

## Indoor Airborne *Penicillium* Species in Taiwan

Ding-Ling Wei,<sup>1</sup> Juei-Hsieh Chen,<sup>2</sup> Shung-Chang Jong,<sup>3</sup> and Horng-Der Shen<sup>2</sup>

<sup>1</sup>Division of Biology, National Yang-Ming Medical College, Taipei, Taiwan, ROC; <sup>2</sup>Department of Medical Research, Veterans General Hospital, Taipei, Taiwan, ROC; and <sup>3</sup>Mycology Department, American Type Culture Collection, Rockville, Maryland, USA

**Abstract.** *Penicillium* species, well-known indoor airborne fungi, are considered to be important causative agents of extrinsic bronchial asthma. For selecting the proper species for clinical investigations, it is important to know which are found indoors. In the present study, 176 plates of modified Mehrlich's Medium were exposed for 20 min in living rooms and bedrooms of 88 homes in Taipei, Taiwan. A total of 5897 colonies of fungi were isolated, varying from one to 329 per house. Of the total, 910 colonies (15.4%) were identified as *Penicillium* spp. The three most frequently encountered species were *P. citrinum*, *P. crustosum* and *P. implicatum*. *Penicillium citrinum* accounted for 40.5% (369 from 28 houses); *P. implicatum*, for 5.2% (47 from three houses); and *P. crustosum*, for 2.1% (19 from seven houses). *Penicillium citrinum* was judged to be the most prevalent airborne fungus in Taiwan. The allergenic significance of this mold is under further investigation.

Airborne fungal spores can cause allergies, and their importance in clinical studies has been recognized for many years. The effect of such allergens on patients suffering with asthma has prompted studies on the kinds of spores found in both indoor and outdoor environments.

The species of several genera, *Alternaria*, *Aspergillus*, *Cladosporium*, *Fusarium*, *Helminthosporium*, *Mucor*, *Penicillium*, *Phoma*, and *Rhizopus*, have been identified as those most often causing immediate skin reactions in allergic individuals [4]. Jones and Gerson [5] reported a correlation between the fungi in patients' homes and skin reactivity to extracts of the same fungi. More recently, species of *Alternaria*, *Aspergillus*, *Cladosporium* and *Penicillium* were shown to be the most frequently found genera associated with allergy [6]. Certain species of Basidiomycetes have also been investigated [7].

*Penicillium* species are well-known indoor fungi and are frequently included in skin test panels [10]. For clinical studies, it is important to know which species are found indoors. Since the *Penicillium* species are considered significant causative agents of extrinsic bronchial asthma, a study was conducted to isolate and identify indoor species in the Taipei area of Taiwan with plate culture technique.

### Materials and Methods

Sterile disposable petri dishes (100 × 15 mm) containing modified Mehrlich's Medium (5 g malt extract, 1 g KH<sub>2</sub>PO<sub>4</sub>, 1 g peptone, 15 g dextrose, 75 g sodium chloride, 15 g agar, 1 L distilled H<sub>2</sub>O) were used [16]. A total of 88 houses in four regions in Taipei were sampled from February to July, 1990 (Fig. 1). Two plates were exposed for 20 min in the living room and bedroom of each house and incubated for 3–14 days at 25°C.

Colonies were identified and counted. If identification was not possible on the original plates, subcultures were made on potato dextrose agar (Difco Laboratories, Detroit, Michigan, USA). All penicillia isolated were subsequently grown for 7 days at 25°C on three standard media for species determination: Czapek yeast extract agar (CYA), malt extract agar (MEA), and 25% glycerol nitrate agar (G25N) [13]. The CYA plates were also incubated at 5°C and 37°C. Classification of the isolated penicillia was based on their microscopic morphology, colony growth rates, and colony morphology, including color of conidia, mycelium, and exudates, colony texture, and the color of the colony's reverse and the medium on which it had been grown.

In addition to light microscopy, scanning electron microscopy was employed for morphological studies. Specimens were prepared according to the following procedure: small pieces (3 × 3 mm) of fungal growth were removed from the culture medium, fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer for 1 h, rinsed twice with 5% sucrose solution in 0.1 M phosphate buffer for 15 min, fixed a second time with 1% osmium tetroxide in 0.1 M phosphate buffer for 1 h, and rinsed twice with 5% sucrose solution in 0.1 M phosphate buffer. The specimens were then dehydrated in a gradient series of ethanol (50%, 70%, 90%, and 95%, 15 min each; 100% twice, 1 h at each step) and finally transferred to 100% acetone for 1 h. They were dried with a

Table 1. Incidence of indoor airborne *Penicillium* species collected from plates exposed in four collection areas of Taipei, Taiwan

| Sampling areas | No. of families | Total mold counts | Total <i>Penicillium</i> counts | Percentage of <i>Penicillium</i> within total mold counts |             |
|----------------|-----------------|-------------------|---------------------------------|-----------------------------------------------------------|-------------|
|                |                 |                   |                                 | Bedroom                                                   | Living room |
| I              | 29              | 1406              | 78(5.5%)                        | 65(4.6%)                                                  | 13(0.9%)    |
| II             | 24              | 1574              | 142(9.0%)                       | 59(3.7%)                                                  | 83(5.3%)    |
| III            | 29              | 2282              | 629(27.6%)                      | 323(14.2%)                                                | 306(13.4%)  |
| IV             | 6               | 635               | 61(9.6%)                        | 35(5.5%)                                                  | 26(4.1%)    |
| Totals         | 88              | 5897              | 910(15.4%)                      | 482(8.2%)                                                 | 428(7.2%)   |

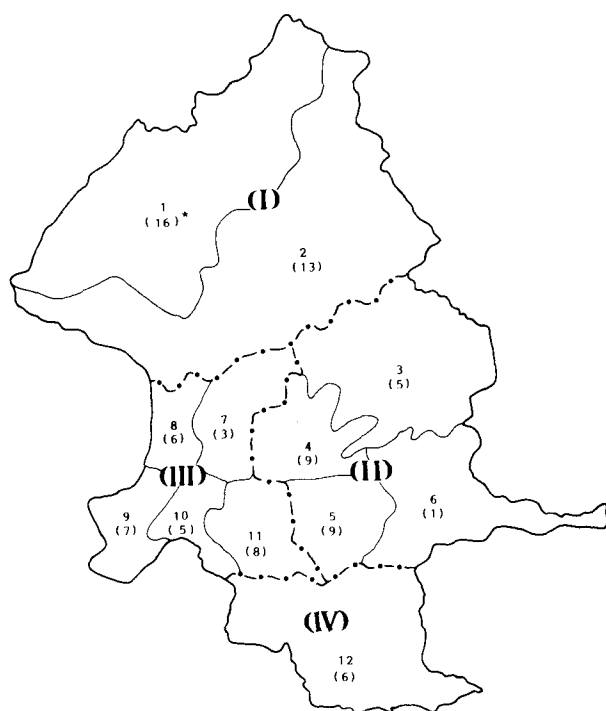


Fig. 1. Sampling areas in Taipei, Taiwan. Region I, including the following districts: 1. Pei-Tou, 2. Shih-Lin. Region II, including 3. Nei-Hu, 4. Sung-Shan, 5. Hsin-I, 6. Nan-Kang. Region III, including 7. Chung-Shan, 8. Ta-Tung, 9. Wan-Hwa, 10. Chung-Cheng, 11. Ta-An. Region IV, 12. Wen-Shen. \*Number(s) in parentheses represent total sampled families.

critical-point dryer, coated with gold, and examined with a Jeol JSM-35C scanning electron microscope.

## Results and Discussion

The presence and types of airborne fungal spores in both indoor and outdoor environments have been

surveyed in many parts of the world [1, 2, 8, 9, 11, 15, 17]. It is generally assumed that the genera most commonly found in air are also the most frequent inhaled allergens [4]. Although molds grow primarily outdoors, Prince and Morrow [14] pointed out that they also are found indoors, especially in damp or poorly ventilated areas. Therefore, in the treatment of asthmatic patients, it is important to consider the conidia found in both places. In Copenhagen, for example, *Penicillium* species were the most prevalent fungi in the bedrooms of eight asthmatic children, while in Manoa, Hawaii, species of that genus were the third most common encountered [11].

Taipei is located in the northern part of Taiwan. The high temperature and humidity of this island favor fungal growth. During 1990 the average daily maximum temperature was 27.4°C; the minimum, 18.6°C. The mean relative humidity varied from 66% to 84%. In a study of outdoor airborne fungi from 1975 to 1979, Han et al. [3] determined daily fungal conidial counts at six different locations in Taipei by the gravity slide method. The average count per year was 5656/cm<sup>3</sup>; with the outdoor plate exposure method, species of *Cladosporium*, *Fusarium*, and *Nigrospora* were the most common molds found. Until this study, no survey of indoor airborne fungi had been undertaken in Taiwan.

In the present study 88 houses in four different regions of Taipei (Fig. 1) were sampled for the presence of *Penicillium* species. Two plates with modified Mehrlich's Medium were exposed in the living room and bedroom of each house for 20 min. Colony numbers varied from 1 to 329 per house. A total of 5897 fungal isolates was obtained (Table 1). The highest counts (2282) were found in Region III, the area with the densest population. Of the total counts, 910 (15.4%) were identified as *Penicillium* spp.,

Table 2. Main characteristics of the three most prevalent indoor airborne *Penicillium* species isolated in Taipei, Taiwan

| Characteristics              | <i>P. citrinum</i>           | <i>P. implicatum</i> | <i>P. crustosum</i>        |
|------------------------------|------------------------------|----------------------|----------------------------|
| Colonies at 25°C for 7 days: |                              |                      |                            |
| Diameter (mm)                |                              |                      |                            |
| CYA <sup>a</sup>             | 20–32                        | 18–22                | 38–45                      |
| MEA <sup>a</sup>             | 13–20                        | 16–21                | 30–45                      |
| G25N <sup>a</sup>            | 13–20                        | 10–16                | 25–30                      |
| Color                        |                              |                      |                            |
| CYA                          | Grayish green                | Dull green           | Dull green                 |
| MEA                          | Dull green                   | Dull green           | Green                      |
| G25N                         | Yellowish green              | Dull green           | Grayish green              |
| Germination at 5°C (CYA)     | No                           | No                   | Germinate                  |
| Growth at 37°C (CYA)         | 10–15 mm                     | No                   | No                         |
| Penicilli                    | Biverticillate               | Monoverticillate     | Terverticillate            |
| Stipe                        |                              |                      |                            |
| Roughness of walls           | Smooth or slightly roughened | Smooth               | Roughened                  |
| Conidia                      |                              |                      |                            |
| Shape                        | Spherical, subspheroidal     | Ellipsoidal          | Ellipsoidal, subspheroidal |
| Size (µm)                    | 2.1–3.0 diam.                | 2.6–3.6 × 2.2–2.5    | 3.0–4.0 × 2.8–3.5          |
| Roughness of walls           | Smooth or finely roughened   | Finely roughened     | Smooth or finely roughened |

<sup>a</sup> Media as described in Materials and Methods.

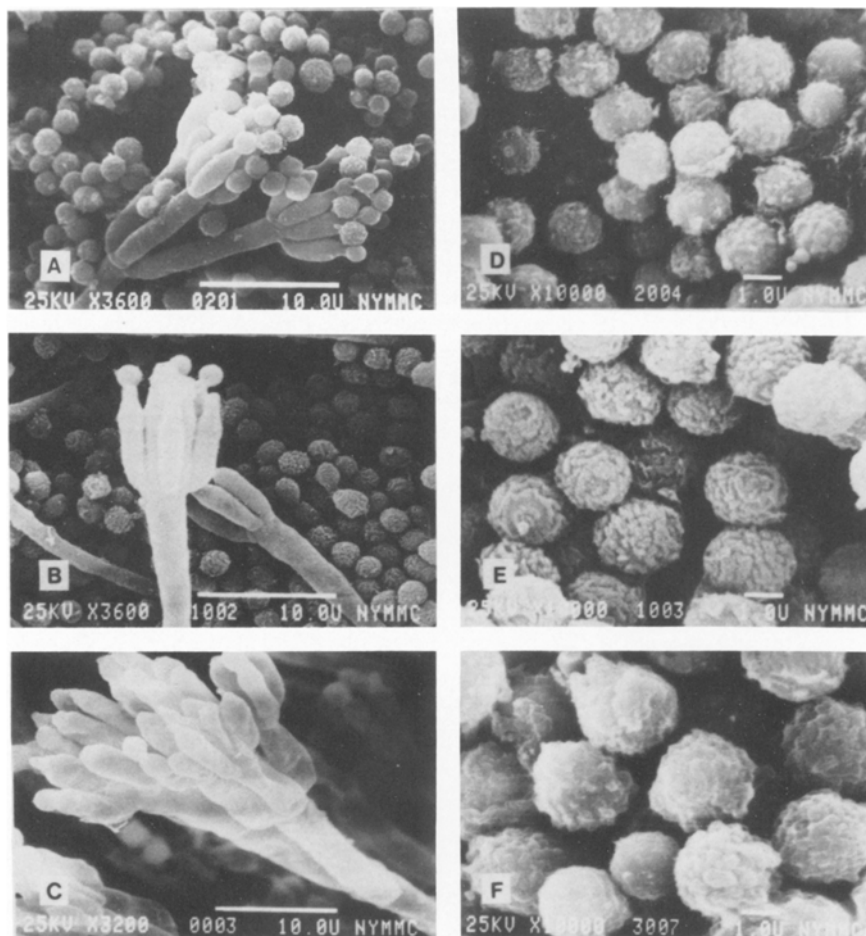


Fig. 2. Scanning electron micrographs of penicilli (A–C) and conidia (D–F) of the three prevalent indoor airborne *Penicillium* species in Taiwan. (A) and (D) *P. citrinum*; (B) and (E) *P. implicatum*; (C) and (F) *P. crustosum*.

53.0% (482/910) of which were obtained in bedrooms and 47.0% (428/910) in living rooms. The occurrence of *Penicillium* spp. in both rooms was similar in all sampling areas, except for Region I (Table 1). An unusually high percentage (66.1%) was found in one house located in Ta-An District of Region III.

Indoor plate sampling revealed a high incidence of *P. citrinum*, *P. crustosum* and *P. implicatum*. Of the 910 *Penicillium* isolates, 369 (40.5% from 28 houses) were *P. citrinum*; 47 (5.2% from three houses) were *P. implicatum*, and 19 (2.1% from seven houses) were *P. crustosum*. Pitt [12] has suggested that *P. citrinum* may well be one of the most common eukaryotic life forms on Earth, flourishing in soil, decaying vegetation, and the air. Muilenberg et al. [10] found that *P. citrinum* was one of the five most frequently recovered species in the living room and outside Topeka homes. Other investigators [6, 8] have reported that *Penicillium* and *Aspergillus* spp. are the dominant indoor molds and that their occurrence is not affected by seasonal changes [4].

The main characteristics of the three *Penicillium* species are summarized in Table 2. Scanning electron micrographs of penicilli and conidia show that *P. citrinum*, *P. implicatum*, and *P. crustosum* are biverticillate, monoverticillate and terverticillate, respectively (Fig. 2A–C); conidial walls are smooth or finely roughened (Fig. 2D–F).

The present investigation demonstrated that *P. citrinum* was the most frequently isolated indoor airborne fungus in the Taipei area of Taiwan. The allergenic significance of this ubiquitous mould is unknown, but is under further investigation.

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