

CASE STUDY

Baker's asthma due to the enzyme xylanase — a new occupational allergen

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Introduction

Xylanases (EC 3.2.1.8) hydrolyse hemicelluloses which belong to non-starch carbohydrates. The latter are cross-linking major structural components of plant cell walls. Fungi and bacteria produce a wide spectrum of xylanases pertaining to two major families of glycosyl hydrolases (families F or 10 and G or 11) which use ion pair catalytic mechanisms and retain their anomeric configuration following hydrolysis. Family 10 xylanases are larger, more complex and produce smaller oligosaccharides; family 11 xylanases are more specific to xylan [1].

Xylanases are industrially used for pulp bleaching, coffee, plant oil and starch extraction and for the degradation of high molecular xylans in baking flour or animal food to make dough rise faster, to improve the quality of bread and to increase the feed conversion efficiency. Most frequently applied xylanases are those from *Aspergillus* spp. and *Trichoderma* spp. Genetically engineered xylanases are of special industrial interest since they have the requested properties thermostability or certain pH optima.

Case report

A 26-year-old non-smoking baker consulted our outpatient department. Since childhood, he has suffered from hay fever annually between March and July. From 1987 to 1991, he worked in a small bakery and afterwards started working in a large bread factory where ≈ 35 colleagues were engaged. For three years he has noticed, with increasing intensity, rhinoconjunctivitis, cough and shortness of breath in the workplace. The symptoms have been regularly provoked by intensive exposure to flour dust, especially during dough

preparation and bread dough flouring. The use of filter masks has significantly reduced, but not totally prevented, symptoms. Different types of rye and wheat flour as well as baking additives have been used in this factory.

Clinical investigations

Both lungs produced inspiratory rouchi. Lung function testing showed a typical obstructive ventilation pattern with FEV₁%VC of 54.6% and increased specific airway resistance (sR_i) of 1.7 kPa $\times$ s (normal range <1.2). P_aO₂ was reduced to 68 mmHg (normal range >80) at rest and normalized during exercise of 50 Watt.

Skin prick testing produced strong positive results with xylanase and cellulase of *Aspergillus niger*, α -amylase of *Aspergillus oryzae*, grass pollen and a weak response to wheat flour (Table 1).

IgE antibody analysis

IgE antibodies to xylanase and cellulase of *Aspergillus niger*, α -amylase of *Aspergillus oryzae*, wheat flour, rye flour, grass pollen and tree pollen were detectable (Table 2).

Nasal challenge test with rye flour extract

A nebulizer was used to apply 0.2 mL of the extract (Bencard, Munich, Germany) [2]. No clinical symptoms occurred, and the nasal resistance measured by rhinomanometry (Rhinoscreen, Jaeger, Wuerzburg) did not change.

Inhalative challenge test by xylanase of *Aspergillus niger*

The challenge test was performed with aerosolized xylanase dissolved in saline [2]. A significant asthmatic reaction after application of $\approx 0.5 \mu\text{g}$ (10 breaths of a xylanase

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Table 1. Results of skin prick testing

Allergen	Manufacturer	Wheal/Flare (diameter, mm)
Xylanase (<i>Asp. niger</i>), 0.1 mg/mL	BASF	10/40
Cellulase (<i>Asp. niger</i>), 1 mg/mL	Sigma	7/30
α -amylase (<i>Asp. oryzae</i>), 1 mg/mL	Novo Nordisk	7/35
Wheat flour	Bencard	3/10
Rye flour	Bencard	2/0
Grass pollen	ALK	9/35
Tree pollen	HAL	3/9
Histamine 10 mg/mL		4/25
Saline—0.4% phenol (diluent)		0/0

concentration of 1 μ g/mL) of the enzyme occurred; the increase of specific airway resistance measured by bodyplethysmography was more than 100% (Fig. 1). No symptoms indicated a late asthmatic response.

The same challenge test performed in two non-exposed asthmatic controls did not produce changes in their lung function parameters.

Discussion

Only two cases of immediate-type sensitization to xylanases have hitherto been described [3]. Two further subjects of the same study sensitized to cellulase of *Aspergillus niger* showed cross-reactivity to xylanase. These patients were laboratory workers engaged in companies where the produced enzymes were analysed. Our patient is the first one shown to develop respiratory sensitization after the application of such an enzyme in the baking industry. We were informed by the manufacturers of regularly used baking additives that these contain natural and probably genetically engineered xylanases. In our study xylanase of *Aspergillus*

niger was used for allergological and bronchial challenge testing. An initial parallel screening of symptomatic bakers showed that $\approx 11\%$ of them have IgE antibodies to this enzyme product [4].

We looked at cross reactivity and found no significant relationship with α -amylase but $\approx 80\%$ vice versa antibody inhibition with the cellulase product of the same mould species. We were informed that the latter enzyme has never been used in the bakery.

Prevalence and incidence of corresponding allergic diseases caused by xylanases are still unknown since they are not included in routine diagnostic testing. Information regarding whether these enzymes are sporadically or already routinely used in certain industrial processes or as food additives is not yet available. Recently, Vanhanen *et al.* [5] quantified xylanases in total dust samples of bakeries and found up to 40 ng/m³. However, the source of the enzyme remained unclear.

Since enzymes behave as potent inhalative allergens [6–11], we strongly recommend application of them as the granulated product. As already shown for detergent

Table 2. Results of IgE antibody analysis.

Method	Allergen	Manufacturer	IgE concentration (kU/L)
EAST ¹	Xylanase (<i>Asp. niger</i>)	BASF	19.20
EAST ¹	Cellulase (<i>Asp. niger</i>)	Sigma	9.53
EAST ¹	α -amylase (<i>Asp. oryzae</i>)	Novo Nordisk	1.67
CAP ²	Wheat flour	Pharmacia	0.92
CAP ²	Rye flour	Pharmacia	1.92
CAP ²	Grass pollen	Pharmacia	41.10
CAP ²	Tree pollen	Pharmacia	3.34

¹Enzyme allergosorbent test; own allergen solid phase preparation [4]. ²CAP system, Pharmacia Uppsala, Sweden; performed according to the manufacturer's instructions.

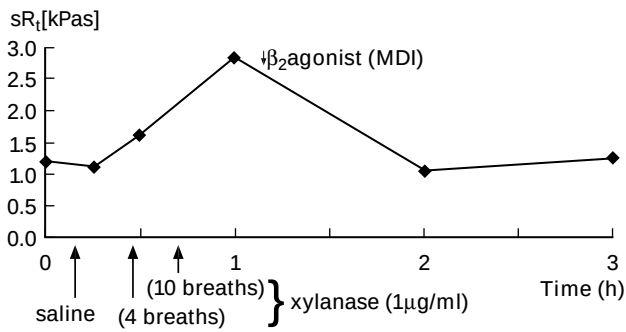


Fig. 1. Inhalative challenge test by xylanase (BASF).

enzymes, granulation can reduce or even eliminate the airborne load and the risk of respiratory sensitization.

Summary

The asthmatic baker showed IgE-mediated sensitization to xylanase of *Aspergillus niger* used as a baking additive. Inhalative challenge with $\approx 0.5 \mu\text{g}$ of the enzyme resulted in an immediate-type asthmatic reaction. This case, as well as a preliminary screening of symptomatic bakers, shows that xylanase is a further relevant type I-sensitizer in the baking industry.

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