

PRECIPITIN RESPONSE OF POTATO PROCESSING WORKERS TO WORK-RELATED MICROBIAL ALLERGENS

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Abstract: Serum samples from 61 potato processing workers and 30 urban dwellers not exposed to organic dusts (as a reference group) were examined in agar-gel precipitation test performed by Ouchterlony double diffusion method with the antigens of 12 microorganisms associated with organic dusts. Each serum was tested twice: not concentrated, and three-fold concentrated, for the detection of low levels of precipitins. The antibody response of workers to the antigen of coryneform bacterium *Agromyces ramosus* was high, at both not concentrated and 3-fold concentrated sera (respectively 29.5% and 45.9%) - significantly greater than in reference group ($p < 0.001$). Workers' response to the antigens of Gram-negative bacterium *Alcaligenes faecalis* and thermophilic actinomycete *Thermoactinomyces vulgaris* was lower (respectively 13.1% and 13.1% at not concentrated sera, 24.6% and 29.5% at 3-fold concentrated sera) but in all cases significantly greater than in reference group ($p < 0.05$ at not concentrated sera, $p < 0.01$ and $p < 0.001$ at 3-fold concentrated sera). The frequency of positive precipitin reactions of potato workers to antigen of *Penicillium citrinum* was high only at 3-fold concentrated sera (55.7%) - significantly higher compared to reference group ($p < 0.001$). The antibody response of potato workers to other antigens was either unspecific or low, showing no significant difference compared to reference group. Twenty eight out of 61 examined potato processing workers (45.9%) reported the occurrence of the work-related pulmonary symptoms. The frequency of positive precipitin reactions to *Agromyces ramosus*, *Alcaligenes faecalis*, *Thermoactinomyces vulgaris*, *Penicillium citrinum* and *Acinetobacter calcoaceticus* was significantly greater in the subgroup of 28 workers reporting work-related pulmonary symptoms compared to 33 asymptomatic workers ($p < 0.05$). Study results suggest that antigens of *Agromyces ramosus*, *Alcaligenes faecalis*, *Thermoactinomyces vulgaris* and *Penicillium citrinum* should be considered as potential occupational allergens, probably stimulating an adverse immunopathological reaction in the exposed potato processing workers.

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INTRODUCTION

Exposure to organic dusts released during handling of various vegetable matter may be a cause of various work-related disorders of respiratory tract, skin and conjunctiva

[2, 21, 22, 31, 43]. They are mostly either allergic diseases (allergic alveolitis, asthma, allergic rhinitis, airborne contact dermatitis) caused by protein and glycoprotein allergens produced by plants, or by bacteria and fungi associated with vegetable dusts, or immunotoxic

diseases (organic dust toxic syndrome, byssinosis, non-allergic dermatitis and conjunctivitis) which are primarily caused by substances of microbial origin (bacterial endotoxin, fungal glucans, peptidoglycan, volatile organic compounds) [4, 6, 20]. The incidence of these diseases may be high among workers of the agricultural industry handling grain, malt, herbs, tobacco and other plant products [3, 4, 9, 21, 22].

Potato (*Solanum tuberosum* L.) is widely cultivated as edible tubers which are among the basic foods for inhabitants of the northern hemisphere. In many countries potatoes are processed in industrial plants for dry flakes, chips, meal, starch, syrup and other products. In Poland, ca. 6,500 people are employed in this industry. During the last decade it has been demonstrated in the Netherlands, the USA and Poland that 16-52% of workers engaged in processing potatoes have reported the presence of work-related respiratory and general symptoms [5, 12, 14, 38, 39, 40, 41, 42]. In our preliminary report on the essential findings of the present work [5] we found an antibody response of potato processing workers to microbial antigens present in the air of the work environment. Hollander *et al.* [14, 15], Zock *et al.* [39], and Zock [40] showed that nearly all workers employed in the Dutch potato processing plants had IgG antibodies against heat-labile potato proteins, mostly of the IgG₄ subclass, but none of them had specific IgE antibodies. The cited authors expressed the opinion that the detected antibodies are not relevant in the etiopathogenesis of work-related acute respiratory symptoms occurring in potato processing workers. According to Zock [40], these symptoms are most likely caused by non-specific airway inflammation caused by bacterial endotoxin. This author has indicated a need for further studies on the incidence of anti-microbial antibodies in potato processing workers [40].

A possibility of work-related allergies in people handling potatoes is documented in reports of clinical cases [1, 13, 25, 26, 29]. They include descriptions of bronchial asthma and rhinitis in housewives [26, 29] and reports of allergic alveolitis in farmers sorting and sieving potatoes [13].

The aim of this work was to determine the frequency of precipitin response of potato processing workers to a wide spectrum of work-related microbial allergens, in order to assess a potential risk posed by these factors in inducing occupational allergic disease in this professional group. Allergens (antigens) for this study were selected on the basis of an earlier microbiological analysis of the air in the examined facility [11], and a questionnaire examination of the workers.

MATERIALS AND METHODS

Examined population. A group of 61 potato processing workers (44 males + 17 females) aged 41.9 ± 12.5 yrs (mean \pm S.D.) were examined. They worked in a big potato processing facility located in eastern Poland in which a total of 140 workers were employed, of these, 90 were directly engaged in the production process. The

group examined in this study consisted only of the workers directly engaged in the production of dried potato flakes and meal (35 individuals) and in the production of potato syrup (26 individuals).

Thirty healthy industrial workers living in a city and not exposed to organic dusts were examined as a reference group. This group comprised 25 males and 5 females, aged 36.2 ± 10.1 yrs (mean \pm S.D.).

All potato processing workers and members of the reference group were examined by the agar-gel precipitation test with saline extracts of the cultures of microorganisms, selected on the basis of earlier microbiological studies of the air performed in the same plant where all the examined potato processing workers were employed [11]. All the subjects were also interviewed with a questionnaire developed at the Institute of Agricultural Medicine in Lublin [7] for the study of work-related symptoms. Human subjects protocols were approved by the Ethics Commission of the Institute of Agricultural Medicine, and all subjects gave informed consent.

Preparation of antigens. The antigens of the following 12 microorganisms, associated with organic dusts were used in the agar-gel precipitation test:

- Gram negative bacteria: *Acinetobacter calcoaceticus*, *Alcaligenes faecalis*, *Pantoea agglomerans* (syn. *Erwinia herbicola*, *Enterobacter agglomerans*);
- Gram-positive bacteria: *Agromyces ramosus*, *Arthrobacter globiformis*, *Bacillus subtilis*, *Microbacterium lacticum*;
- Actinomycetes: *Saccharopolyspora rectivirgula* (syn. *Micropolyspora faeni*, *Faenia rectivirgula*), *Thermoactinomyces vulgaris*;
- Fungi: *Aspergillus fumigatus*, *Aspergillus niger*, *Penicillium citrinum*.

The majority of the microbial species used as a source of antigens were reported as causative agents of allergic and/or immunotoxic respiratory disease induced by occupational exposure to organic dusts [3, 10, 20, 21, 23]. All species, except for *Saccharopolyspora rectivirgula* and *Penicillium citrinum*, were found in the air of the examined potato processing plant. Extracts of *S. rectivirgula* and *P. citrinum* were included in the test, as these microorganisms are known to be a common cause of allergic alveolitis. The antigens of *Agromyces ramosus*, *Microbacterium lacticum*, and *Aspergillus niger* were prepared from the strains isolated in the examined potato processing plant, while the other antigens were prepared from the reference strains used for research and diagnostic purposes in the Institute of Agricultural Medicine in Lublin [3, 8, 9, 35, 36].

The lyophilised saline extracts of bacterial or fungal mass, produced in the Institute of Agricultural Medicine in Lublin, were used as antigens. In the case of Gram-negative and Gram-positive bacteria, the mass was harvested from nutrient agar cultures, while in the case of actinomycetes and fungi - from sugar broth cultures. The mass was then homogenised and extracted in saline

Table 1. Precipitin reactions of potato processing workers to microbial antigens occurring in the air of work environment.

Antigen, group	Persons showing positive reaction (number, percent)			
	Potato processing workers (N = 61)		Reference group (N = 30)	
	Sera not concentrated	Sera 3-fold concentrated	Sera not concentrated	Sera 3-fold concentrated
Gram-negative bacteria				
<i>Acinetobacter calcoaceticus</i>	0	6 (9.8%)	0	1 (3.3%)
<i>Alcaligenes faecalis</i>	8 (13.1%)*	15 (24.6%)**	0	0
<i>Pantoea agglomerans</i>	0	0	0	1 (3.3%)
Gram-positive bacteria				
<i>Agromyces ramosus</i>	18 (29.5%)*	28 (45.9%)*	0	1 (3.3%)
<i>Arthrobacter globiformis</i>	0	0	0	0
<i>Bacillus subtilis</i>	0	0	0	0
<i>Microbacterium lacticum</i>	2 (3.3%)	6 (9.8%)	0	1 (3.3%)
Actinomycetes				
<i>Saccharopolyspora rectivirgula</i>	0	0	0	3 (10.0%)
<i>Thermoactinomyces vulgaris</i>	8 (13.1%)*	18 (29.5%)*	0	0
Fungi				
<i>Aspergillus fumigatus</i>	1 (1.6%)	4 (6.6%)	0	0
<i>Aspergillus niger</i>	27 (44.3%)	36 (59.0%)	22 (73.3%)	24 (80.0%)
<i>Penicillium citrinum</i>	3 (4.9%)	34 (55.7%)*	0	2 (6.7%)

* - ***: significantly greater compared to reference group; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

(0.85% NaCl) in the proportion 1:2 for 48 hrs at 4°C, with intermittent disruption of cells by 10-fold freezing and thawing. Afterwards, the supernatant was separated by centrifugation, dialysed against distilled water for 24 hrs, concentrated by evaporation to 0.1–0.15 of previous volume and lyophilised. The antigens were used in agar-gel precipitation test at the concentration 30 mg/ml [9, 23, 35, 36].

Agar-gel precipitation test. The test was performed by Ouchterlony double diffusion method in purified 1.5% Difco agar. The worker's serum was placed in the central well, and antigens dissolved in 0.85% NaCl in the peripheral wells. Each serum was tested twice: not concentrated, and three-fold concentrated, for the detection of low levels of precipitins. The plates were incubated for 6 days at room temperature, then washed in saline and in 5% sodium citrate solution (to prevent false positive reactions), and stained with azocarmine B [9, 23, 27, 35, 36].

Statistical analysis. The obtained results were analysed by the Student's t-test, assuming $p < 0.05$ as the significance level. The incidence of precipitin reactions in potato processing workers was compared with the occurrence of work-related pulmonary symptoms, described in a separate paper [24]. Comparison was carried out by Pearson's correlation test.

The study was performed mostly during the years 1989-1991, and continued during 1999-2002. Preliminary results have been reported elsewhere [5].

RESULTS

Frequency of precipitin reactions. The frequency of positive precipitin reactions to 12 microbial antigens associated with organic dusts in the group of potato processing workers and reference group is shown in Table 1. The antibody response of the workers to antigen of coryneform bacterium *Agromyces ramosus* was high, both at not concentrated and 3-fold concentrated sera (respectively 29.5% and 45.9%) - significantly greater than in reference group ($p < 0.001$) (Tab. 1). The workers' response to the antigens of Gram-negative bacterium *Alcaligenes faecalis* and thermophilic actinomycete *Thermoactinomyces vulgaris* was lower (respectively 13.1% and 13.1% at not concentrated sera, 24.6% and 29.5% at 3-fold concentrated sera), but in all cases significantly greater than in reference group ($p < 0.05$ at not concentrated sera, $p < 0.01$ and $p < 0.001$ at 3-fold concentrated sera). The frequency of positive precipitin reactions of potato workers to antigen of *Penicillium citrinum* was low at not concentrated sera (4.9%), but very high at 3-fold concentrated sera (55.7%) - significantly higher compared to the reference group ($p < 0.001$) (Tab. 1).

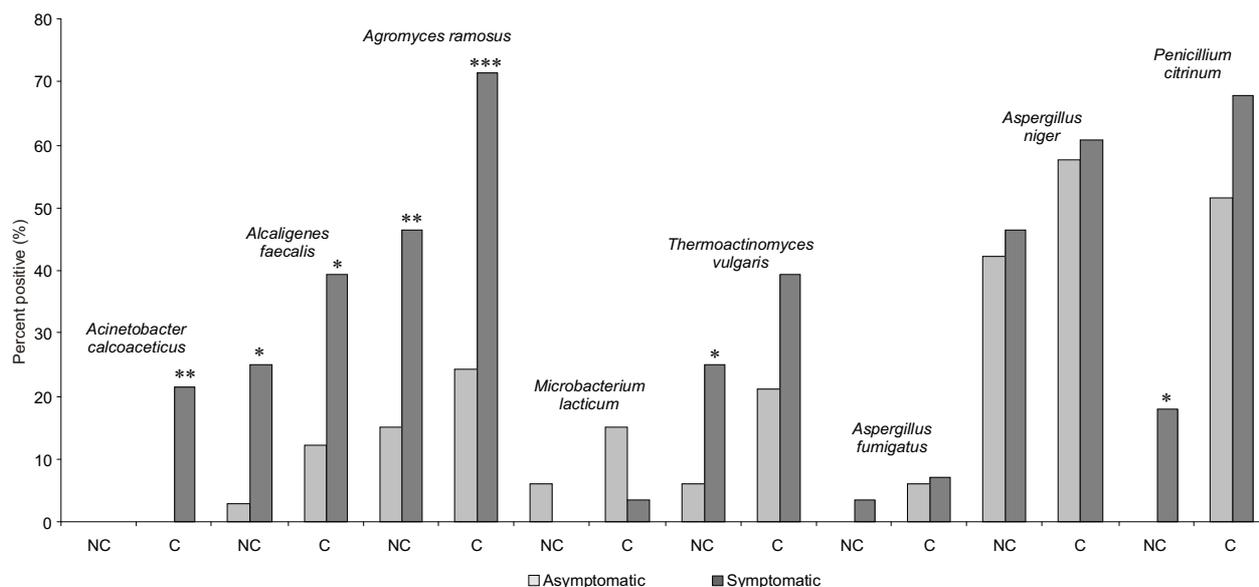


Figure 1. Frequency of positive precipitin reactions to extracts of 8 environmental microbes in the subgroups of asymptomatic (N = 33) and symptomatic (N = 28) potato processing workers. NC = sera not concentrated, C = sera 3-fold concentrated. Results with the remaining 4 antigens used (*Arthrobacter globiformis*, *Bacillus subtilis*, *Pantoea agglomerans*, *Saccharopolyspora rectivirgula*) are not shown - they were negative in all cases. *-***: significantly greater compared to asymptomatic workers; * p < 0.05, **p < 0.01, ***p < 0.001.

The highest frequency of positive reactions (44.3% at not concentrated sera, 59.0% at 3-fold concentrated sera) was noted to the antigen of *Aspergillus niger*. Nevertheless, even higher response was recorded in the reference group (respectively 73.3% and 80.0%), which suggests that these reactions were unspecific and not related to exposure. The antibody response of potato workers to other antigens was low and did not show a significant difference compared to the reference group (Tab. 1).

Relationship between the occurrence of work-related pulmonary symptoms and allergic reactions. Twenty eight out of 61 examined potato processing workers (45.9%) reported the occurrence of the work-related pulmonary symptoms. The frequency of positive precipitin reactions with particular antigens in the subgroups of 28 symptomatic workers and 33 asymptomatic workers is presented in Figure 1.

As seen in Figure 1, the antibody response of symptomatic workers to 5 antigens was significantly greater compared to those who were asymptomatic. The frequency of positive reactions to *Agromyces ramosus* was 3 times greater in symptomatic workers, both at not concentrated and 3-fold concentrated sera, and the differences were statistically significant (respectively p < 0.01 and p < 0.001) (Fig. 1). Similarly, symptomatic workers reacted significantly more frequently to *Alcaligenes faecalis*, both at not concentrated and 3-fold concentrated sera (p < 0.05). The frequency of positive reactions to *Thermoactinomyces vulgaris* and *Penicillium citrinum* was significantly greater in symptomatic workers only in the case of not concentrated sera (p < 0.05), while to *Acinetobacter calcoaceticus* - only in the case of 3-fold concentrated sera (p < 0.05) (Fig. 1).

DISCUSSION

The results of this study suggest that antigens of *Agromyces ramosus*, *Alcaligenes faecalis*, *Thermoactinomyces vulgaris* and *Penicillium citrinum* should be considered as potential occupational allergens that may stimulate an adverse immunopathological reaction in the exposed potato processing workers. This is indicated by the fact that the frequency of the positive precipitin reactions to these 4 antigens was significantly greater, not only in the total group of exposed potato workers compared to the not exposed reference group, but also in the workers who reported work-related pulmonary symptoms compared to the asymptomatic ones. In addition, a possible role of *Acinetobacter calcoaceticus* as a potential occupational allergen may be purported as its antigen reacted significantly more frequently with the sera of symptomatic workers compared to asymptomatic ones.

On the basis of this study, *Agromyces ramosus* seems to be the most important source of occupational allergen in the potato industry as more than 70% of persons who reported work-related pulmonary symptoms showed antibody response to the extract of this coryneform bacterium. The difference in the frequency of positive antibody response to *A. ramosus* between the subgroups of symptomatic and asymptomatic workers attained a very high level of significance, similar to that between the groups of potato processing workers and referents. *Agromyces ramosus* is a little known soil bacterium [16] capable of developing in potato parenchyma [11]. Large quantities of these bacteria are released into the air of a potato processing plant after the peeling of potatoes, and reach maximal level after cutting, blanching, and drying of the potatoes [11]. A possible role of this species in

causing work-related disorders among the workers of the potato industry deserves further study.

Alcaligenes faecalis is another bacterial species that should be considered as a source of potentially pathogenic allergens and endotoxin in the potato industry. This Gram-negative bacterium was consistently recovered from the air of a potato processing plant [11]. The frequency of positive antibody response to *A. faecalis* was distinctly and significantly greater in potato processing workers compared to referents and in symptomatic workers compared to asymptomatic ones. It has been experimentally demonstrated that this species revealed strong allergenic and endotoxic properties [34]. *Alcaligenes faecalis* was identified as a cause of allergic alveolitis in a Polish farmer [23]. A study by Kuś *et al.* [18, 19] indicated that this species could be a cause of the work-related pulmonary disorders in a group of 23 female herb processing workers. Twelve workers of this group (52.2%) showed a positive response in the inhalation challenge with the diluted extract of *Alcaligenes faecalis*.

Thermoactinomyces vulgaris is a thermophilic actinomycete that abundantly develops in overheated fodders and is widely recognised as one of the most important agents causing farmer's lung, a specific form of allergic alveolitis (hypersensitivity pneumonitis). Moulds of the genus *Penicillium* are often implicated in the etiology of allergic alveolitis [20, 21] and *Penicillium citrinum* was identified as a potential occupational allergen among sawmill workers [8]. Although the latter 2 organisms were either rare (*Th. vulgaris*) or absent (*P. citrinum*) in the air samples taken in the potato processing plant [11] they might possibly develop there in other seasons, or in places which were not examined during this study. *Acinetobacter calcoaceticus*, a coccoid Gram-negative bacterium frequently occurring in organic dusts, exhibits strong allergenic and endotoxic properties [32, 33]. This organism has been isolated from the air of the examined potato processing plant.

The highest percentage of positive precipitin reactions among potato workers was noted with the antigen of *Aspergillus niger*, a fungus dominant in the air of the potato processing plant [11]. However, these reactions were even more common in the unexposed reference group, and were not correlated with the occurrence of work-related pulmonary symptoms. Thus, these reactions are most probably unspecific and their clinical significance is unclear. *Aspergillus niger* is often reported as a causative agent of allergic, immunotoxic or infectious disease [17, 20, 30, 37].

The frequencies of positive precipitin reactions of potato processing workers to the antigens of work-related microorganisms was similar to those found by our group in earlier seroepidemiological studies of farmers, grain elevator workers, maltworkers, sawyers, and herb processing workers [3, 8, 9, 35, 36]. However, in the present work, contrary to the earlier studies, the highest frequency of the specific precipitin reactions was noted not with the antigens of Gram-negative bacteria, but with the antigen of a coryneform bacterium *Agromyces ramosus*.

The presence of precipitins to an environmental microbe is regarded as proof of exposure rather than of clinical impairment. The original view of Pepys [28], postulating the basic role of the precipitin-mediated, type III reaction in inducing allergic alveolitis, was revised, and currently the main role of the T-lymphocyte dependent, type IV reaction in etiopathogenesis of this disease is widely accepted [20, 21]. Nevertheless, precipitins are regarded as an important measure of exposure, probably implicated to some extent in the pathogenesis of the disease [4]. Therefore, the finding in the present study of a significant relationship between the presence of precipitins against some environmental microbes, and the occurrence of work-related pulmonary symptoms among potato processing workers, suggests that besides the non-specific, immunotoxic reactions evoked by endotoxins, specific, allergic reactions to work-related microorganisms should also be considered as a possible cause of occupational disorders among this professional group. The clarification of this problem needs further study.

CONCLUSION

The frequency of the positive precipitin reactions to the antigens of *Agromyces ramosus*, *Alcaligenes faecalis*, *Thermoactinomyces vulgaris* and *Penicillium citrinum* was significantly greater among exposed potato processing workers compared to referents, who were not exposed and in the workers who reported work-related pulmonary symptoms compared to the asymptomatic ones. This suggests that these microorganisms should be considered as potential occupational allergens that probably stimulate an adverse immunopathological reaction in the exposed potato processing workers.

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