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Penicillium and *Aspergillus* species in the habitats of allergy patients in the Tulsa, Oklahoma area

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Abstract

Penicillium and *Aspergillus* have been recognized as important aeroallergens for more than 30 years, and are especially significant in indoor environments. There are over 400 species of *Penicillium* and *Aspergillus* combined, but there is little information on which species occur most frequently in the environment, or if each exhibits unique allergenic properties. A preliminary study showed no overlap between those species isolated from an outdoor site in Tulsa, Oklahoma and the species used in immunotherapy at allergy clinics in the Tulsa area. Pursuing this line of research, air samples were collected as three seasonal samples (over a 6 month period) in the homes or offices of ten allergy patients known to be allergic to *Penicillium* and/or *Aspergillus*. Twenty three species of *Penicillium* and 12 species of *Aspergillus* were identified from these samples through isolation, macroscopic, and microscopic examination. *Penicillium corylophilum*, *P. glabrum*, *Aspergillus niger*, and *A. flavipes* were the most abundant species isolated, supporting the data obtained in a preliminary study. At least in the Tulsa area, it appears that atopic patients are being tested and treated with extracts of *Penicillium* and *Aspergillus* species that are either not present or not abundant in the local indoor or outdoor environments. Additional research is necessary to determine if the environmental isolates share allergens with those species used in immunotherapy. © 1997 Elsevier Science Ireland Ltd.

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1. Introduction

Since the 1920s, fungal spores have been recognized as important aeroallergens (Feinberg, 1935). Some of the more common allergenic asexual fungi include *Alternaria*, *Aspergillus*, *Candida*, *Cladosporium*, *Curvularia*, *Drechslera*, *Epicoccum*, *Fusarium*, *Penicillium*, and *Trichoderma*, with only a few of these genera having been extensively studied (Salvaggio and Aukrust, 1981). Investigation of *Penicillium* and *Aspergillus* is of great importance due to the wide range of allergic diseases that are associated with exposure to these fungi. Asthma, rhinitis, bronchopulmonary aspergillosis, and hypersensitivity pneumonitis, or extrinsic allergic alveolitis are diseases that can be triggered by exposure to *Penicillium* and *Aspergillus* (Samson, 1985; Mishra et al., 1992; Chang et al., 1995). In previous studies, *Penicillium* and *Aspergillus* spores have not only been linked to asthmatic reactions, but also to the passage of their small spores into lung alveoli (Rijckaert and Broers, 1980; Salvaggio and Aukrust, 1981).

It has been estimated that people in modern society spend approximately 90% of their time in indoor environments (Cordasco et al., 1995), therefore, indoor air quality has become an important health concern. In recent studies, as described by Levetin (1995), indoor concentrations of fungal spores have been reported to be as high as 20 000 colony-forming units per cubic meter (CFU/m^3). Considering the fact that an allergic reaction may occur with exposure to minute concentrations of an allergen (Salvaggio and Aukrust, 1981), high

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indoor concentrations of molds could easily create health risks for atopic individuals occupying such a building. Conditions that determine the prevalence of fungal spores in indoor environments include outdoor spore concentration, type and rate of ventilation, indoor humidity, and activity within a building (Levetin, 1995; Burge, 1985).

Species of *Penicillium* and *Aspergillus* have historically been considered soil-borne saprophytes, but in recent studies have shown preferential growth in indoor niches (Burge, 1990). Indoor concentrations are frequently greater than outdoor, because a wide variety of organic substrates (including upholstery, carpets, potted plants, and tile grout) may be colonized by *Penicillium* or *Aspergillus* (Burge, 1985). This endless supply of organic matter allows growth in virtually any building environment. While high relative humidity and a suitable substrate for growth are necessary for the establishment of most *Penicillium* and/or *Aspergillus* species, several species of both genera are capable of growing in xeric environments, such as house dust (Burge, 1990). The spores of both genera are small (2–8 μm) and can therefore remain aloft in air currents for long periods of time (Pitt, 1991; Klich and Pitt, 1992). *Penicillium* and *Aspergillus* were chosen for this study because of their recognition as important indoor contaminants, as well as significant sources of aeroallergens.

For current immunotherapy, doctors and patients rely on a standard set of pharmaceutical extracts that usually contain one to half a dozen *Penicillium* or *Aspergillus* species (Table 1), out of the estimated 250 *Penicillium* and 150 *Aspergillus* species. In preliminary studies, *Penicillium* and *Aspergillus* species isolated from an outdoor site at The University of Tulsa differed from the species used to prepare extracts for the diagnosis and treatment of allergy patients at a local clinic. If species of *Penicillium* and *Aspergillus* differ antigenically, this finding suggests that extracts currently used may be inappropriate for treating some mold allergy patients. This project was undertaken to identify the species of *Penicillium* and *Aspergillus* found in the homes of allergy patients hypersensitive to these fungi and determine if there is an overlap with the species used for diagnosis by local allergists.

Table 1
Penicillium and *Aspergillus* species used in allergy clinics in the Tulsa area

Penicillium species:	*Aspergillus* species:
P. camemberti	*A. fumigatus*
P. chrysogenum	*A. nidulans*
P. digitatum	*A. niger*
P. expansum	*A. terreus*
P. glaucum	
P. notatum [= *P. chrysogenum*] (Pitt, 1991)	
P. roqueforti	

Note: The species listed above combines all species tested for from all allergy clinics in Tulsa, OK.

2. Materials and methods

2.1. Testing sites

Ten sites were sampled for indoor and outdoor viable fungi. All sampling sites were the homes or offices of allergy patients being treated at the Allergy Clinic of Tulsa. Only patients who were known to be sensitive to *Penicillium* and/or *Aspergillus*, as determined by a skin testing reaction of 3+ to 4+, were considered for this study. Along with this requirement, the specific qualifications required for patients to volunteer their home (or office) included:

1. Patient must live/work in the same house/office as when the allergy was diagnosed.
2. Patient must have lived/worked in that home/office for at least 1 year.

The patients agreed to three seasonal samples (September, November, and February) in their home or work. Samples were collected in those rooms in which the patient spent the most time, usually including a living room, kitchen, bedroom, and bathroom.

2.2. Culture media

Three types of media were used in the collection and maintenance of *Penicillium* and *Aspergillus* colonies. Difco malt extract agar (MEA) and dichloran-glycerol (DG-18) medium (Hocking and Pitt, 1980) were used in viable sample collections. MEA is a broad spectrum medium that supports the growth of most fungi. DG-18 is a low water activity medium that was used to allow isolation of xerophilic species that may be unable to grow on MEA. Difco Czapeks's solution agar (CSA) was used to subculture and maintain *Penicillium* and *Aspergillus* colonies.

2.3. Viable air samples

Duplicate air samples for viable fungi were collected on DG-18 and MEA with two single stage (N-6) Andersen samplers running simultaneously. The flow rate for DG-18 samples was 35 l/min and 37 l/min for MEA. Indoor samples were collected during a 2 min sampling time, and outdoor samples were taken over a 1 min period. These sample durations were used to avoid sample overload. Samples were taken at the level of breathing height for each room ranging from 1 to 1.5 m. The plates were incubated at room temperature (25 $\pm$ 1°C) for 7–14 days before colonies present were counted and identified to the genus level. Colony

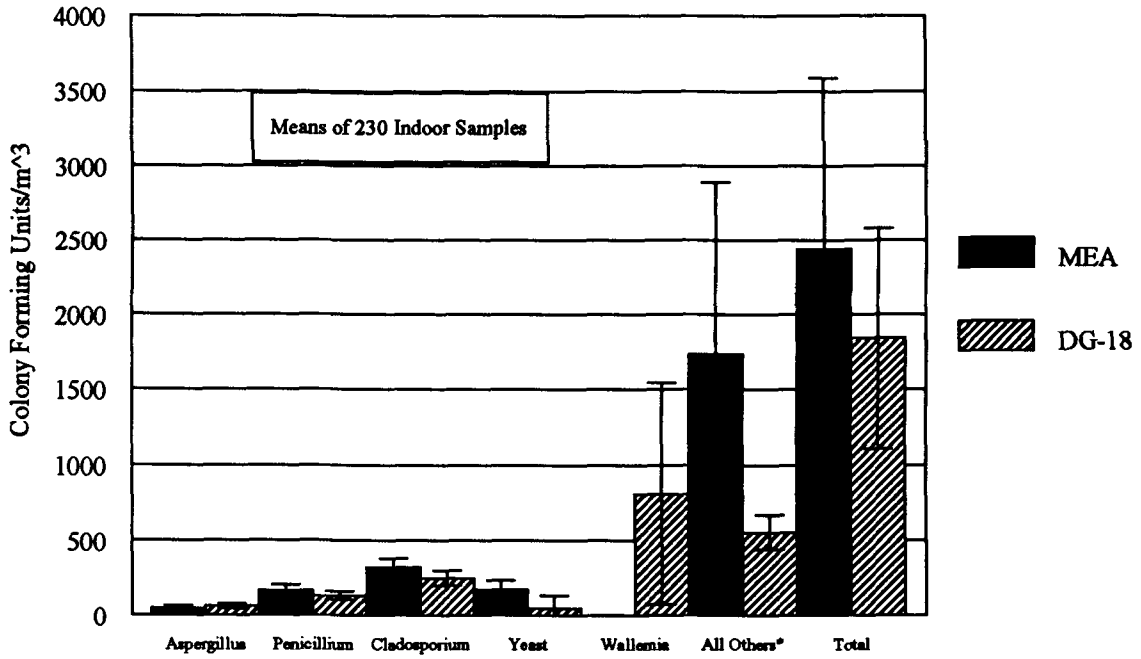

Fig. 1. Mean indoor concentrations of *Aspergillus*, *Penicillium*, and other taxa. Comparison of mean indoor concentrations of taxa identified from malt extract agar (MEA) and dichloran-glycerol agar (DG-18). Note the relatively small concentrations of *Aspergillus* and *Penicillium*. *All others include other identified taxa, unknowns, and non-sporulating fungi.

counts were converted into airborne concentration and expressed as colony-forming units per cubic meter (CFU/m^3).

2.4. Species identification

Each of the *Penicillium* and *Aspergillus* colonies was subcultured onto CSA medium and described according to macroscopic and microscopic characteristics of each colony. These were then identified to the species level primarily using keys by Pitt (1991) and Klich and Pitt (1992). Additional keys (Ramirez, 1982; Raper and Fennell, 1965) were also used. Following identification, colony counts were converted to atmospheric concentrations and means were determined for each species identified.

All allergy clinics in the Tulsa area were surveyed to identify those species of *Penicillium* and *Aspergillus* used in immunotherapy treatments.

3. Results

A survey of nine Tulsa allergists indicated that extracts of six species of *Penicillium* and four species of *Aspergillus* were used for skin testing and immunotherapy (Table 1). In several instances, mixed extracts of several of the listed *Penicillium* species were used.

During the air sampling phase of the investigation, 230 indoor samples and 52 outdoor samples were collected using single-stage Andersen samplers. Several genera of fungi were isolated as common indoor air contaminants, including *Alternaria*, *Aspergillus*, *Cladosporium*, *Penicillium*, and *Wallemia*, with *Cladosporium* being the most abundantly occurring genus. Fig. 1 shows the mean concentrations of *Aspergillus* and *Penicillium* compared to other fungi isolated during this study. Mean concentrations of different taxa isolated on both MEA and DG-18 are shown in Fig. 1, displaying the differing results of these two media. Even though *Penicillium* and *Aspergillus* were not the most abundant of the fungi isolated, they comprised an important component: *Penicillium* colonies were isolated in 100% of the homes/offices sampled, and *Aspergillus* colonies were isolated in 90%. *Penicillium* concentrations at all test sites ranged from 0 to 297 CFU/m^3 (Table 2) and *Aspergillus* concentrations ranged from 0 to 327 CFU/m^3 (Table 3). The mean concentrations of all *Penicillium* and *Aspergillus* species appear low due to the inclusion of all 230 samples for calculation of these concentrations.

Altogether 301 *Penicillium* and *Aspergillus* isolates were subcultured from the original air samples. The 229 *Penicillium* isolates included 23 species, and the 81 *Aspergillus* isolates included 12 species. The three most abundant *Penicillium* species identified from all indoor sites were *Penicillium corylophilum*, *P. glabrum*, and *P. verrucosum*, with indoor concentrations of 9.57, 5.39, and 3.66 CFU/m^3, respectively (Table 2). The outdoor mean concentrations of these species were *P.*

Table 2
Penicillium species identified from all testing sites

Species name	Mean indoor concentration (CFU/m^3)	Percent indoor occurrence*	Maximum concentration (CFU/m^3)	Medium (DG-18, MEA, or both)	Mean outdoor concentration (CFU/m^3)
P. corylophilum	9.6	18	297.3	Both	22.3
P. glabrum	5.4	16	139.4	Both	12.7
P. verrucosum	3.7	6	192.9	Both	22.8
P. decumbens	3.2	7	281.8	Both	0.6
P. oxalicum	2.8	6	137.6	Both	—**
P. citreonigrum	2.7	6	100.7	Both	0.5
P. simplicissimum	2.1	3	131.5	Both	2.6
P. fellutanum	2.0	4	115.5	Both	—
P. minioluteum	1.7	2	269.0	MEA	—
P. spinulosum	1.5	4	60.0	Both	2.2
P. rugulosum	1.1	3	98.3	Both	—
P. variabile	1.0	2	72.3	Both	—
P. citrinum	0.6	3	38.5	Both	1.0
P. crustosum	0.4	1	84.9	Both	1.0
P. camembertii	0.4	<1	86.2	MEA	—
P. miczynskii	0.3	2	27.0	MEA	3.9
P. chrysogenum	0.2	1	38.5	DG-18	—
P. raistrickii	0.2	1	14.3	Both	—
P. commune	0.1	<1	27.6	DG-18	—
P. waksmanii	0.1	1	14.3	Both	0.6
P. lividum	0.1	<1	13.5	MEA	—
P. olsonii	0.1	<1	13.5	MEA	—
P. sclerotiorum	—	—	—	MEA	1.1

* The percentage of a species occurring on indoor samples on which the species occurred.
** Not detected.

verrucosum at 22.8 CFU/m^3, *P. corylophilum* at 22.3 CFU/m^3, and *P. glabrum* at 12.7 CFU/m^3. *Penicillium chrysogenum*, a species used widely in immunotherapy, had a mean indoor concentration of only 0.23 CFU/m^3 and was not found at any outdoor site.

Aspergillus versicolor, *A. flavipes*, and *A. niger* made up the three most abundant *Aspergillus* species from all indoor sites, with mean indoor concentrations of 3.54, 2.63, and 2.07 CFU/m^3, respectively (Table 3). *A. niger* (7.16 CFU/m^3), *A. fumigatus* (2.56 CFU/m^3), and *A. terreus* (1.51 CFU/m^3) were the three most abundant species isolated at outdoor sites. Indoor concentrations of *A. fumigatus* and *A. terreus*, both of which are used in immunotherapy, were 0.18 CFU/m^3 for *A. fumigatus*, while *A. terreus* was not isolated indoors in this study.

4. Discussion

This study demonstrated that the species of *Penicillium* and *Aspergillus* isolated from patient environments did not correlate with the species used for allergy diagnosis and immunotherapy in Tulsa. When the five most abundant species identified in this research (*Penicillium corylophilum*, *P. glabrum*, *P. verrucosum*, *P. decumbens*, and *P. oxalicum*) are compared with other studies, there is an overlap with data collected from Topeka, Canada, and Denmark. Mishra et al. (1992) reported the summer indoor and outdoor occurrence of *P. oxalicum*, *P. brevicompactum*, *P. chrysogenum*, *P. citrinum*, and *P. spinulosum* as the five most abundant species in Topeka, Kansas. A recent Canadian study (Escamilla et al., 1994) found *P. glabrum*, *P. aurantiogriseum*, *P. simplicissimum*, and *P. chrysogenum* as the most abundant species, while a study of Danish schools (Frisvad and Gravesen, 1994) listed *P. glabrum*, *P. corylophilum*, and *P. chrysogenum* as those species occurring most abundantly. However, there appears to be little or no overlap with similar studies performed in other geographical locations. Rosas et al. (1993) identified *P. aurantiogriseum*, *P. chrysogenum*, *P. crustosum*, and *P. spinulosum* as commonly occurring *Penicillium* species in areas of Mexico City, while in Taiwan, Wei et al. (1993) showed the most numerous species as *P. citrinum*, *P. crustosum*, and *P. implicatum*. This variation in the occurrence of certain *Penicillium* species may be due to differences in geographical distribution, environmental differences, and/or problems in species identification. This disparity becomes important when considering that pharmaceutical companies distribute the same standard set of mold allergy extracts worldwide (personal communication with a Miles Co. representative).

Table 3
Aspergillus species identified from all testing sites

Species name	Mean indoor concentration (CFU/m^3)	Percent indoor occurrence[a]	Maximum concentration (CFU/m^3)	Medium (DG-18, MEA, or both)	Mean outdoor concentration (CFU/m^3)
A. versicolor	3.5	4	326.7	Both	—[b]
A. flavipes	2.6	5	162.0	Both	0.5
A. niger	2.1	8	80.5	Both	7.2
A. caespitosus	2.0	4	231.0	Both	—
A. ochraceous	1.2	2	227.9	Both	—
A. carneus	0.9	2	93.7	Both	0.5
A. sydowii	0.9	2	81.0	Both	—
A. flavus	0.5	2	30.2	Both	—
A. fumigatus	0.2	1	28.4	MEA	2.6
A. niveus	0.1	1	14.2	Both	0.8
A. oryzae	0.1	<1	13.5	MEA	1.0
A. terreus	—	—	—	DG-18	1.5

[a] The percentage of a species occurring on indoor samples on which the species occurred.
[b] —, not detected.

The present study was confined to the homes or offices of ten patients and took place during a 6-month period (September–February). Due to this limited sampling, it is possible that additional species may occur in the Tulsa area, especially during the months that were not sampled.

Because many fungal extracts used in immunotherapy, especially *Penicillium*, have been chosen with little knowledge of the allergenic properties (Mishra et al., 1992) or geographic distribution, it is not surprising that many patients afflicted with mold allergies do not respond well to immunotherapy. One reason for patients not responding as expected to fungal extracts may be that the species being used are not representative of those the patients are exposed to in their daily environment. When the list of species from environmental isolates in this study is compared with those species used for immunotherapy, there is little similarity. For example, the most abundant *Penicillium* species identified in this study, *Penicillium corylophilum*, *P. glabrum*, and *P. verrucosum*, are not used in skin testing by any Tulsa area allergists. In fact, none of these most commonly isolated species is even produced as an extract by a large, US-based pharmaceutical company (Miles, 1995). It is also noteworthy that the only *Penicillium* species overlapping with current extracts used in the Tulsa area was *P. chrysogenum*, isolated as two colonies from one indoor site. Compared to other species of *Penicillium* isolated, *P. chrysogenum* was ranked as the 17th most abundant species at 0.23 CFU/m^3, out of 23 species identified.

There was greater overlap between the *Aspergillus* species isolated in this study and those used in immunotherapy than with *Penicillium*. *Aspergillus niger*, *A. fumigatus* and *A. terreus* occur in both lists. This greater overlap could be because the allergenic properties of *Aspergillus* are better known than *Penicillium* (Salvaggio and Aukrust, 1981), or because there are only 150 *Aspergillus* species, compared to the estimated 250 *Penicillium* species.

With Tulsa allergy patients being exposed to a wide range of *Penicillium* and *Aspergillus* species in their everyday environments but diagnosed with a different range, the question of cross-reactivity is raised. Do the species used in allergy extracts share common allergens with those species isolated from allergy patients' environments? If there is sufficient cross-reactivity, then the current extracts are probably adequate. However, because the allergenic properties of some species are quite different (Mishra et al., 1992), a serious re-evaluation of the standard procedures is necessary. With so much physical variation between species in each genus, it is possible for the allergenic properties to be as diverse as the physical properties (Levetin, 1995). In understanding these relationships more fully, immunotherapy could become a more effective method of treating allergic diseases.

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