.

Our studies indicate that a number of fungal enzymes seem to have substantial reactivity to IgE antibodies in the sera from mold-allergic individuals. These fungal enzymes vary in terms of the magnitude of their reactivity and the number of sera to which they react. Clearly, not all mold-allergic individuals react to these preparations but a number of them do, indicating that these could be important sources of fungal allergens for *in vitro* diagnosis. Furthermore, it is notable that manufacture of these particular fungal products could be a potential occupational hazard.

When reactivities to our extracts are used in certain combinations, the sensitivity and specificity for detecting a fungus-allergic individual approaches 100% (Table 2). Testing with this combination of fungal preparations could be used clinically as an inexpensive screening tool for atopic patients. The use of fungal enzyme preparations may be an efficient way to screen allergic patients for reactivity to fungi. In coming decades, our arsenal of testing will improve in depth and breadth to incorporate specific fungal enzymes.³³ Until then, however, the expense of polymerase chain reaction cDNA cloning remains an inhibiting factor to routine enzyme-extract production. Furthermore, recombinant enzyme production produces an enzyme based on DNA sequence only; posttranslational modifications are not considered. Purified enzymes procured

using this technique, then, may not actually be concordant with the allergenic enzyme expressed by a particular fungus. Testing with our combination of fungal preparation is an important screening tool for detecting fungus allergy and represents a transition from current, more insensitive methods to the future of specific allergen production.

In conclusion, these studies certainly support the suggestion that commercial fungal enzymes are potentially important, readily available source materials for identification of fungal allergens, and indicate that further studies are warranted. Using commercially produced fungal enzyme extracts as source material for clinical allergen testing may be less expensive than current techniques, and therefore of manufacturing interest. Certainly, these sources should help improve diagnostic and therapeutic fungal reagents as well as provide immunogens for making monoclonal antibodies, preparing peptides, and providing treatment of mold-allergic subjects.

REFERENCES

1. Kendrick B. Eumycotan fungi—The mainstream. In *The Fifth Kingdom*. Waterloo, ON, Canada: Mycologue Publications, 1985. 27–30.
2. Horner WE, Helbling A, Salvaggio JE, et al. Fungal allergens. *Clin Microbiol Rev* 8:161–179, 1995.
3. Bennett JW. Mycotoxin research: 1989. *Mycopathologia* 107:65–66, 1989.
4. Bush RK. Fungal extracts in clinical practice. *Allergy Proc* 14: 385–390, 1993.
5. Bowyer P, Fraczek M, and Denning D. Comparative genomics of fungal allergens and epitopes shows widespread distribution of closely related allergen and epitope orthologues. *BMC Genomics* 7:251, 2006.
6. Baur X, and Czuppon AB. Allergic reaction after eating alpha-amylase (Asp o 2)-containing bread. A case report. *Allergy* 50: 85–87, 1995.
7. Lehrer SB, Aukrust L, and Salvaggio JE. Respiratory allergy induced by fungi. *Clin Chest Med* 4:23–41, 1983.
8. Sandiford CP, Tee RD, and Taylor AJ. The role of cereal and fungal amylases in cereal flour hypersensitivity. *Clin Exp Allergy* 24:549–557, 1994.
9. Kurup VP, Horng-Der S, and Vijay H. Immunobiology of fungal allergens. *Int Arch Allergy Immunol* 129:181–188, 2002.
10. Mudarri D, and Fisk WJ. Public health and economic impact of dampness and mold. *Indoor Air* 17:226–235, 2007.
11. Esch RE. Manufacturing and standardizing fungal allergen products. *J Allergy Clin Immunol* 113:210–215, 2004.
12. Simon-Nobbe B, Probst G, Kajava AV, et al. IgE-binding epitopes of enolases, a class of highly conserved fungal allergens. *J Allergy Clin Immunol* 106:887–895, 2000.
13. Schneider PB, Denk U, Richter K, et al. *Alternaria alternata* NADP-dependent mannitol dehydrogenase is an important fungal allergen. *Clin Exp Allergy* 36:1513–1524, 2006.
14. Ceska M, and Lundkvist U. A new and simple radioimmunoassay method for the determination of IgE. *Immunochemistry* 9:1021–1030, 1972.
15. Akiyama K, Shida T, Yasueda H, et al. Atopic asthma caused by *Candida albicans* acid protease: Case reports. *Allergy* 49:778–781, 1994.

16. Kanny G, and Moneret-Vautrin DA. alpha-Amylase contained in bread can induce food allergy. *J Allergy Clin Immunol* 95: 132–133, 1995.
17. Quirce S, Cuevas M, Diez-Gomez M, et al. Respiratory allergy to *Aspergillus*-derived enzymes in bakers' asthma. *J Allergy Clin Immunol* 90:970–978, 1992.
18. Breitenbach M, Simon B, Probst G, et al. Enolases are highly conserved fungal allergens. *Int Arch Allergy Immunol* 113:114–117, 1997.
19. Baldo BA. Allergenic crossreactivity of fungi with emphasis on yeasts: Strategies for further study. *Clin Exp Allergy* 25:488–492, 1995.
20. Liengswangwong V, Salvaggio JE, Lyon FL, et al. Basidiospore allergens: Determination of optimal extraction methods. *Clin Allergy* 17:191–198, 1987.
21. Butcher BT, O'Neil CE, Reed MA, et al. Basidiomycete allergy: Measurement of spore-specific IgE antibodies. *J Allergy Clin Immunol* 80:803–809, 1987.
22. Pepys J. Immunological and clinical findings in workers and consumers exposed to enzymes of *Bacillus subtilis*. *Proc R Soc Med* 66:930–932, 1973.
23. Pepys J, Wells ID, D'Souza MF, et al. Clinical and immunological responses to enzymes of *Bacillus subtilis* in factory workers and consumers. *Clin Allergy* 3:143–160, 1973.
24. Baur X, Chen Z, and Sander I. Isolation and denomination of an important allergen in baking additives: Alpha-amylase from *Aspergillus oryzae* (Asp o II). *Clin Exp Allergy* 24:465–470, 1994.
25. Cullinan P, Cook A, Jones M, et al. Clinical responses to ingested fungal alpha-amylase and hemicellulase in persons sensitized to *Aspergillus fumigatus*? *Allergy* 52:346–349, 1997.
26. Houba R, Heederik DJ, Doekes G, et al. Exposure-sensitization relationship for alpha-amylase allergens in the baking industry. *Am J Respir Crit Care Med* 154:130–136, 1996.
27. Hewitt CR, Foster S, Phillips C, et al. Mite allergens: Significance of enzymatic activity. *Allergy* 53:60–63, 1998.
28. Martinez J, Eraso E, Palacios R, et al. Enzymatic analyses of house dust mite extracts from *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* (Acari: Pyroglyphidae) during different phases of culture growth. *J Med Entomol* 36:370–375, 1999.
29. Deleuran M, Ellingsen AR, Paludan K, et al. Purified Der p and p 2 patch tests in patients with atopic dermatitis: Evidence for both allergenicity and proteolytic irritancy. *Acta Dermato-Venerol* 78:241–243, 1998.
30. Stewart GA, Bird CH, Krska KD, et al. A comparative study of allergenic and potentially allergenic enzymes from *Dermatophagoides pteronyssinus*, *D. farinae* and *Euroglyphus maynei*. *Exp Appl Acarol* 16:165–180, 1992.
31. Cambra O, and Berrens L. Monoclonal antibodies against *Dermatophagoides* group I allergens as pseudo-cystatins blocking the catalytic site of cysteine proteinases. *Immunol Lett* 50:173–177, 1996.
32. Robinson C, Kalsheker NA, Srinivasan N, et al. On the potential significance of the enzymatic activity of mite allergens to immunogenicity. Clues to structure and function revealed by molecular characterization. *Clin Exp Allergy* 27:10–21, 1997.
33. Kurup VP, and Banerjee B. Fungal allergens and peptide epitopes. *Peptides* 21:589–599, 2000. □

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.