

Occupational Sensitization to Fungal Enzymes Used in Animal Feed Industry

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Key Words

Allergy · Asthma · Basophil activation · Industrial enzyme · Flow cytometry · Immunoglobulin E · Immunoblotting · Occupational pathology · Rhinitis

Abstract

Background: Industrial enzymes cause the increasing prevalence of occupational hypersensitivity. Our objective was to study workers occupationally exposed to fungal enzymes in 2 animal feed factories to determine if the sensitization originated in the enzymes or was caused by the microorganism used to produce the enzymes. **Methods:** Eighty-six consenting workers were studied by skin prick tests with extracts from the enzymatic products handled in their factories. Positive workers were then studied by IgE immunoblotting and basophil activation was measured by flow cytometry. **Results:** Eight of the 86 workers analysed (9%) tested positive and were more frequently sensitized to phytase from *Trichoderma* and *Peniophora*. Glucanase and α -amylase from *Bacillus amyloliquefaciens* did not cause sensitization in any worker. No cross-reactions were observed between *Trichoderma* and *Peniophora* sp. phytases. Workers were sensitized to the product that they handled. **Conclusions:** Fungal enzymes cause occupational hypersensitivity in animal feed industries. Immunoblotting and basophil activation are useful to evaluate the effects of handling enzymes as part of the

medical surveillance of enzyme-exposed workers. We describe *Peniophora* sp. 6-phytase as a new allergen and enzymes from *Trichoderma* as strong sensitizers.

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Introduction

The widespread use of enzymes in industrial processes is increasing the prevalence of occupational hypersensitivity. Workers in pharmacy, bakery, paper, detergent, textile or animal feed industries frequently handle enzymes [1].

Fungal enzymes or those enzymes produced in molds, have been described as relevant allergens. A β -xylosidase produced in *Aspergillus niger* mold was demonstrated to be an occupational allergen (Asp n 14) in the baking industry [2, 3]. Xylanase and cellulase enzymes contained in baking additives caused baker's asthma [4]. The pectinase and glucanase enzymes used in fruit and salad processing were also described as asthma inductors [5]. Specific IgE to mixed fungal enzymes was demonstrated in 6% of bakery workers presenting with significant nasal symptoms [6].

The animal feed industry uses cellulase, β -glucanase, protease and α -amylase enzymes to facilitate the digestibility of cereals such as wheat, rye and barley and there-

Table 1. Data of the 8 workers with positive SPTs with the products handled in their factories

Factory	Worker	Age	Sex	Smoking	Time exposed, years	Position	Symptoms at work	Positive SPT to other allergens
A	1	26	M	No	3	Packing, filling silos, weigher	Asthma	No
	2	40	F	Yes	9	Administration office	Asthma, rhinoconjunctivitis	Dog dander
	3	40	M	No	1	Packing, filling silos, weigher	Asthma, rhinoconjunctivitis	No
	4	31	M	Yes	5	Packing, filling silos, weigher	Asthma, rhinitis	<i>Platanus</i>
	5	44	M	No	10	Packing, filling silos, weigher	Rhinitis	No
B	1	55	M	No	29	Production operator	Asthma	Grass pollen, <i>Olea</i>
	2	29	M	Yes	5	Production operator	Asthma	Grass pollen, dog and cat dander
	3	40	M	Yes	18	Production operator	Asthma	Grass, <i>Olea</i> and <i>Platanus</i>

by improve the nutrition of poultry and swine [7]. Phytase produced in *A. niger*, added to increase the bioavailability of phosphors of plant origin, was described as an occupational allergen in feed additives causing a high sensitization when handled in powdered form [8, 9]. Powdered phytase and β -glucanase have also been shown to cause occupational asthma [10]. Powdered enzymes evaluated by skin prick test (SPT) and radioallergosorbent test showed a 7% rate of sensitization in 4 factories in the animal feed industry [7].

Our objective was to study workers occupationally exposed to fungal enzymes in 2 animal feed factories to determine if the sensitization originated in the enzymes or was caused by the microorganism used to produce the enzymes.

Material and Methods

Subjects

A total of 86 consenting workers from 2 animal feed factories, 48 from factory A and 38 from factory B, were studied by SPTs with extracts from the enzymatic products handled in their factories. Workers testing positive (table 1) were then studied by IgE immunoblotting and basophil activation was measured by flow cytometry.

Source of Enzymes

A total of 8 enzymatic products were included in the study, 4 from each factory. Workers handled these products mixing them into the animal food. Three products used in factory A were produced in *Trichoderma reesei* in powdered form and the fourth in *Bacillus amyloliquefaciens* in granulated form. Products used in factory B were produced as follow: 1 product was produced in *Aspergillus aculeatus* in powdered form, 2 products in *Aspergillus oryzae* in liquid form and the fourth in *Trichoderma longibrachiatum* in liquid form. One of these products from *A. oryzae* was genetically modified and, although produced in *A. oryzae*, its active phytase was derived from a gene of the basidiomycete class of

fungus *Peniophora* sp. as the donor organism. Table 2 contains the activity and microbiological origin of all products studied.

Crude Extracts Preparation

Before use, an extraction process was performed with products in powdered form. These products were diluted in PBS (10% w/v) and shaken for 24 h at room temperature. Products in liquid form were diluted 1:20 in a buffer containing 50% glycerol and 0.9% NaCl. These mixtures were then centrifuged at 8,000 g for 30 min and the pellets discarded, the supernatants were filtered through a 0.22- μ m pore membrane producing the crude extracts.

Skin Prick Tests

SPTs were carried out in all workers with the crude extracts prepared as described. Commercial allergenic extracts used for screening SPTs were provided by ALK-Abelló (Madrid, Spain). The routine panel of aeroallergens used for screening included: *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Alternaria alternata*, cat and dog dander, *Parietaria judaica*, grass mix (*Dactylis*, *Festuca*, *Lolium*, *Phleum*, *Poa*), *Olea europaea*, *Plantago lanceolata*, *Platanus hybrida*, *Artemisia vulgaris* and cypress. These allergens are the most common causative agents of respiratory allergy in our geographic area. We also carried out SPTs to the molds *Penicillium notatum*, *Aspergillus fumigatus*, *Cladosporium herbarum* and *Mucor mucedo*. Histamine phosphate was used as positive control and saline solution as negative control. An SPT response was scored as positive if the largest wheal diameter was 3 mm or greater than that produced by the negative control.

Basophil Activation with the Crude Extracts

The basophil activation test was performed as previously described [11]. A sample of 100 μ l of heparinized whole blood was incubated for 15 min at 37°C with 20 μ l of stimulation buffer containing IL-3 (60 ng/ml; Pharmingen, BD Biosciences, Erembodegem, Belgium) and an enzyme extract (1:1,000 dilution of the crude extract used in the SPTs). Negative and positive controls were performed by incubating blood without allergen and 10 μ l of anti-IgE (10 μ g/ml; Pharmingen), respectively. Basophil activation was stopped adding 10 μ l EDTA (20 mM; Sigma-Aldrich, Madrid, Spain) at room temperature for 5 min.

Table 2. Activity and biological origin of all the products studied

Factory	Product	Enzymatic activity and form	Host organism (production organism)	Donor organism
A	1	Endo-1,3(4)- β -glucanase (powdered form)	<i>T. reesei</i>	
	2	α -Amylase Endo-1,3(4)- β -glucanase (granulated form)	<i>B. amyloliquefaciens</i>	
	3	Endo-1,3(4)- β -glucanase Endo-1,4- β -xylanase (powdered form)	<i>T. reesei</i>	
	4	3-phytase (powdered form)	<i>T. reesei</i>	
B	1	Endo-1,3(4)- β -glucanase (powdered form)	<i>A. aculeatus</i>	<i>Peniophora</i> sp.
	2	6-phytase (liquid form)	<i>A. oryzae</i>	
	3	Endo-1,4- β -xylanase (liquid form)	<i>A. oryzae</i>	
	4	Endo-1,4- β -glucanase Endo-1,3(4)- β -glucanase Endo-1,4- β -xylanase (liquid form)	<i>T. longibrachiatum</i>	

To analyze basophil activation, cells were stained with 20 μ l CD63(IgG1)-FITC/CD123(IgG1)-PE/HLA-DR(IgG2a)-PerCP reagent mixture (BD Biosciences) for 20 min in the dark on ice. Samples were then incubated with 2 ml FACS lysing solution (BD Biosciences) for 15 min at room temperature. After centrifugation (300 g, 5 min) and washing with PBS, cells were resuspended with 300 μ l 1% paraformaldehyde (Sigma) and acquired in a FACScan flow cytometer (BD Biosciences).

To analyze the percentage of allergen-activated cells, the basophil population was gated using side scatter and CD123 to eliminate most of the debris and select CD123+ cells. At least 500 CD123+ cells were analyzed. Then, gated cells were displayed in a CD123/HLA-DR dot plot to eliminate HLA-DR+ cells. Subsequently, CD63 expression was detected in the CD123+ HLA-DR-basophils. In the CD63/CD123 dot plots, the quadrants were set with the negative control of the test, and in order to identify activated basophils by high CD63 expression. The percentage of activated basophils was calculated by subtracting spontaneous CD63 expression (negative control) from the data obtained with enzyme extract stimulation.

SDS-PAGE and Specific IgE Immunoblotting

Crude extracts from the enzymatic products studied were highly concentrated when analyzed by SDS-PAGE. For this reason extracts were normalized by dilution in sample buffer without 2-mercaptoethanol (nonreducing conditions) in different proportions depending on the product to adjust the intensity of protein bands. The mixtures were then placed in individual wells of 12% acrylamide minigels and subjected to electrophoresis (150 V) in standard SDS-PAGE conditions. After electrophoresis, proteins were transferred by simple diffusion to 2 nitrocellulose sheets (Bio-Rad Trans-Blot, supported nitrocellulose membrane 0.45 μ m) for 18 h. Nitrocellulose sheets were individually incubated overnight (room temperature and continuous shaking) with 500 μ l of serum from each worker diluted in 10 ml of incubation buffer (10 mM Tris-saline pH 7.4, 1% Tween 20). The detection of IgE-binding proteins was performed with a 1:1,000 dilution of a monoclonal anti-IgE antiserum (Ingenasa, Madrid,

Spain) in incubation buffer (10 mM-Tris saline pH 7.4, 1% Tween 20, 5% fetal bovine serum) and a 1:2,500 dilution of an alkaline phosphatase-labeled goat anti-mouse antiserum (Biosource International, Camarillo, Calif., USA). Finally, the membranes were washed, and the signal was visualized by an alkaline phosphatase 5-bromo-4-chloro-3-indolyl phosphate/4-nitroblue tetrazolium system (Amresco, Solon, Ohio, USA) for 30 min as described [12].

Immunoblot Inhibition Assay

To investigate the existence of cross-reactivities among the enzymatic products, an immunoblot inhibition assay was performed with sera from 2 workers from factory A who recognized 2 products from factory B. Ten microliters of each enzymatic product was added separately to 200 μ l of the serum from each worker (A2 and A4), and incubated for 2 h before overnight incubation with the nitrocellulose. The immunoblotting procedure was followed as previously described.

Oxidation of Sugar Residues

To study if carbohydrate groups were involved in the allergenicity and cross-reactivity, possible carbohydrate structures from the antigens studied were treated with periodate as described by Vieths et al. [13], following the protocol according to Moneo et al. [14]. A new SDS-PAGE in 12% acrylamide minigel was performed with those extracts which were recognized by workers from each factory. After transference to 2 nitrocellulose sheets, a periodate oxidation of the transferred proteins was performed and the treated and untreated membranes were then developed with the sera by IgE immunoblotting.

Statistical Analysis

Statistical analysis was performed with the SPSS 10.0 software (SPSS Inc., Chicago, Ill., USA). Continuous variables were not normally distributed according to the Shapiro-Wilk test and were described by the median and range. Bivariate correlations were evaluated with Spearman's rho. $p < 0.05$ was considered statistically significant.

Table 3. Results corresponding to the 8 workers with positive SPTs to some of the enzymatic products handled

Factory	Worker	Endo-1,3(4)- β -glucanase <i>T. reesei</i>			α -Amylase Endo-1,3(4)- β -glucanase <i>B. amyloliquefaciens</i>			Endo-1,3(4)- β -glucanase Endo-1,4- β -xylanase <i>T. reesei</i>			3-phytase <i>T. reesei</i>		
		SPT	ACT	BLT	SPT	ACT	BLT	SPT	ACT	BLT	SPT	ACT	BLT
A	1	8 $\times$ 5	74	–	–	–	–	9 $\times$ 6	74	+	–	–	+
A	2	15 $\times$ 10	44	++	8 $\times$ 6	–	–	21 $\times$ 15	43	++	28 $\times$ 14	42	++
A	3	20 $\times$ 8	42	+	–	–	–	10 $\times$ 15	49	+w	6 $\times$ 3	48	+w
A	4	12 $\times$ 9	25	++	–	–	–	10 $\times$ 10	34	++	13 $\times$ 9	37	++
A	5	–	–	–	–	–	–	–	–	–	8 $\times$ 5	–	+
B	1			–			–			–			–
B	2			–			–			–			–
B	3												

Values for SPTs are presented as wheal diameters expressed in millimeters. Values for basophile activation are percentages. ACT = Basophil activation; BLT = immunoblotting; – = negative result; + = positive; ++ = strong positive; +w = weakly positive; ND = not determined.

Results

Skin Prick Tests

Eight of the 86 workers studied (9%) showed positive SPTs with extracts from the enzymatic products handled in their factories and were then studied by IgE immunoblotting and basophil activation. Table 3 contains the results corresponding to the 3 tests. No workers with a positive SPT to an industrial fungal extract but without either asthma or rhinitis was found in our study at the concentrations used. All workers studied tested negative to the molds *P. notatum*, *A. fumigatus*, *C. herbarum* and *M.ucedo*.

Basophil Activation

Basophil activation tests were first performed with samples from individuals with no history of allergy to evaluate the existence of nonspecific basophil activation induced by the enzyme extracts. Results obtained with the different enzyme extracts were below 5% of activated basophils. Furthermore, basophil activation tests were performed in 5 exposed, asymptomatic workers from factory A with the enzymes to which they were exposed. They showed negative SPT and <7% of activated basophils with the enzyme extracts. Workers with at least 1 positive SPT to an occupational fungal extract were assayed for basophil activation with all enzymes to which they were exposed. The percentage of basophils activated in negative and positive controls of the assay was 2.0% (0.0–14.0) and 55.5% (31.0–78.0), respectively. Basophil

activation in workers yielded 46.0% (1.1–92.0) of basophils activated on challenge with the enzyme extract involved in their sensitization and 2.4% (0.0–4.7) with the other enzymes to which they were exposed but not sensitized. Two workers with positive SPTs were negative for the basophil activation test on challenge with α -amylase, endo-1,3(4)- β -glucanase from *B. amyloliquefaciens* (worker A2) and 3-phytase from *T. reesei* (worker A5). Correlation analysis showed no association between the percentage of basophils activated and the size of the wheal or worker age. However, a significant correlation was found between flow-cytometric data and the time of exposure to the industrial enzymes ($r = 0.5$, $p = 0.02$). Furthermore, the proportion of overall agreement between SPT and basophil activation assay was 95% and a κ coefficient of 0.91 was obtained.

SDS-PAGE and Specific IgE Immunoblotting

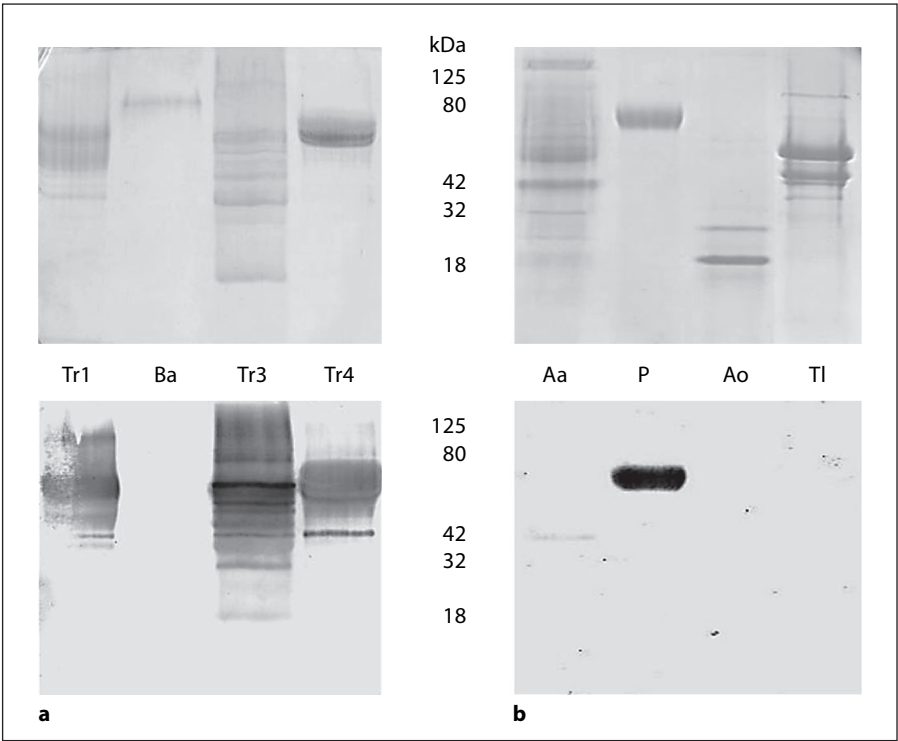
The first SDS-PAGEs performed with the extracts prepared with the products from each factory showed that some of them were poorly purified and several protein bands were visualized (fig. 1).

When specific IgE immunoblotting was performed individually for each worker with the products handled, overall agreement with the previous results obtained by SPTs was 84% (κ coefficient = 0.66), and the following findings were observed.

In factory A, phytase derived from *T. reesei* (product 4) was the enzyme most frequently recognized among workers (5/7), while nobody had specific IgE to the

Endo-1,3(4)- β -glucanase <i>A. aculeatus</i>			6-phytase <i>Peniophora</i> sp.			Endo-1,4- β -xylanase <i>A. oryzae</i>			Endo-1,4- β -glucanase Endo-1,3(4)- β -glucanase Endo-1,4- β -xylanase <i>T. longibrachiatum</i>		
SPT	ACT	BLT	SPT	ACT	BLT	SPT	ACT	BLT	SPT	ACT	BLT
		+			-			-			++
		+			-			-			++
9 $\times$ 9	85	+	11 $\times$ 9	85	++	-	ND	-	15 $\times$ 9	87	+w
6 $\times$ 7	ND	+w	14 $\times$ 9	87	++	15 $\times$ 7	61	-	17 $\times$ 17	23	-
8 $\times$ 6	81	+	9 $\times$ 6	87	+	-	ND	-	-	ND	-

Fig. 1. SDS-PAGE (top) and IgE immunoblotting (bottom) performed with extracts from the enzymatic products used by workers in factory A (**a**) and factory B (**b**). Tr1 = Glucanase (*T. reesei*); Ba = amylase + glucanase (*B. amyloliquefaciens*); Tr3 = glucanase + xylanase (*T. reesei*); Tr4 = phytase (*T. reesei*); Aa = glucanase (*A. aculeatus*); P = phytase (*Peniophora* sp.); Ao = xylanase (*A. oryzae*); Tl = glucanase + xylanase (*T. longibrachiatum*).



mixture amylase+glucanase from *B. amyloliquefaciens* (product 2). Two workers showed intense recognition of the 3 products (1, 3 and 4) derived from *T. reesei*. The molecular weights of the IgE-binding components of the products ranged from 18 to 125 kDa. Figure 1a shows SDS-PAGE with enzymatic products used by workers

from factory A and an IgE immunoblot carried out with the serum from 1 of the 2 workers (A4), with more intensive recognition of the 3 products derived from *T. reesei*.

In factory B, the 3 workers studied showed weak specific IgE to a glucanase from *A. aculeatus* (product 1) and stronger recognition of phytase from *Peniophora* sp.

Fig. 2. Specific IgE detection of all the extracts by 2 workers from each factory. Immunoblots of workers from factory A (**a**) and factory B (**b**). Tr1 = Glucanase (*T. reesei*); Ba = amylase + glucanase (*B. amylo-liquefaciens*); Tr3 = glucanase + xylanase (*T. reesei*); Tr4 = phytase (*T. reesei*); Aa = glucanase (*A. aculeatus*); P = phytase (*Peniophora* sp.); Ao = xylanase (*A. oryzae*); Tl = glucanase + xylanase (*T. longibrachiatum*).

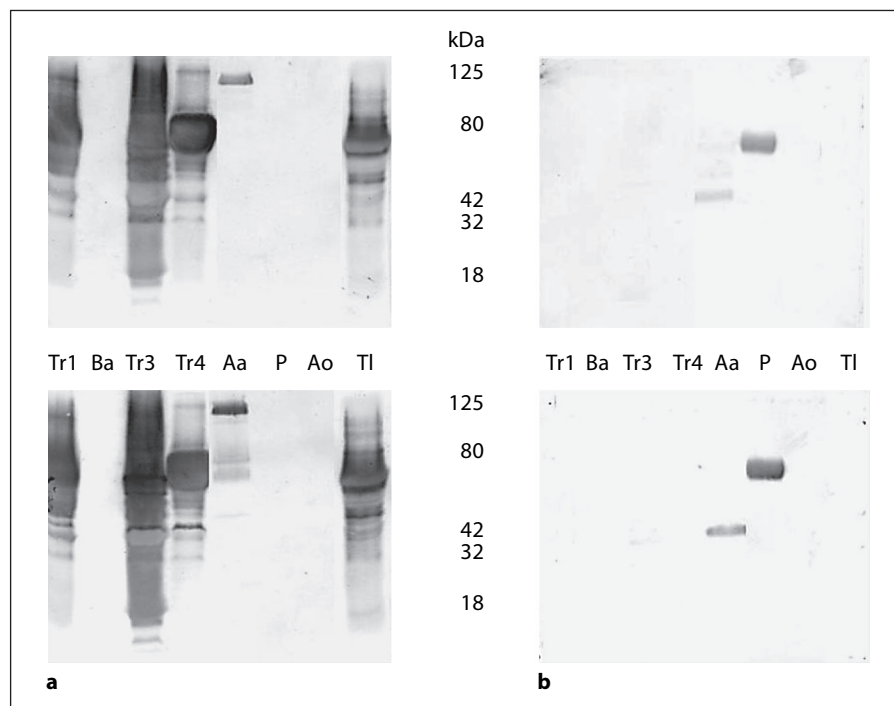


Fig. 3. Immunoblotting inhibition assay performed with the serum from 1 of the workers from factory A who recognized 2 products from factory B. Tr1 = Glucanase (*T. reesei*); Ba = amylase + glucanase (*B. amylo-liquefaciens*); Tr3 = glucanase + xylanase (*T. reesei*); Tr4 = phytase (*T. reesei*); Aa = glucanase (*A. aculeatus*); Tl = glucanase + xylanase (*T. longibrachiatum*). Inhibitions were performed by preincubation with Tr1, Ba, Tr3 and Tr4 products.

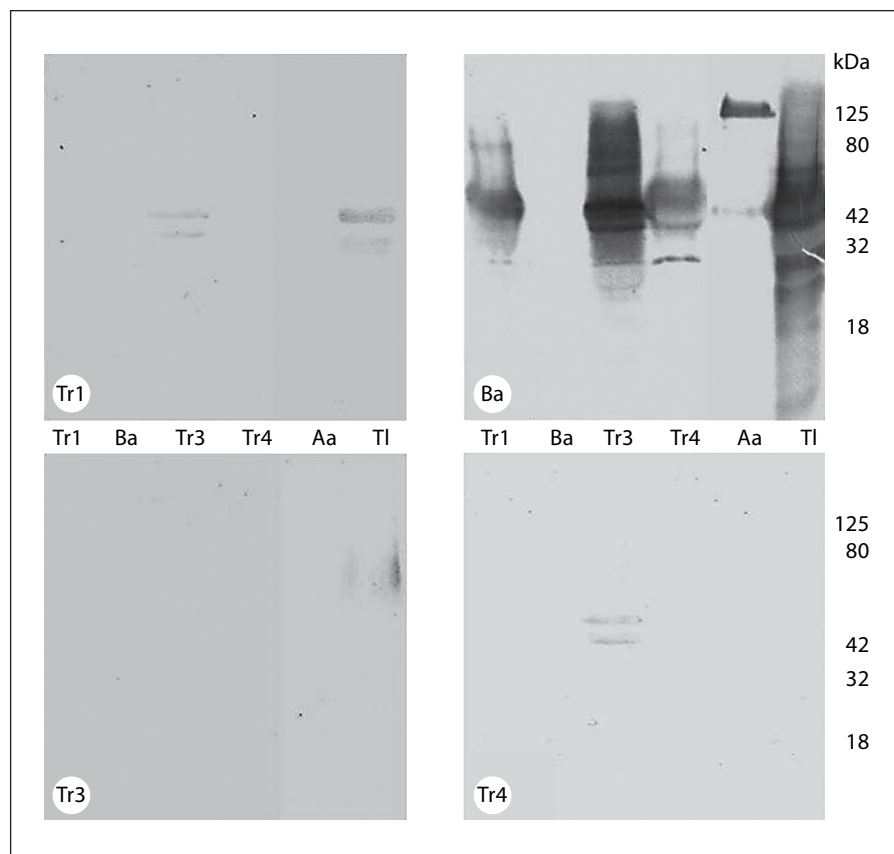
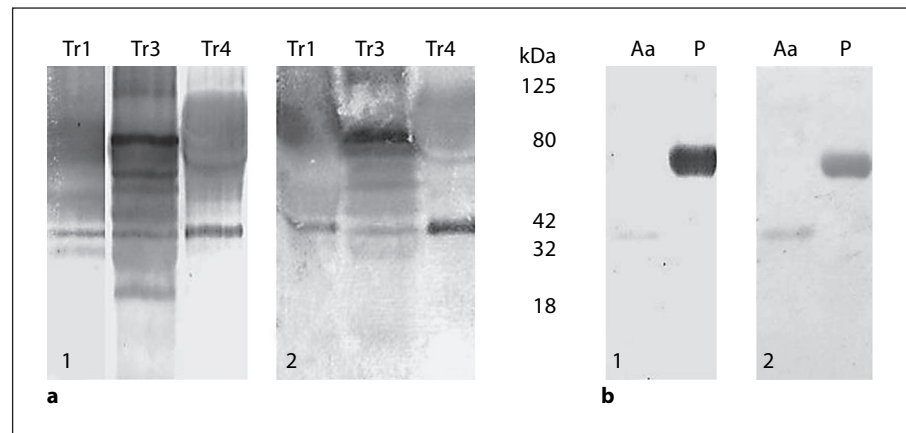


Fig. 4. Oxidation of sugar residues of transferred proteins from enzymatic products. **a** Immunoblots of a worker from factory A with untreated (1) and after periodate treatment (2) of the follow products. Tr1 = Glucanase (*T. reesei*); Tr3 = glucanase + xylanase (*T. reesei*); Tr4 = phytase (*T. reesei*). **b** Immunoblots of a worker from factory B with untreated (1) and after periodate treatment (2) of the follow products. Aa = Glucanase (*A. aculeatus*); P = phytase (*Peniophora* sp.).



(product 2). Figure 1b shows SDS-PAGE with products from factory B and an IgE immunoblot carried out with the serum from 1 of these workers (B1).

Specific IgE Immunoblotting with the Extracts from Both Factories

Two workers from each factory (A2, A4, B1 and B2) were analyzed by IgE immunoblotting to study the recognition of allergenic proteins contained in all extracts from both factories.

As demonstrated in figure 2, workers from factory A showed intense specific IgE detection of proteins contained in products derived from molds belonging to the *Trichoderma* (*reesei* and *longibrachiatum*), even though they were not exposed to the product derived from *T. longibrachiatum* used in factory B. Also, they showed recognition of the nonexposed product from *A. aculeatus* used in factory B. Workers from factory B did not recognize any product from factory A, but only detected a protein contained in the product derived from *A. aculeatus* and another in a product derived from *Peniophora* sp. Nobody showed recognition of the extract from *B. amyloliquefaciens*, including the exposed workers from factory A.

Immunoblot Inhibition Assay

Cross-reactions among enzymatic products from the 2 species belonging to *Trichoderma* and between products from *Trichoderma* with *A. aculeatus* were demonstrated. Figure 3 shows the result corresponding with worker A2, the same used in the immunoblot of fig. 2a (top).

Oxidation of Sugar Residues

The involvement of carbohydrate groups in the allergenicity of the enzymatic products was investigated to

distinguish specific IgE directed to cross-reactive carbohydrate determinants from other periodate-resistant epitopes. After periodate treatment, membranes were developed with sera from the same workers of each factory (A4 and B1) used for IgE immunoblotting shown in figure 1. In our study, periodate treatment did not result in a loss of allergenicity of the products studied (fig. 4).

Discussion

We studied workers occupationally exposed to fungal enzymes in 2 animal feed factories to determine if the sensitization originated in the enzymes or caused by the microorganism used to produce the enzymes. Previously, we described α -amylase hypersensitivity in nonexposed millers [15]. Although there was no occupational contact with the enzyme, workers were sensitized to *A. oryzae* α -amylase caused by a sensitization to *Aspergillus* and other molds contaminating cereal grains or flour. A literature review also showed that a sensitization to *A. niger* phytase may occur as a consequence of exposure to *A. niger* [8]. In a study carried out in a factory producing animal feed additives, the authors observed that the phytase used did not cross-react with common mold extracts, except for those extracts from *A. niger*.

Analyzing the sensitization to the enzymes studied in our work, no cross-reactions between *T. reesei* and *Peniophora* sp. phytases were observed in the 4 workers selected to be studied by IgE immunoblotting. Workers were only sensitized to the product that they handled.

We have demonstrated the allergenicity of a *Peniophora* sp. 6-phytase for the first time. A phytase with a molecular weight of 71 kDa was recently described as a gene

product of *Peniophora lycii* expressed in industrial strains of *A. oryzae* [16]. This 71-kDa protein, as its molecular weight suggests, could correspond with the 6-phytase recognized by the workers from factory B.

The specific allergen responsible for the sensitization to the glucanases studied was not determined because these products were not purified preparations, as shown by SDS-PAGE (products A1 and B1) or because the glucanases were mixed with xylanases (products A3 and B4). However, cross-reactions among products from the 2 species belonging to the *Trichoderma* and between products from the *Trichoderma* and *A. aculeatus* were observed. This fact could explain why workers from factory A not exposed to *Aspergillus* recognized proteins from a product used in factory B, a glucanase from *A. aculeatus* (product B1).

In relation to the xylanases studied, the 2 products containing xylanases which workers recognized (A3 and B4) were also mixtures containing glucanases. Therefore, a comparison between the *Trichoderma* and *Aspergillus* xylanases was not possible. However, the inhibition assay suggests that the weak bands remained when the inhibition was performed with the glucanase-xylanase mixtures (fig. 3, Tr1 and Tr4). This could correspond with the xylanase contained in these products because these bands disappeared when the inhibition was performed with the product containing only a xylanase (fig. 3, Tr3).

Although cases of occupational asthma caused by bacillary amylase were described in the detergent industry [17], in our study, glucanase and α -amylase from *B. amyloliquefaciens* did not cause sensitization in any worker, not even in the 5 exposed workers from factory A.

We evaluated occupationally exposed workers by SPTs, basophil activation and IgE immunoblotting, test-

ing in vivo, ex vivo and in vitro. An overall agreement between SPT and basophil activation of 95% was found. The overall agreement between SPT and IgE-immunoblotting was lower (84%) and could be attributed to the existence of conformational epitopes. This finding suggests that basophil activation measured by flow cytometry could be included as a tool to evaluate the effects of handling the enzymes in the medical surveillance of enzyme-exposed workers.

A review article found that *T. reesei* was considered a safe production organism and also a safe host for other harmless gene products [18]. A second article concluded that a β -glucanase preparation obtained from *T. reesei* was safe for use in animal feed, but some occupational health precautions should be taken to avoid skin contact and inhalation [19]. We found that powdered *T. reesei* products caused strong sensitization. One worker from factory A working in the office, presented frequently with asthma and rhinoconjunctivitis symptoms and was demonstrated to have strongly positive IgE immunoblots for all extracts from *Trichoderma*, even for the product derived from *T. longibrachiatum* not used in her factory. Therefore, allergen concentrations in surface dust should be measured in addition to the usual airborne dust sampling, both in production and nonproduction areas [20]. Occupational exposure controls should be performed to reduce the incidence of work-related sensitizations.

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