

Allergenic and mutagenic characterization of 14 *Penicillium* species[★]

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Abstract

Extracts of 14 *Penicillium* species, *P. aurantiogriseum*, *P. brevicompactum*, *P. citrinum*, *P. chrysogenum*, *P. expansum*, *P. glabrum*, *P. hirsutum*, *P. italicum*, *P. janthinellum*, *P. melini*, *P. oxalicum*, *P. purpurescens*, *P. simplicissimum*, and *P. viridicatum* were investigated by total protein, specific enzyme determinations, isoelectric focusing (IEF), sodium dodecylsulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting using pooled human atopic IgE. Considerable variation was observed between the *Penicillium* species with respect to protein yield and the number of distinct protein bands resolved in IEF. Using the Api-Zym system, the most common activities observed among the extracts included acid and alkaline phosphatase, phosphodiesterase and β -glucosaminidase. The number of discrete atopic IgE-reactive bands in immunoblots of *Penicillium* extracts ranged from 1 (*P. chrysogenum*) to 9 (*P. viridicatum*). Certain allergens showed potential for cross-reactivity between species, including 52 and 54 kDa proteins in *P. citrinum*, *P. purpurescens*, *P. viridicatum* and 40 kDa proteins in several species. The extracts were also nonmutagenic when tested with the Ames assay using *Salmonella* strains TA98 and TA100. The results tend to indicate that *P. viridicatum*, *P. janthinellum*, *P. oxalicum*, *P. brevicompactum* and *P. italicum*, which are highly immunogenic as well as allergenic, could possibly be good candidates for allergen cloning studies through the construction of cDNA libraries. The extracts were non-mutagenic and can be used safely for skin testing.

1. Introduction

Penicillium is one of the most prevalent airborne indoor fungi (Salvaggio and Aukrust, 1981; Beaumont et al., 1984; Gravesen, 1985; Licorish et al., 1985) and their spores have been identified as potential respiratory allergens (Al-Doory and Domson, 1984; Horner et al., 1994). Different species in the genus *Penicillium* could be associated

with clinical allergy. Results from skin tests have also demonstrated that *Penicillium* is one of the molds that frequently elicits positive skin reaction in allergic individuals (Hsieh, 1984; Santilli et al., 1990). More than 100 different species have been identified in this prevalent airborne fungal genus (Pitt, 1988). Muilenberg et al. (1990) have reported that *P. citrinum*, together with *P. chrysogenum* (notatum), *P. oxalicum*, *P. brevicompactum*, and *P. spinulosum* were the five most frequently recovered species of *Penicillium* in Topeka KS. *P. citrinum* was the most prevalent

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Penicillium species reported from Taiwan (Wei et al., 1993). However, allergens in prevalent *Penicillium* species and human responses to major known allergens have not been well characterized. In order to determine whether specific *Penicillium* species may be significant to the study of asthma epidemiology, the allergenic proteins from 14 *Penicillium* species (*P. aurantiogriseum*, *P. brevicompactum*, *P. chrysogenum*, *P. citrinum*, *P. expansum*, *P. glabrum*, *P. hirsutum*, *P. italicum*, *P. janthinellum*, *P. melini*, *P. oxalicum*, *P. purpureescens*, *P. simplicissimum*, and *P. viridicatum*) were examined by biochemical and immunological assays. As a preliminary characterization for adverse health effects, should the extracts prove useful in the clinical diagnosis of allergy, the extracts were also assayed for mutagenicity using the Ames *Salmonella*/microsome *in vitro* test (Schrader et al., 2001, 2002). These species were selected because they were the most abundant and frequently observed in the indoor air of the Montreal and Vancouver (Canada) metropolitan areas (Escamilla Garcia et al., 1994).

2. Materials and Methods

2.1. Culture of *Penicillium* species

Initial collection of the isolates of *Penicillium* species was made from indoor atmosphere on Sabouraud dextrose agar solid medium (Difco, Detroit, Michigan, USA). Pure strains were identified and kept on malt extract agar (MEA) solid medium (Difco). A new culture was grown on MEA for 7 days, exposed for 12 h every day to UV light. A small portion of this culture (1 mm³) was used to start every new culture on liquid medium BBL malt extract broth 1.5% (pH 4.7). Cultures were grown in 300 ml pyrex flasks, containing 100 ml BBL medium (Difco) and incubated at 26 °C for three weeks. The pellicles were collected and washed with saline followed by acetone and air-dried.

2.2. Preparation of mycelial extracts

The defatted mold pellicles were homogenized and extracted while stirring on a magnetic plate, with 1:10 w/v double distilled water at 4 °C for 48 h. The suspension was centrifuged at 1000 *g* at 4 °C for 30 min, followed by 30,000 *g* at 4 °C for

20 min. The resulting supernatant was concentrated using an Amicon YM-5 membrane (Millipore, Etobicoke, Ont., Canada). The retentate was filtered through a 0.45 µm filter and lyophilized. All *Penicillium* species extracts were prepared using the same protocol.

2.3. Biochemical assays

Protein contents of the extracts were determined in triplicate by a commercial dye-binding assay (Bio-Rad Laboratories, Mississauga, Ont., Canada), using bovine serum albumin as standard (Bradford, 1976). The extracts were screened for the determination of 19 enzyme activities with the Api-Zym system; including alkaline and acid phosphatases, three lipases, five peptidases, phosphodiesterase and eight glycosidases as reported earlier (Vijay et al., 1985). Analytical isoelectric focusing (IEF) was carried out using polyacrylamide gel plates with a pH ranges 2–6 and 3.5–9.5 and Coomassie brilliant blue (CBB) R-250 staining.

2.4. Immunological assays

Previously reported procedures (Vijay et al., 1981) were followed for the production of antisera to the mycelial extracts in New Zealand white rabbits (3 animal per antigen) and the screening of the antisera by immunodiffusion (Vijay et al., 1981, 1984). The IgE antibody preparation was a mixture of equal volumes of sera from 6 patients highly allergic to *Penicillium* as judged by skin test reaction of 3+ to 4+ and direct RAST titer of 2+ to 3+ using discs coupled with specific species of *Penicillium* extract >5 kDa (Vijay et al., 1985).

2.5. SDS-PAGE and western blotting

SDS-PAGE was carried out in 12% polyacrylamide gel in discontinuous buffer (Laemmli, 1970). Aliquots of the samples corresponding to 12 µg of total protein were loaded. Proteins were visualized on the membrane by staining with Coomassie brilliant blue R-250. For immunoblotting, protein bands separated on SDS-PAGE gel were electrotransferred onto PVDF membranes in a Trans-blot cell (Bio-Rad Laboratories). Free protein sites on the membrane were blocked with 1% bovine serum albumin at 4 °C overnight (Towbin et al., 1979).

Proteins binding to specific human IgE antibodies against *Penicillium* species were detected by incubation with atopic human sera (6-patient pool) followed by incubation with alkaline phosphatase conjugated goat anti-human IgE as reported by Curran et al. (1993). The membranes were washed and stained with 5-bromo-4-chloro-3-indolyl phosphate and nitro blue tetrazolium. Controls were included in which the atopic serum was replaced by a serum pool from 5 non-allergic individuals.

2.6. Salmonella/microsome mutagenicity assay

Extracts were dissolved in DMSO and tested for mutagenic activity in the Ames *Salmonella* test, following standard procedures previously described (Maron and Ames, 1983). The assays were performed using *S. typhimurium* tester strains TA98 and TA100. Fresh cultures of tester strains were grown to approximately 10^9 cells/ml in 5 ml Oxoid Nutrient Broth No. 2 (Oxoid, Ottawa, Ont.) in 15 ml sterile culture tubes. The cultures were grown for 12 h at 37 °C shaking at 200 RPM in a G24 Environmental Shaker (New Brunswick Scientific Company, Edison, NY). For metabolic activation, the S9 mix contained 10% S9 (50 μ l S9 per plate), which was prepared from male Sprague Dawley rats induced with Aroclor 1254 (Moltox, Boone, NC).

The plate incorporation test included various concentrations of test compound in 0.1 ml DMSO, 0.1 ml of overnight bacterial strain cultures and either 0.5 ml of 0.1 M phosphate buffer pH 7.4 or 0.5 ml of 10% S9 mix if required for metabolic activation combined with 2 ml top (soft) agar that contained 0.5 mM histidine/biotin. After mixing, the contents were poured onto 25 ml Vogel–Bonner minimal agar plates. Each sample was tested in triplicate. Positive and negative controls were included for each strain in each experiment. Plates were incubated for 48–72 h at 37 °C.

After incubation, the revertant colonies were counted using an Accucount 1000 Colony Counter (Biologics, Gainesville, VA). Means of triplicate plates, standard errors and curve fitting were then calculated (GraphPad Prism, San Diego, CA). In this assay, a positive response was defined as a dose-related increase, at least two times above negative controls, in histidine revertant colonies in any one of strain/activation combination. The

background lawn of bacterial growth was also examined as an aid in determining toxicity of the test chemical (Maron and Ames, 1983).

3. Results

3.1. Extraction of 14 *Penicillium* species

There was a significant variability in the amount of soluble material (148–463 mg) obtained from 5.0 g of dried pellicles (Table 1). The total protein contents also varied from <0.1 to 10%. *P. citrinum* had the highest (10%) protein content followed by *P. hirsutum*, *P. janthinellum*, *P. italicum*, and *P. brevicompactum* (5.3, 5.24, 5.2, and 4.0% respectively) (Table 1). *P. aurantiogriseum* and *P. chrysogenum* gave less than 0.1% protein.

3.2. Enzyme screening

All the extracts contained large amounts of acid phosphatases while eight species contained alkaline phosphatases and leucine aminopeptidase (Table 2). Phosphodiesterase and β -glucosaminidase were also present in most of the species. In contrast, α -mannosidase and α -fucosidase were largely absent except for *P. citrinum*, *P. purpureum*, and *P. melini* which contained large amounts of α -fucosidase. Table 2 indicates that enzyme profiles among the extracts can be grouped by similarity, i.e. *P. aurantiogriseum*, *P. glabrum*, *P. chrysogenum*, and *P. expansum* as well as *P. simplicissimum* and *P. viridicatum*, all appear to lack detectable levels of alkaline phosphatase, C4 and C8 esterase, β -galactosidase and α -glucosidase, whereas the second group (*P. brevicompactum*, *P. citrinum*, *P. italicum*, *P. janthinellum*, *P. hirsutum*, *P. purpureum*, *P. melini*, and *P. oxalicum*) tend to exhibit positive levels of these enzymes.

3.3. Isoelectric focusing

Most of the extracts had similar IEF patterns i.e., 22–31 bands with pI values between 3.5–5.85 and 1–6 bands with pI values between 5.85 and 8.65. However, *P. expansum*, *P. aurantiogriseum*, *P. glabrum*, and *P. chrysogenum*, had only 4–11 bands with pI values between 3.5–5.85 and one or fewer bands in the region of 5.85–8.65 (Figure 1a, 1b). These results can be seen clearly in Table 3.

Table 1. Protein yields in extracts of 14 *Penicillium* species

Extract	Soluble material (mg) per 5 gm mould dry weight	Protein (%)
<i>P. aurantiogriseum</i>	1620	< 0.1
<i>P. glabrum</i>	1557	0.5
<i>P. chrysogenum</i>	1544	< 0.1
<i>P. expansum</i>	1463	1.0
<i>P. brevicompactum</i>	1482	4.0
<i>P. citrinum</i>	1685	10
<i>P. italicum</i>	1178	5.2
<i>P. hirsutum</i>	1783	5.3
<i>P. janthinellum</i>	2316	5.24
<i>P. purpurescens</i>	848	2.1
<i>P. simplicissimum</i>	712	1.07
<i>P. viridicatum</i>	848	2.0
<i>P. melini</i>	741	1.8
<i>P. oxalicum</i>	1093	1.7

3.4. Immunogenicity of the extracts

All the extracts were found strongly immunogenic when the rabbit antisera were screened by immunodiffusion tests.

3.5. SDS-PAGE and immunoblotting analysis

The allergenic components of the extracts were characterized by SDS-PAGE followed by immunoblotting with atopic serum pool and anti-human IgE conjugated to alkaline phosphatase. The immunoblots showed that there was a considerable variation in the number of allergenic proteins in the extracts. *P. chrysogenum* had only one band at 40 kDa whereas *P. viridicatum* showed 9 allergenic proteins at approximately 97, 76, 59, 54, 50, 47, 45, 31, and 27 kDa (Figure 2). The highest frequency of IgE binding proteins appeared to be at approximately 40, 54 and 76 kDa. In most species, a broad region from 50 to 82 kDa also showed diffused reaction with atopic

Table 2. Enzymatic activities in extracts of 14 *Penicillium* species

Enzymes	Score														
	A	G	C	E	B	Ct	I	J	H	P	S	V	M	O	
Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Alkaline phosphatase	0	0	0	0	5	5	5	5	5	5	0	0	5	5	
Esterase (C4)	0	0	0	0	1	1	1	0	1	0	0	1	1	1	
Esterase (C8)	0	0	0	0	1	1	1	1	1	0	0	0	1	0	
Lipase (C14)	0	0	0	0	0	0	0	0	1	0	0	0	0	0	
Leucine aminopeptidase	0	0	0	0	5	5	5	5	5	2	0	0	4	3	
Valine aminopeptidase	0	0	0	0	0	1	1	1	1	0	0	0	2	1	
Cystine aminopeptidase	0	0	0	0	0	0	1	0	1	0	0	0	0	0	
Trypsin	0	0	1	0	0	0	1	0	1	0	0	0	0	0	
Chymotrypsin	0	0	0	0	0	1	5	2	1	0	0	0	1	0	
Acid phosphatase	3	5	4	1	5	5	5	5	5	5	5	5	5	3	
Phosphodiesterase	3	4	1	1	1	5	5	5	4	1	4	3	5	5	
α -galactosidase	0	0	0	0	1	4	1	0	1	0	0	0	5	1	
β -galactosidase	0	0	0	0	0	2	1	3	4	2	0	1	5	2	
β -glucuronidase	0	0	0	0	1	1	0	0	0	0	0	0	3	0	
α -glucosidase	0	0	0	0	2	4	4	4	1	4	0	1	5	2	
β -glucosidase	1	0	0	0	3	4	3	1	1	2	1	4	5	4	
β -glucosaminidase	4	3	0	0	5	5	5	5	5	0	1	5	2	3	
α -mannosidase	0	0	0	0	0	1	1	0	1	0	0	0	1	0	
α -fucosidase	0	0	0	0	1	5	0	0	1	5	0	0	5	0	

A – *P. aurantiogriseum*, G – *P. glabrum*, C – *P. chrysogenum*, E – *P. expansum*, B – *P. brevicompactum*, Ct – *P. citrinum*, I – *P. italicum*, J – *P. janthinellum*, H – *P. hirsutum*, P – *P. purpurescens*, S – *P. simplicissimum*, V – *P. viridicatum*, M – *P. melini*, O – *P. oxalicum*. 0: Negative – 5: Maximum intensity.

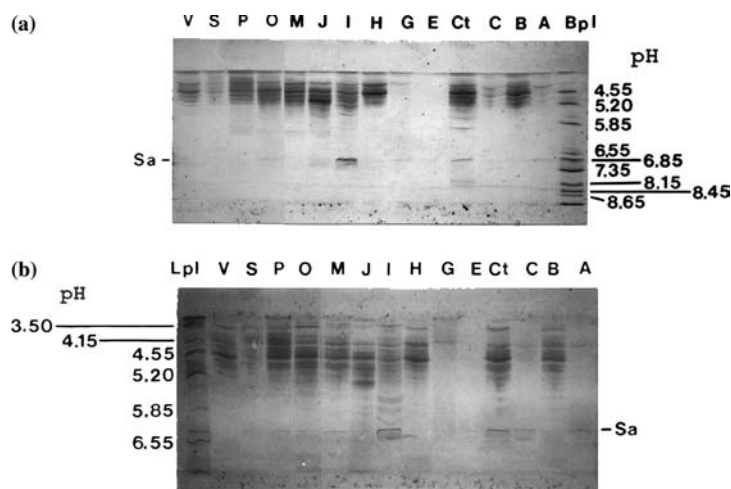


Figure 1. Broad range (a) and low range (b) analytical IEF of extracts of 14 *Penicillium* species, V – *P. viridicatum*, S – *P. simplicissimum*, P – *P. purpurescens*, O – *P. oxalicum*, M – *P. melini*, J – *P. janthinellum*, I – *P. italicum*, H – *P. hirsutum*, G – *P. glabrum*, E – *P. expansum*, Ct – *P. citrinum*, C – *P. chrysogenum*, B – *P. brevicompactum*, and A – *P. aurantiogriseum*. The standards pI markers were run in the lane marked BpI (Broad pI) and LpI (Low PI) and their values are given at the side. The anode is at the top. Sa– Sample application site.

IgE. Strongly reactive proteins are indicated in italics (Table 4). Certain allergens show potential for cross-reactivity between species as demonstrated by a 54 kDa protein in *P. citrinum*, *P. viridicatum*, *P. purpurescens* and 40 kDa protein in *P. citrinum*, *P. brevicompactum*, *P. melini*, and *P. chrysogenum*. Six species, *P. citrinum*, *P. janthinellum*, *P. brevicompactum*, *P. hirsutum*, *P.*

italicum, and *P. viridicatum* have strongly reactive protein at 76 kDa. Molecular weights of allergenic components of all 14 *Penicillium* species are shown in Table 4.

3.6. Mutagenicity test

The extracts were examined for mutagenicity with the Ames assay using *Salmonella* strains TA98 (Base shift mutation) and TA100 (Base pair mutation) with or without rat liver S9 for activation. Representative data presented in Table 5 demonstrate that *P. chrysogenum* and *P. brevicompactum* appear to be non-mutagenic. Similar results were obtained with the rest of the extracts studied.

4. Discussion

Mold allergens are probably the least studied causative allergens of human beings. The strains and species employed in mold allergenic studies, as well as culture conditions and extraction methods, all contribute to variations in the results obtained. In the present study, 14 *Penicillium* species (*P. aurantiogriseum*, *P. brevicompactum*, *P. oxalicum*, *P. purpurescens*, *P. viridicatum*, *P. simplicissimum*, *P. chrysogenum*, *P. citrinum*,

Table 3. Number of resolved proteins in broad and narrow range IEF of 14 *Penicillium* species extracts

Organism	No. of bands of pI	
	3.50–5.85	5.85–8.65
<i>P. aurantiogriseum</i>	6	0
<i>P. glabrum</i>	7	0
<i>P. chrysogenum</i>	11	1
<i>P. expansum</i>	4	0
<i>P. brevicompactum</i>	28	2
<i>P. citrinum</i>	31	3
<i>P. italicum</i>	28	4
<i>P. hirsutum</i>	25	1
<i>P. janthinellum</i>	30	6
<i>P. purpurescens</i>	26	6
<i>P. simplicissimum</i>	15	0
<i>P. viridicatum</i>	22	2
<i>P. melini</i>	28	4
<i>P. oxalicum</i>	25	3

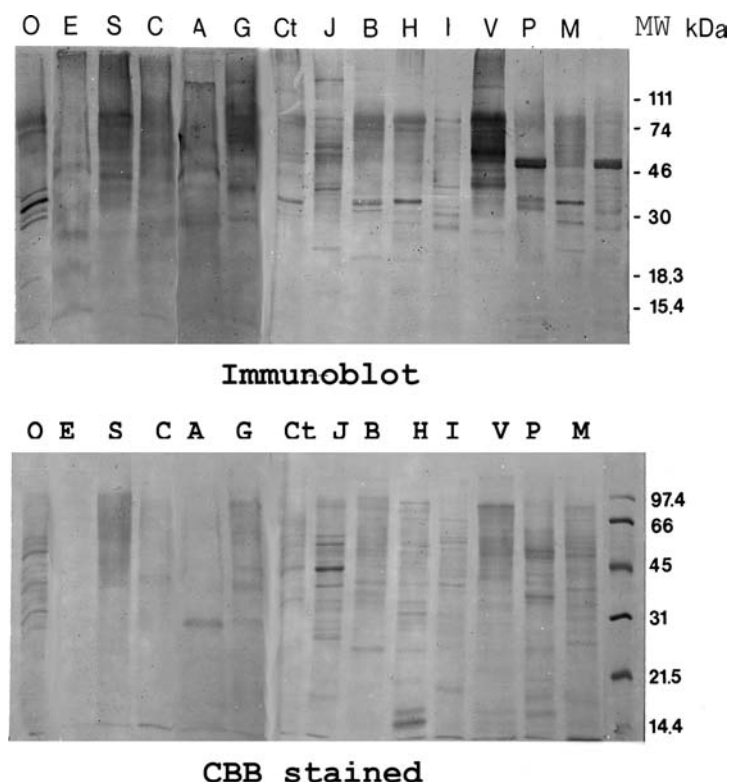


Figure 2. SDS-PAGE followed by Coomassie brilliant blue R-250 staining (Bottom) and Immunoblots (Top) of extracts from 14 *Penicillium* species, O – *P. oxalicum*, E – *P. expansum*, S – *P. simplicissimum*, C – *P. chrysogenum*, A – *P. aurantiogriseum*, G – *P. glabrum*, Ct – *P. citrinum*, J – *P. janthinellum*, B – *P. brevicompactum*, H – *P. hirsutum*, I – *P. italicum*, V – *P. viridicatum*, P – *P. purpurescens*, M – *P. melini*. Molecular weight standards are shown on the right.

P. expansum, *P. glabrum*, *P. citrinum*, *P. hirsutum*, *P. italicum*, and *P. janthinellum*, *P. melini*) were investigated by total protein, specific enzyme

determination, IEF, SDS-PAGE and immunoblots using pooled human atopic serum (IgE) from *Penicillium* sensitive individuals.

Table 4. Number and M_r of allergens in extracts of 14 *Penicillium* species

Species (No. of allergens)	M_r in thousands									
<i>P. citrinum</i> (6)	76	73	56	54	40	36				
<i>P. janthinellum</i> (8)	102	76	61	56	53	44	27	19		
<i>P. brevicompactum</i> (6)	76	70	40	39	37	25				
<i>P. hirsutum</i> (3)	76	70	39							
<i>P. italicum</i> (6)	76	44	42	38	35	32				
<i>P. viridicatum</i> (9)	97	76	59	54	50	47	45	31	27	
<i>P. purpurescens</i> (4)	54	52	40	38						
<i>P. melini</i> (5)	63	53	40	34	28					
<i>P. oxalicum</i> (5)	93	86	44	40	37	33	22			
<i>P. expansum</i> (4)	33	29	22	16						
<i>P. simplicissimum</i> (5)	73	67	56	50	46					
<i>P. chrysogenum</i> (1)	40									
<i>P. aurantiogriseum</i> (4)	97	59	50	43						
<i>P. glabrum</i> (4)	73	61	42	33						

Note: In most species, a broad region from 50 to 82 kDa also shows diffuse IgE reactivity. Strongly reactive proteins are in Italics.

Table 5. Representative Ames *Salmonella* test results for *P. chrysogenum* and *P. brevicompactum* (Revertants/plate)

Extract ($\mu\text{g}/\text{plate}$)	<i>P. chrysogenum</i>				<i>P. brevicompactum</i>			
	TA98		TA100		TA98		TA100	
	–S9	+S9	–S9	+S9	–S9	+S9	–S9	+S9
0	33.0 ± 2.5	34.3 ± 2.0	139.0 ± 7.4	145.3 ± 7.3	23.3 ± 1.5	24.7 ± 3.4	138.3 ± 12.2	134.7 ± 9.9
1	20.0 ± 0.6	18.3 ± 1.5	101.7 ± 9.2	131.0 ± 12.0	19.0 ± 2.0	24.7 ± 2.2	141.7 ± 4.8	143.3 ± 7.7
10	27.3 ± 1.8	24.3 ± 2.3	122.0 ± 1.0	138.8 ± 2.7	25.0 ± 2.5	18.0 ± 2.6	145.3 ± 1.9	137.7 ± 5.0
100	30.3 ± 4.4	22.0 ± 2.1	138.7 ± 12.1	142.0 ± 3.2	24.7 ± 0.9	21.3 ± 1.2	101.7 ± 3.8	147.3 ± 1.2
500	24.0 ± 2.3	22.3 ± 2.4	156.3 ± 0.9	146.7 ± 10.1	19.3 ± 2.3	21.0 ± 4.9	151.3 ± 1.2	141.7 ± 14.1

Revertants are presented as means of triplicate plates $\pm$ standard error Aroclor-induced rat liver S9 was used (50 $\mu\text{l}/\text{plate}$), for metabolic activation. Results are representative of those found for other *Penicillium* species extracts.

Extracts of these species exhibited considerable variability with respect to protein yield and number of distinct protein bands in IEF. It is interesting to note that other investigators (Shen et al., 1998, 1999) have shown production of significant protein bands, which are also allergenic, at pI 6.5 for *P. chrysogenum*. The discrepancy in the results could be due to the type of isolate, culture, and extraction conditions. It has been shown for other fungi such as *Alternaria alternata* (Vijay et al., 1984) and *Cladosporium herbarum* (Vijay et al., 1991) that many variables influence the production of allergens in culture and hence the potency of allergen extracts.

The extracts were highly immunogenic in rabbits. Therefore, the rabbit antisera could be used for cross-reactivity studies among other mold allergens. These antisera also could be used for initial screening of the cDNA library of these *Penicillium* species. The results with Api-Zym system showed that in general, high levels of acid phosphatase were evident in these *Penicillium* species, while alkaline phosphatase and β -glucosaminidase were also present in most species. It is apparent from SDS-PAGE immunoblot data that the number of allergens in these extracts ranges from 1 (*P. chrysogenum*) to 9 (*P. viridicatum*) and that certain allergens show potential for cross-reactivity between species (approximately 40 kDa in several species). Several allergenic proteins of similar molecular weight were found in different *Penicillium* species, particularly of 40 and 76 kDa

and to a lesser extent, a 52 and 54 kDa doublet. Although, it is premature to conclude that such proteins are species-specific homologs, it remains possible that certain allergens once purified in a large quantity may represent useful immunotherapeutic agents, potentially effective against a wide variety of *Penicillium* species. Moreover, these allergens could be used as standard allergy markers for the detection of *Penicillium* fungi in indoor air.

Allergens purified using conventional procedures are often obtained in low yield and tend to be of inconsistent quality. In particular, fungal allergens are subject to considerable variation between isolates of the same species, since molds rapidly adapt in response to a new environment. Molecular characterization of these allergenic proteins is essential, not only to better understand the pathogenesis of atopic diseases, but also to develop specific diagnostic and immunotherapeutic treatments. Stable and consistently pure allergenic proteins can be prepared using recombinant DNA techniques. Expression of these proteins in a *Pichia pastoris* based expression system would yield allergens containing similar posttranslational modifications to those of natural allergens. Recently, several *Penicillium* allergens have been characterized at molecular level. Among the *Penicillium* allergens, the 32–34 kDa alkaline and/or vacuolar serine protease were identified as the major allergens of *P. citrinum*, *P. brevicompactum*, *P. chrysogenum*, and *P. oxalicum* (Shen et al., 1997). They have been designated as Group 13 for alkaline

serine protease and Group 18 for vacuolar serine protease allergens as recommended by the Allergen Nomenclature Subcommittee (Shen et al., 1999). Immunoblotting data showed that IgE antibodies against components of these prevalent *Penicillium* species could be detected in the sera of 16–20% of asthmatic patients (Shen et al., 1999). Majority of the positive serum samples tested showed IgE binding to the 32–34 kDa serine proteinase(s) with a frequency >80% in different fungal species tested. The cDNA of the alkaline serine protease allergens from *P. citrinum* (Pen c 13) and *P. chrysogenum* (Pen ch 13), and the vacuolar serine proteases from *P. citrinum* (Pen c 18), *P. oxalicum* (Pen o 18), and *P. chrysogenum* (Pen ch 18) have recently been cloned. The IgE cross-reactivity between the allergens in *Penicillium* and *Aspergillus* has been detected (Shen et al., 1998, 2001; Su et al., 1999; Chou et al., 2002). It seems unlikely that the approximately 40 kDa immunoreactive protein seen in our results for *P. citrinum*, *P. brevicompactum* and *P. oxalicum* may correspond to the already cloned 32–34 kDa alkaline and/or vacuolar serine protease major allergens. Since the isolation of mould allergens from a variety of species is known to depend upon culture conditions and isolation procedures, the present results may indicate the presence of a different allergens. The effect of different culture conditions and batch-to-batch comparisons will be made as the production of allergens in individual species is examined in more detail.

5. Conclusion

In the present study, the findings suggest that *P. viridicatum*, *P. janthinellum*, *P. brevicompactum*, *P. oxalicum*, and *P. italicum* could be candidates for cloning studies since these species offer the largest number of strongly immunoreactive bands in Western blots. No mutagenicity was associated with any of the extracts tested up to 500 µg/plate in the Ames *Salmonella* test using strains TA98 (frameshift mutations) and TA100 (base pair mutations) with and without metabolic activation.

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