

ASSESSMENT OF INDOOR FUNGI IN ALLERGIC PATIENTS' HOUSES AND THEIR BIOCHEMICAL CHARACTERIZATION

Murlidhar Mishra¹, Priyanka Sinha² and Md. Minhaj Alam³.

Key words : Allergen, *Aspergillus flavus*, *Rhizopus nigricans*, SDS-PAGE.

Allergy, which is an altered immune response, has become a common health problem with variable consequences, some being fatal in severe cases. About 10-20% of human population suffers from one or the other form of allergy, particularly respiratory diseases. The fungal allergens consisting of fungal spores and mycelia are of significance with respect to their allergenic manifestations. They are also present in the indoor environment. Assessment of indoor fungi and their biochemical characterization included *Aspergillus flavus* and *Rhizopus nigricans*. The characterization was done with the help of SDS-PAGE which revealed presence of allergenic proteins inciting immune response. The present investigation clearly indicates that the indoor fungal allergens are one of the major causes of allergies, particularly in atopic individuals.

INTRODUCTION

Allergic diseases are one of the most important problems of the present human pathology all over the world. About 12-20% of the world population is known to suffer from different allergic disorders such as bronchial asthma, rhinitis and urticaria (Smith & Salvin, 1998; Chhabra *et al.*, 1998; Charpin *et al.*, 2000). Allergy is an altered immune response. Fungi are also responsible for causing allergy in human beings. The fungal spores and mycelia contain allergenic proteins which are responsible for inciting immune response. The indoor fungi in bed dust and in indoor air are very active in causing allergies particularly in atopic allergic patients. The air inside building is often contaminated with particles and chemicals that adversely affect the health of occupants. Indoor allergens are mainly dust mites, animal dander and fungi. Assessment of indoor fungi has been done from different areas of New Delhi to know the fungal species responsible for causing allergies. Besides this, biochemical characterization of the isolated fungi was also done. Isolation of fungi was done from indoor air and bed dust. This work is the part of the project work undertaken at Institute of Genomics and Integrative Biology (IGIB), Delhi University new campus, New Delhi.

MATERIAL & METHODS

The aerobiological survey was done in allergic patients' houses belonging to the age group of 4-16 years. Sampling was performed in fifteen houses. Both air sampling and dust sampling were done to evaluate relationship between dust borne and air borne fungi. The air sampling was done by using Andersen Six Stage-Volumetric Sampler (Fig. 1) and dust sampling was done by using a vacuum cleaner (Euroclean Jet Vacuum Cleaner, Eureka Forb's Limited, India). It was followed by isolation and identification of fungi. The media used for air borne fungi was Sabourad's agar and DG-18 medium for dust borne fungi. Out of the various fungal colony types isolated from the indoor environment of the asthmatic patients, only dominant and frequently occurring types have been analyzed in detail for their periodic distribution over a period of two months. For detailed biochemical investigation mass culture of *Rhizopus nigricans* and *Aspergillus flavus* were done by preparing Sabourad's agar liquid media as per

composition. After complete sporulation of *Rhizopus nigricans* and *Aspergillus flavus* mass culture, the media was decanted and collected for antigen preparation. Biochemical characterization of extracted mycelial protein was done by using SDS-PAGE. The present study was started in the month of February and was carried upto the month of April.

OBSERVATION

The indoor fungi which were observed during aerobiological survey included various species of *Aspergillus* like *A. flavus*, *A. niger*, *A. fumigatus*, *A. ripens* (Fig. 2) and *Rhizopus nigricans*. *Aspergillus niger* was conspicuous throughout the sampling but considerably high concentration was observed in the month of February. Besides *Aspergillus spp.* and *Rhizopus spp.*, *Alternaria spp.* and *Epicoccum spp.* were also found with highest count during April. Comparative analysis between DG-18 media and Sabourad's media shows that *Aspergillus amstelodemi* was present only in DG-18 media with percentage contribution of 3.58%. The biochemical characterization of *Aspergillus flavus* showed bands in the molecular range of 14.4 kD to 112.2 kD. The bands of 14.4, 19.9, 36.3, 41.6, 112.2, 97.7 and 112.2 kD were most prominent (Fig. 3). The remaining bands were thin and resolved at molecular weight 72.4 and 25.7 kD. While the two bands of mycelial extracts of *Rhizopus nigricans* by SDS-PAGE had weight 83.1 kD and 89.1 kD, both the bands were prominent. Details of fungi reported during our survey have been given in Tables 1 and 2.

DISCUSSION

The indoor fungi are highly responsible for causing allergies in the human beings (Ragab and Samaka, 2013 and Mark and Anna, 2011). Allergens are usually proteins or glycoproteins with molecular weight ranging between 10 to 90 kD. In the present investigation the antigenic proteins having molecular weight ranging in between 14.4 kD to 112.2 kD were found to be more potent in inciting type-I mast cell mediated hypersensitivity reaction. From air samples, nineteen fungal types were isolated which belonged to ten genera of which *Aspergillus* was represented by seven species. From the dust sample 21 fungal types were isolated of which *Aspergillus flavus* and *Rhizopus nigricans* were found to be causing allergic disorders in immune suppressed individuals.

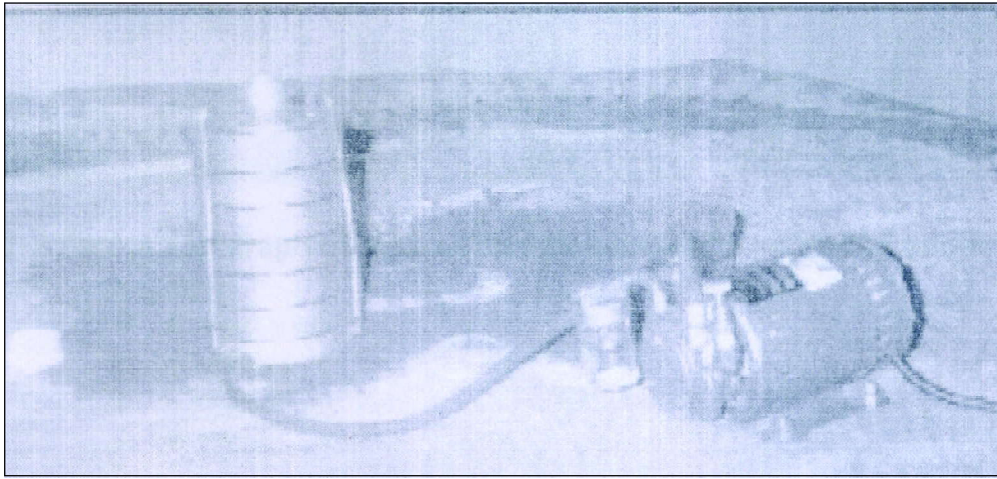


Fig. 1 : Photograph showing Andersen six Stage Sampler with an unexposed petriplate containing Sabourad's agar medium

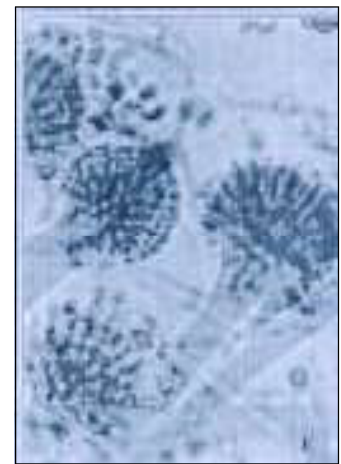
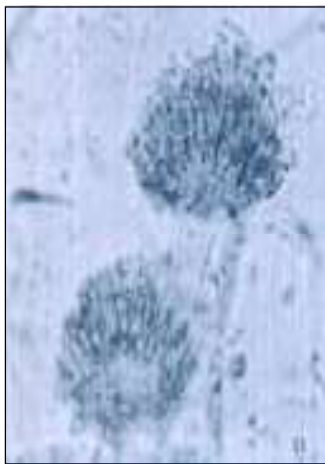
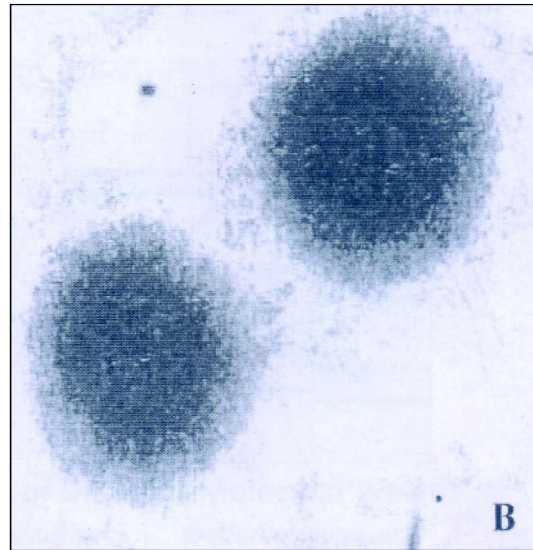
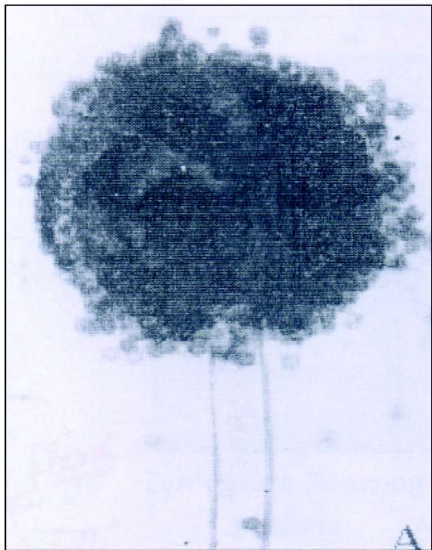


Fig. 2 : Photomicrograph of different fungal types isolated during sampling.

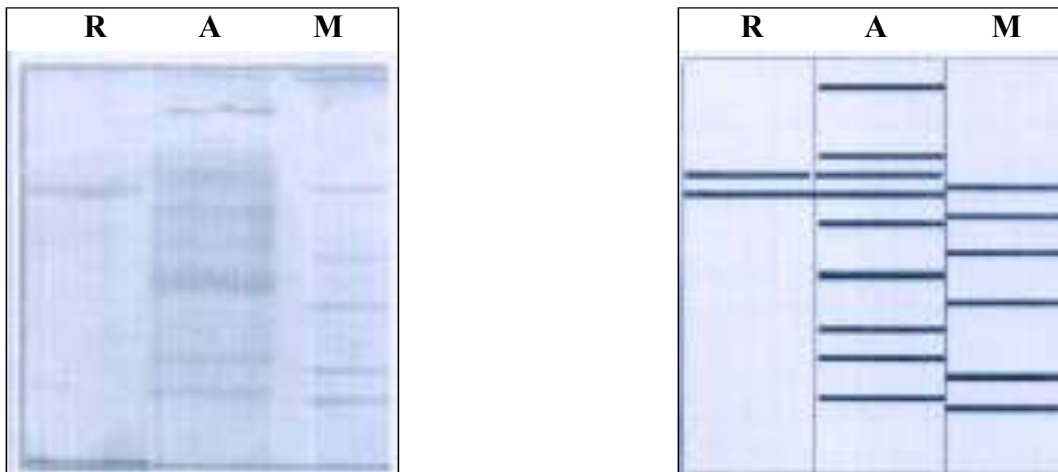
A. *A. niger*

B. *Aspergillus* spp.

C. *A. versicolor*

D. *A. terreus*

E. *A. repens*



Zymogram showing bands of different molecular weight

M=Marker,	A= <i>Aspergillus flavus</i>	R= <i>Rhizopus nigricans</i>
R	A	M
89.1 kD	112.2 kD	97.0 kD
83.1 kD	97.7 kD	66.0 kD
	91.2 kD	45.0 kD
	72.4 kD	30.0 kD
	41.6 kD	20.1 kD
	36.3 kD	14.4 kD
	25.7 kD	
	19.9 kD	
	14.4 kD	

TABLE-1 : Airborne fungal types isolated from indoor air of asthmatic patients over a period of two months (Feb. and April) survey twice a month.

Fungal types	S	C
1. <i>Alternaria spp.</i>	+	+
2. <i>Acremonium</i>	+	+
3. <i>A. flavus</i>	+	+
4. <i>A. fumigatus</i>	+	+
5. <i>A. granulosis</i>	+	-
6. <i>A. niger</i>	+	+
7. <i>A. repens</i>	+	+
8. <i>A. terreus</i>	-	+
9. <i>A. versicolor</i>	+	+
10. <i>Aspergillus sp.</i>	+	+
11. <i>Cladosporium</i>	+	+
12. <i>Dreschlera</i>	+	+
13. <i>Epicoccum</i>	+	+
14. <i>Fusarium</i>	+	+
15. <i>Paecilomyces</i>	+	-
16. <i>Penicillium</i>	+	+
17. <i>Spicariaedulis</i>	+	-
18. <i>Sporotricum</i>	+	+
19. <i>Stacchybotrys</i>	+	+

S=Indoor

C=Control

TABLE-2 : Dust borne fungal types isolated from asthmatic patients over a period of two months at rate as colony forming units.

Fungal types	S	C
1. <i>Alternaria spp.</i>	+	+
2. <i>A. amstelodami</i>	+	+
3. <i>A. awamori</i>	+	-
4. <i>A. candidus</i>	+	+
5. <i>A. flavus</i>	+	+
6. <i>A. fumigatus</i>	-	+
7. <i>A. nidulans</i>	+	-
8. <i>A. niger</i>	+	-
9. <i>A. niveus</i>	-	-
10. <i>A. repens</i>	+	+
11. <i>A. terreus</i>	-	+
12. <i>A. versicolor</i>	+	+
13. <i>Cladosporium</i>	+	+
14. <i>Dreschlera</i>	+	+
15. <i>Epicoccum</i>	+	+
16. <i>Fusarium</i>	+	+
17. <i>Paecilomyces</i>	+	-
18. <i>Penicillium</i>	+	+
19. <i>Spicariaedulis</i>	+	-
20. <i>Sporotricum</i>	+	+
21. <i>Stachybotrys</i>	+	+
22. <i>Syncephalastrum</i>	+	-

S=Indoor

C=Control

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