

Allergenic properties of a feed protein of microfungal origin

K. KAUPPINEN, OUTI KUULIALA* and F. BJÖRKSTÉN

Hospital for Allergic Diseases, Helsinki University Central Hospital, and *Central Public Health Laboratory, Helsinki, Finland

(Received 15 January 1982; accepted for publication 8 February 1982)

Summary

We studied thirty-five process and laboratory workers occupationally exposed to *Paecilomyces varioti* protein. The protein, used as an animal feed, is prepared industrially by fermentation in pulp waste liquor. Skin tests and radioallergosorbent tests (RAST) to detect specific IgE antibodies showed positive results to *Paecilomyces* antigen among the workers and among the atopic control persons not occupationally exposed to *Paecilomyces* protein. Also IgG antibodies to *Paecilomyces* protein could be detected in workers and in controls in similar amounts in both groups. In no worker could clinical symptoms due to *Paecilomyces* protein allergy be found. We conclude that positive skin tests and RASTs indicate that immediate hypersensitivity to *Paecilomyces* antigens occurs. Clinical allergy probably has been prevented by the closed manufacturing process, which keeps occupational exposure at a low level.

Introduction

For many years yeasts and other microfungi have been commercially cultivated in carbohydrate-rich pulp mill waste liquors. Usually the purpose has been to produce high protein products for use as animal feeds. Only a few occupational allergy problems due to proteins derived from microfungi has been reported.

In Finland a protein production unit, in which the yeast, *Candida utilis*, is cultivated, has recently been found to cause major allergy problems both to workers and to people in the vicinity of the factory. The plant started in 1974, and in the following year allergic rhinitis and asthma appeared in some employees. Later, other persons living in the vicinity were affected. The manufacturing process was open, resulting in much airborne dust dispersed indoors in the plant, and dust was released through chimneys into the neighbourhood. This dust was shown to be a potent allergen (Nordman *et al.*, 1981). Improvements in the process subsequently decreased the allergy problems.

Correspondence: Dr K. Kauppinen, Hospital for Allergic Diseases, Helsinki University Central Hospital, SF-00250 Helsinki 25, Finland

The intention of the present study was to investigate the risk of allergy occurring in persons working on a related process, in which a mould, *Paecilomyces varioti*, is used in the fermentation. At the time of the study the protein plant had been operated by the United Paper Mills Ltd in Jämsänkoski, Finland, for 5 years. Due to the closed nature of the process only small amounts of dust escape. The workers still come into contact with *Paecilomyces* protein, as some dust will escape from the complete product and from plant equipment during service work. The particle size of *Paecilomyces* protein dust varies greatly. On an average the particles are rather large, about 100 μm .

Patients and methods

Workers exposed to Paecilomyces protein

All the twenty-seven employees working at the protein plant, most of them in the process area, were examined by the factory physician. Twenty-two of them had been working in the plant for 5 years. We also studied eight laboratory workers who had been employed in the development of the process and irregularly exposed. All had been in contact with *Paecilomyces* protein.

Control groups

Skin tests with *Paecilomyces* protein were made on 115 atopic patients from the Hospital for Allergic Diseases, and IgE antibodies specific to *Paecilomyces* protein were determined in sera from ten atopic patients of the same hospital. Controls for determination of specific IgG antibodies were sera from thirty pregnant women originally taken for antenatal Rh-testing.

Skin tests

The scratch-chamber method was used (Hannuksela & Lahti, 1977). *Paecilomyces* protein (the factory product) was moistened, placed in a small aluminium chamber (Finn Chamber®, Epitest Ltd) and applied to a scratch by taping the chamber on the skin. Chambers containing 10 g/l histamine dihydrochloride and 9 g/l sodium chloride absorbed into filter paper were applied similarly to the skin. The result was recorded after 15 min. It was considered positive if *Paecilomyces* protein induced a weal with a diameter at least half of that caused by the histamine.

Paecilomyces protein extract

Paecilomyces protein was extracted with Coca's solution (1:10 w/v) in our laboratory. The extract contained 0.9 g/l protein and it was used in the assays IgE and IgG antibodies.

Determination of serum IgE specific to Paecilomyces protein

For the radioallergosorbent test (RAST), the proteins in the *Paecilomyces* protein extract were coupled to cyanogenbromide-activated Munktell OOH filter paper discs (Ceska & Lundkvist, 1972). Other reagents were obtained from Phadebas RAST kits (Pharmacia Diagnostics, Sweden). A semi-quantitative RAST score (0–4) based on commercial reference system was used for the interpretation of the results. Scores 1–4 were considered positive.

Determination of serum IgG specific to Paecilomyces protein

An enzyme-linked immunosorbent assay (ELISA) was used. To adsorb antigen to

tubes, the *Paecilomyces* protein extract was first diluted 100-fold with 50 mmol/l carbonate-bicarbonate buffer (pH 9.2) which contained 1 mmol/l $MgCl_2$. Next, 300 μ l diluted extract was added to polystyrene test tubes (Mekalasi, Finland), which were incubated for 20 hr at 37°. The solution was sucked off, 300 μ l of a solution containing 5 g/l bovine serum albumin (BSA) in the same buffer was added, and the tubes were incubated for 2 hr at 37°. The tubes were washed three times with phosphate-buffered saline (PBS) to which 2 g/l Tween 20 had been added. The PBS contained 150 mmol/l NaCl and 150 mmol/l phosphate (pH 7.3). The tubes were used for assays immediately.

In the assay sera were diluted 100-fold with PBS containing 5 g/l BSA. The 250 μ l of each dilution was added to tubes in duplicate, and these were incubated overnight at room temperature. The samples were sucked off and tubes were washed three times as described above. Anti-human IgG conjugated with alkaline phosphatase (Orion Diagnostica, Finland) was diluted 100-fold with PBS containing 5 g/l BSA. Of this dilution, 250 μ l was added to the tubes, and they were incubated for 5 hr at room temperature. Phosphate substrate (Sigma, U.S.A.) was dissolved to obtain a solution of 1 g/l substrate in 50 mmol/l carbonate-bicarbonate buffer (pH 9.8) containing 1 mmol/l $MgCl_2$. Substrate solution (250 μ l) was added to the tubes, and they were incubated for 30 min at room temperature. The enzymatic reaction was stopped by adding 50 μ l 3 mol/l NaOH. The absorbance was measured at 405 nm.

Results

Three out of the twenty-seven process workers in the *Paecilomyces* protein plant had atopic dermatitis, and one of them also had allergic rhinitis. One out of eight laboratory workers, who had been in contact with *Paecilomyces* protein, had atopic dermatitis. In not one person had symptoms become worse during the time the subjects had worked at the plant or in the laboratory, and there was no indication in the history of an occupational cause or their disorder.

Paecilomyces protein elicited a positive skin test reaction in three workers and the sera of two of these contained IgE antibodies specific to *Paecilomyces* protein (Table 1). Positive skin test results were also observed in a control group with an incidence not significantly different from that in the exposed workers (Table 2). One third of the control group were atopic children all of whom showed positive skin test reactions.

IgG antibodies specific to *Paecilomyces* protein were found both in sera from exposed workers and in sera from controls who were healthy adult women. Mean concentrations in the two groups were not significantly different (Fig. 1).

Table 1. Workers with positive skin tests or specific IgE to *Paecilomyces* protein (none had symptoms of occupational allergy due to *Paecilomyces* protein)

Worker	Work	<i>Paecilomyces</i> protein				
		Atopic dermatitis	Allergic rhinitis	Skin test	Specific IgE (RAST score)	Specific IgG (absorbance)
K.R.	Plant	+	+	++	2	1.38
P.S.	Plant	+		+	0	0.98
M.V.	Laboratory	+		++	1	1.15

Table 2. Occurrence of positive skin tests and specific IgE to *Paecilomyces* protein in exposed workers and control groups (no significant difference between the groups could be detected)

Group	n	Positive tests results	
		Skin test	Specific IgE
Plant and laboratory workers	35	3 (9%)	2 (6%)
Controls (atopic patients)	115	7 (6%)	
Plant workers	27		1 (4%)
Controls (atopic patients)	10		0

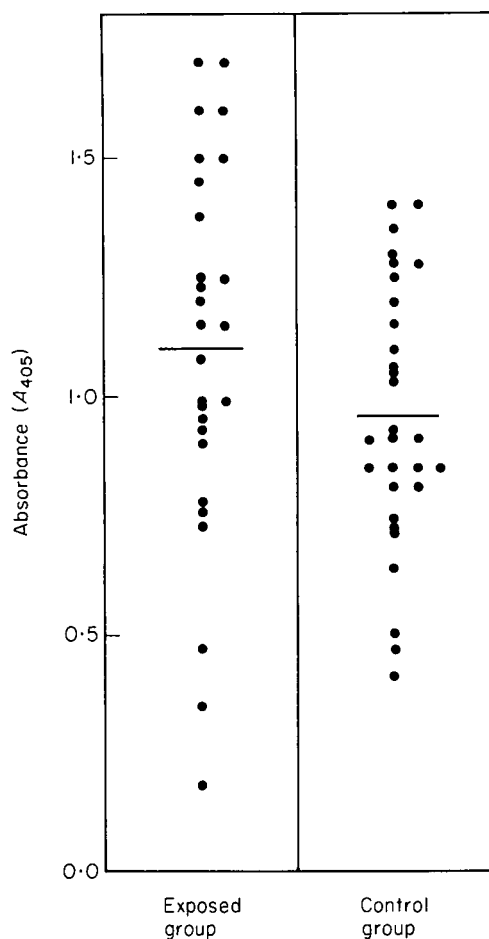


Fig. 1. The concentration of IgG antibodies to *Paecilomyces varioti* protein in 27 sera from exposed factory workers and in thirty sera from controls. Enzyme-linked immunosorbent assay (ELISA) was used, and results are given in arbitrary (absorbance) units. The difference in mean concentration (horizontal lines) was not significant (*t* test, $P=0.121$).

Discussion

Protein dusts, including moulds and other microfungi, can cause allergic occupational respiratory disease, e.g. allergic rhinitis, asthma and alveolitis (Pepys & Davies, 1978). Causal agents include proteolytic enzyme dust from *Bacillus subtilis* (Belin & Norman, 1977; How *et al.*, 1978), *Candida utilis* dust (Nordman *et al.*, 1981) and flour dust (Järvinen *et al.*, 1979). *Candida utilis* has also caused allergic diseases in non-employees in the neighbourhood of a factory using the yeast for fermentation (Nordman *et al.*, 1981).

In this study we examined workers at a factory fermenting pulp mill waste liquor with the mould *Paecilomyces varioti*. We could find no clinical symptoms of allergy which could be related to occupational exposure, neither is the factory known to have caused allergic diseases among the people living in the vicinity.

On the other hand, we did find that 9% of plant and laboratory workers reacted with positive skin tests to *Paecilomyces* protein and 4% of the workers had IgE antibodies to the same material. Positive skin test results were also observed at a similar rate in a control group (Tables 1 and 2). We conclude, that people can become sensitized to naturally occurring *Paecilomyces* or to some cross-reacting fungal antigen. Whether sensitization is more common among the workers studied than among normal people is unknown, since the control group was comprised of hospital patients with allergic disorders. Thus type I reactivity to *Paecilomyces* protein occurs, and it is probably that under adverse conditions the protein has potential to cause clinical allergy.

Serum IgG antibodies to antigens encountered occupationally (How *et al.*, 1978, Pepys & Davies, 1978) is evidence of exposure to the antigens but does not reflect allergic sensitization. In allergic alveolitis caused by microfungi, specific IgG antibodies are frequently found at increased levels. In the present study, we observed the presence of IgG antibodies to *Paecilomyces* protein both in sera from exposed workers and in sera from control persons. The mean level in sera from workers was higher, but not significantly so (Fig. 1).

These observations lead us to conclude that *Paecilomyces* protein is a potential allergen, though we observed no clinical allergy to *Paecilomyces* protein in employees engaged in the manufacture. This appears to be due to the closed, dust free process, which keeps exposure down to a safe level.

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