

Short communication

Enumeration of airborne molds in some indoor environments of Madras city (India) by cultural and non-cultural volumetric samplers

Balumuri Pandu Ranga Vittal^{*a}, Shaik Khaja Rasool^b

^aCentre for Advanced Studies in Botany, University of Madras, Guindy Campus, Madras 600 025, India

^bBotany Department, The New College, Madras-600 014, India

Received 25 September 1993; revision received 21 March 1995; accepted 5 April 1995

Abstract

The air mycoflora of six indoor environments in Madras city (India) has been investigated by sampling air with an Andersen sampler and a Burkard personal sampler. Forty-eight species assignable to 24 genera were recorded by Andersen sampler. Spores belonging to 14 genera in addition to *Penicillium* and *Aspergillus* were identified from Burkard trap slides. Species of *Aspergillus*, *Penicillium*, *Mucor* and *Rhizopus* were most frequently isolated in considerable numbers. As a single genus *Aspergillus* ranked first followed by *Penicillium* at some sites, and *Cladosporium* at some other sites. The predominance of *Penicillium* and *Aspergillus* was also confirmed by Burkard trap data. Spores belonging to *Ganoderma*, *Nigrospora*, *Epicoccum*, and *Tetraploa* were recorded only by Burkard sampler, thereby suggesting the necessity of using two complimentary spore traps, cultural and non-cultural, in any aerobiological investigation.

Keywords: Airborne molds; Indoor environments; Andersen sampler; Burkard personal sampler

1. Introduction

The outdoor fungal airspora has been enumerated and identified from many parts of the world, including India, mostly by non-volumetric samplers or occasionally by using either a cultural or non-cultural volumetric sampler, but rarely both. The airborne molds of Madras, the fourth largest city in India, and its suburbs were monitored earlier with a Burkard 7-day sampler (Vittal and Krishnamoorthy, 1981, 1988, 1989). The present paper describes the results of a comparative study of airborne fungi in six indoor environments of Madras city, monitored by using cultural and non-cultural volumetric samplers.

2. Methods

Investigations were carried out in six indoor environments, viz. bakery, school, dormitory, finished leather

store house, college library (non-air conditioned), outpatient room of a hospital and poultry house. Air sampling was carried out by using together an Andersen (Andersen, 1958) cultural (microbial) sampler (Andersen Instruments Inc., Atlanta, Georgia, USA) and a Burkard personal volumetric sampler (Burkard manufacturing Co. Ltd. Rickmansworth, Hertfordshire, England). Five samples at approximately 3-week intervals were taken at each of the six sites between February and April 1989. Petriplates containing Potato-dextrose agar were used for exposure in the sampler and the duration of sampling was 5 min. The sampler was placed on a tin of 30 cm height so that the intake orifice pointing upwards was at 50 cm above ground level. Colony counts and identifications were made after 5 days of incubation at room temperature and the colony counts were not transformed for multiple impactions. The number of colonies were converted to per cubic metre of air and the data expressed as Colony forming units (CFU).

the Burkard personal sampler was operated with its

* Corresponding author.

orifice at 50 cm above ground level for 5–15 min, depending on the site, at a flow rate of 10 l/min. The exposed slides were mounted in 'Gelvatol' (supplied by Burkard manufacturing Co., UK) and allowed to dry

for 48 h before being scanned. Spore types were identified up to the generic level. As spores of *Aspergillus* and *Penicillium* could not be distinguished on the basis of morphology, they were grouped collectively under the

Table 1

Species of fungi isolated and their mean number of CFU/m³ in different indoor environments

Species	Bak.	Dorm.	L.G.	Lib.	Hos.	P.H.
Zygomycotina						
<i>Absidia glauca</i>	—	—	—	3	1	—
<i>Circinella simplex</i>	—	—	1	3	—	—
<i>Cunninghamella echinulata</i>	—	—	1	—	—	3
<i>Mucor racemosus</i>	4	7	11	5	7	7
<i>Rhizopus nigricans</i>	17	18	20	21	20	29
<i>Syncephalastrum racemosum</i>	—	8	—	4	3	—
Deuteromycotina						
<i>Alternaria alternata</i>	—	20	—	14	6	14
<i>A. longipes</i>	—	3	—	—	—	—
<i>A. tenuissima</i>	—	14	—	—	—	—
<i>Aspergillus flavipes</i>	—	—	6	—	—	—
<i>A. flavus</i>	737	58	81	88	78	138
<i>A. fumigatus</i>	3	21	120	88	43	16
<i>A. japonicus</i>	—	3	3	1	6	3
<i>A. nidulans</i>	133	18	14	6	16	4
<i>A. niger</i>	3040	271	367	460	454	130
<i>A. ornatus</i>	—	3	—	—	—	—
<i>A. sydowii</i>	—	—	1	—	—	—
<i>A. terreus</i>	16	30	30	60	34	77
<i>A. versicolor</i>	3	26	11	245	10	11
<i>A. wentii</i>	—	1	—	—	—	3
<i>Cephalosporium sp.</i>	—	1	—	—	—	3
<i>Chrysosporium sp.</i>	—	—	—	3	—	—
<i>Cladosporium oxysporum</i>	—	3	13	—	—	—
<i>C. cladosporioides</i>	—	4	—	—	1	133
<i>C. herbarum</i>	—	87	—	14	—	117
<i>Curvularia lunata</i>	—	6	—	10	3	3
<i>C. pallescens</i>	—	—	1	8	4	—
<i>C. eragrostidis</i>	—	8	—	—	—	—
<i>Drechslera australiensis</i>	3	63	—	4	4	6
<i>D. hawaiiensis</i>	—	44	10	3	3	—
<i>Fusarium semitectum</i>	—	8	—	—	—	8
<i>Gliocladium roseum</i>	6	14	—	88	3	4
<i>Humicola fuscoatra</i>	—	—	—	—	—	71
<i>Microsporium gypseum</i>	—	4	—	—	—	—
<i>Paecilomyces fusisporus</i>	—	—	—	—	—	3
<i>P. variotii</i>	—	6	—	—	—	—
<i>Penicillium citrinum</i>	11	16	53	20	14	—
<i>P. chrysogenum</i>	7	24	77	63	20	93
<i>P. funiculosum</i>	—	—	1	—	—	—
<i>P. implicatum</i>	—	—	3	—	—	—
<i>P. javanicum</i>	—	—	1	—	—	—
<i>Phoma sp.</i>	—	3	—	—	—	—
<i>Pithomyces atrovivaceus</i>	—	1	—	—	1	24
<i>Scopulariopsis brevicaulis</i>	—	—	—	—	—	86
<i>Stachybotrys parvispora</i>	—	—	—	—	1	—
<i>Trichoderma viride</i>	1	7	—	214	—	—
<i>T. harzianum</i>	—	1	—	1	—	—
Non-sporulating	1	44	8	30	23	47

Bak.: Bakery, Dorm.: Dormitory, L.G.: Leather store house, Lib.: Library, Hos.: Hospital, P.H.: Poultry House, and — : indicates absence.

category 'Pen./Asp.'. The counts are expressed as number per cubic meter of air.

3. Results

3.1. Andersen sampler

Levels of fungus colony forming units (CFU) isolated from the six indoor environments sampled are presented in Table 1. The 48 species isolated in the course of the present study are distributed over 24 genera. The largest number (33 spp.) were recovered from a dormitory and the least 13 spp. from a bakery. *Aspergillus flavus*, *A. fumigatus*, *A. nidulans*, *A. niger*, *A. terreus* and *A. versicolor*, *P. chrysogenum*, *M. racemosus* and *R. nigricans* were recovered from all six environments and these appear to be the most common of the environments sampled. A high percentage of species were infrequently isolated with very low CFU/m³ values.

Aspergillus niger was numerically the most abundant in all the six environments, except at the poultry house. Its contribution was highest in the bakery and least in the dormitory. *A. flavus*, which was ranked first in the poultry house, was second in the bakery and hospital, and third in the dormitory, while *C. cladosporioides* was second in the poultry house. *T. viride* showed high frequency in the library atmosphere, while *A. fumigatus* showed relatively high frequency in the hospital.

3.2. Burkard personal sampler

Spores belonging to 14 genera, viz. *Alternaria*, *Botryodiplodia*, *Cladosporium*, *Curvularia*, *Drechslera*, *Epicoccum*, *Fusarium*, *Ganoderma*, *Nigrospora*, *Periconia*, *Pithomyces*, *Scopulariopsis*, *Tetraploa* and *Torula*, in addition to 'Pen./Asp.', were identified from the exposed slides. Maximum number of spore types were recovered from the poultry house. Spores of *Alternaria*, *Cladosporium*, *Curvularia*, *Drechslera*, *Nigrospora*, *Pithomyces* and 'Pen./Asp.' were recorded at all the sites. 'Pen./Asp.' were found to be most abundant in the air of the bakery (95.1%), poultry house (39.8%) and leather store house (20.9%). On the other

Table 3

Analysis of variance of air sampling data (Burkard sampler)

Sources of variation	df	Sum of squares (10 ⁷)	F-value
Among samplings	4	9.78	0.33 ns
Among spore types	6	240.75	5.36 ^a
Among sites	5	181.2	4.84 ^a
Residual	194	1451.55	
Total	209	1883.34	

ns — non-significant

^asignificant at $P < 0.0001$

hand, *Cladosporium* has emerged as the predominant component in the air of the hospital (28%), while *Pithomyces* was dominant in the library (24.6%) and dormitory (37.4%). *Cladosporium* ranked second in the order of dominance in the air of the leather store house and dormitory. 'Pen./Asp.' ranked second in the hospital, *Scopulariopsis* in the poultry house and *Curvularia* in the library.

3.3. Statistical analysis

To explore the statistical significance of differences between samples from the same site, among different sites as well as among different species/spore types, an analysis of variance test was carried out (Tables 2 and 3). ANOVA test showed that there were no significant differences between samples taken from the same site. However, the mean effects among species/spore types as well as among sites differed significantly ($P < 0.0001$). Further, the Duncan Multiple Range Test (DMRT) at 5% level indicated that *A. niger* in Andersen sampler catches and 'Pen./Asp.' in Burkard sampler catches differed significantly from other species which fell into one group. Similarly, the bakery environment was significantly different as compared to others.

4. Discussion and conclusions

The data obtained in the present study emphasise the value of using two trapping methods, cultural and non-cultural, in an aerobiological investigation. That neither sampling approach alone is adequate even in sampling the most prevalent deuteromycete taxa has been emphasized by Burge et al. (1977).

Forty-eight species of fungi, mostly belonging to *Deuteromycetes* (85% of identified species), were isolated and identified from the six indoor environments by Andersen sampler which included many potential allergens. Species of *Aspergillus*, *Penicillium*, *Cladosporium*, *Alternaria*, *Drechslera* and *Curvularia* were most frequently isolated, with significant differences ($P < 0.0001$) among sites. Relatively large number of taxa including those characteristic of outdoor air were isolated.

Table 2

Analysis of variance of air sampling data (Andersen sampler)

Sources of variation	df	Sum of squares (10 ⁵)	F-value
Among samplings	4	3.13	0.46 ns
Among species	8	149.84	10.93 ^a
Among sites	5	50.64	5.91 ^a
Residual	252	431.84	
Total	269	635.45	

ns — non-significant

^asignificant at $P < 0.0001$

ted from all the sites except the bakery. The numbers and types of fungus spores in indoor air seem to depend on air exchange with the outside and the presence of indoor spore sources (Lacey 1981; Caretta, 1992). The recovery of *Microsporum gypseum*, a human pathogen, from the dormitory points to the availability of a local source. Dermatophyte infections are known to be common in adults and school children living in old age homes and orphanages, respectively, in Madras city.

Aspergillus species turned out to be most frequent and abundant in all the indoor environments, with highest frequency in the bakery. The genus *Penicillium* showed a tendency to occur invariably next to *Aspergillus* with a maximum in the leather store house. *Cladosporium* was recovered in large numbers in the poultry house, which was surrounded by dense vegetation. Living or dead vegetation is known to act as the main source of the air-spores (Gregory 1973). The predominance of *Aspergillus* and *Penicillium* in the air of indoor environments was established by earlier workers (Noble and Clayton, 1963; Chute and Barden, 1964; Alteras and Lehrer, 1977; Burge et al, 1978; Jayaprakash, 1978; Rati et al. 1980). Their predominance in the air of the bakery, poultry house and leather store house has also been confirmed by Burkard sampler data. Similarly, the high frequency of *Cladosporium* in the dormitory and hospital was also revealed by Burkard sampler. Basidiospores of *Ganoderma* and conidia of *Nigrospora*, *Tetraploa*, and *Epicoccum* found on Burkard trap slides could not be recovered on the plates of Andersen sampler. While *Aspergillus* and *Penicillium* provide a larger proportion of the catch on culture plates, the dominance of conidial fungi such as *Cladosporium* and *Nigrospora* is normally revealed only on slides exposed in traps such as the Burkard sampler. Thus, it may be necessary to use both cultural as well as direct examination methods to get a total picture of the air mycoflora of any environment.

Acknowledgements

The authors are thankful to the Director, CAS in Botany, for facilities. The first author (B.P.R.V) is extremely thankful to the Society for General Microbiology, UK, and Dr. J. Lacey, Rothamsted Experimental Station, Harpenden, UK for donating the Burkard personal sampler. Thanks is also due to various agencies and authorities for permission to take air samples in their respective organisations.

References

- Alteras, M.D. and Lehrer, N. (1977) Fungal flora in the air of a large hospital. *Castellania* 11, 217–219.
- Andersen, A.A. (1958) New sampler for the collection, sizing and enumeration of viable airborne particles. *J. Bacteriol.* 76, 471–484.
- Burge, H.P., Boise, J.R., Rutherford, J.A. and Solomon, W.R. (1977) Comparative recoveries of airborne fungus spores by viable and non-viable modes of volumetric collection. *Mycopathologia* 61, 27–33.
- Burge, H.P., Boise, J.R., Solomon, W.R. and Bandra, E. (1978) Fungi in libraries. An aerometric survey. *Mycopathologia* 64, 67–72.
- Caretta, G. (1992) Epidemiology of allergic diseases: the fungi. *Aerobiologia* 8, 438–445.
- Chute, H.L. and Barden, E. (1964) The fungus flora of chick hatcheries. *Avian Dis.* 8, 13–19.
- Gregory, P.H. (1973) *Microbiology of the atmosphere*. New York, John Wiley & Sons.
- Jayaprakash, K.B. Rati, E. and Ramalingam, A. (1978) *Aspergillus flavus* in the air of working environments. *Curr. Sci.* 47, 920–921.
- Lacey, J. (1981) The aerobiology of conidial fungi. In: G.T. Cole and B. Kendrick (Eds.), *Biology of conidial fungi*. Vol II. London, Academic press, pp. 373–416.
- Noble, W.C. and Clayton, Y.M. (1963) Fungi in the air of hospital wards. *J. Microbiol.* 32, 397–402.
- Rati, E., Jayaprakash, K.B. and Ramalingam, A. (1980) Air spora of a poultry shed at Mysore. *Ind. J. Microbiol.* 20, 6–11.
- Vittal, B.P.R. and Krishnamoorthy, K. (1981) Air spora of an agricultural farm in Madras, India. *Grana* 20, 61–64.
- Vittal, B.P.R. and Krishnamoorthy, K. (1988) A census of airborne mold spores in the atmosphere of the city of Madras, India. *Ann. Allergy* 60, 99–101.
- Vittal, B.P.R. and Krishnamoorthy, K. (1989) Circadian and seasonal periodicities of some mold allergens in the atmosphere of Madras city, India. *Proc. Ind. Acad. Sci. (Pl. Sci.)* 99, 247–251.