

Airborne viable fungi in Riyadh and allergenic response of their extracts

Luftbürtige Pilze in Riad und allergene Potenz ihrer Extrakte

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Schlüsselwörter. Luftbürtige Pilze, Allergenität, Antigenität, Prick-Test, Saudi-Arabien.

Summary. The allergenicity and antigenicity of various airborne fungi isolated from the atmosphere of Riyadh were studied. Protein nitrogen contents were estimated and found to range from 0.9 mg ml⁻¹ for *Cladosporium* to 2.1 mg ml⁻¹ for *Aspergillus* extracts. Sodium dodecyl sulphate–polyacrylamide gel electrophoresis analysis for those extracts exhibited a number of protein bands of higher molecular weight between 13 and 80 kDa for *Alternaria*, *Ulocladium*, *Penicillium*, *Aspergillus* and *Cladosporium*. Extracts in both aqueous and lyophilized forms were sterilized and tested for diagnostic skin prick test in 100 consecutive patients having bronchial asthma and allergic rhinitis. Overall, 13% of patients reacted positively to fungal extracts, revealing allergic sensitization to these fungi. These findings necessitate further investigation as regards the purification and characterization of these local extracts for better diagnostic use in patients in Saudi Arabia.

Zusammenfassung. Es wurde die Allergenität und Antigenität mehrerer luftbürtiger Pilze untersucht, die aus der Luft von Riad isoliert worden waren. Hierzu wurden Pilzextrakte hergestellt, deren Proteinstickstoffgehalt zwischen 0.9 mg ml⁻¹ bei *Cladosporium* und 2.1 mg ml⁻¹

bei *Aspergillus* lag. Die SDS–PAGE-Analyse zeigte eine Anzahl von Proteinbanden höheren Molekulargewichts zwischen 13 und 80 kDa für *Alternaria*, *Ulocladium*, *Penicillium*, *Aspergillus* und *Cladosporium*. Die sowohl wässrigen wie lyophilisierten Extrakte wurden sterilisiert und an 100 unausgewählten Patienten mit Bronchialasthma und allergischer Rhinitis im Pricktest getestet. Insgesamt 13% der Patienten reagierten auf die Extrakte positiv, was für eine allergische Sensibilisierung gegen diese Pilze spricht.

Introduction

Different fungal spores can act as sensitizing agents for the development of allergic diseases. Two examples are *Aspergillus* [1] and *Penicillium*, which cause bronchopulmonary allergic diseases in man [2–5]. Most studies of fungi as aeroallergens have been limited to the fungi imperfecti, with little information available concerning the possible allergenicity of members of other fungal classes [6]. However, the importance of atmospheric mold spores in clinical allergy has been well established for many years. Respiratory allergic reactions to fungi were quite common in clinical patients [7–9]. Goldstein and Chai [10] reported a reduced specific bronchial sensitivity in a few patients treated with *Cladosporium* or *Alternaria* extracts [11,12]. Agarwal *et al.* [13] studied the relationship between the air spores and skin prick test reaction of patient and documented the importance of airborne fungi in allergy [14]. Lumpkins *et al.* [15] found a correlation between fungi (e.g. *Cladosporium*, *Alternaria*, *Penicillium*, etc.) in the home and skin testing of patients to extracts from the same fungal genera.

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The aim of the present study was to evaluate the response of extracts from various fungal isolates from the environment in Riyadh by conducting immunoglobulin E (IgE)-mediated skin prick tests in allergic patients in the same city.

Materials and methods

Preparation of fungal extracts (antigens)

The major common allergens, such as *Cladosporium* spp., *Aspergillus* spp., *Penicillium* spp., *Alternaria* spp. and *Ulocladium* spp., were inoculated in 1-l flasks containing 250 ml of Czapek semi-synthetic liquid media (Oxoid, Basingstoke, UK). Cultures were incubated at room temperature for 3–10 weeks depending on the growth of fungi. The mycelia were harvested, separated from the liquid medium and defatted in chloroform and methanol (2:1) (v/v) for 24 h on a rotary shaker. The defatted mycelia were filtered through Whatman filter paper no. 1 and dried at room temperature for 24 h. Dried fungal mats were weighed and ground to a fine powder with pestle and mortar. The powders were extracted in 1:50 (w/v) of phosphate buffered saline (PBS) pH 8, containing 0.4% (w/v) phenol crystals as preservative. The extracts were shaken for 72 h at 4 °C in the cold room and centrifuged at 20 000 g for 30 min. Centrifugation was repeated for 30 min at 4 °C [16], and the supernatant was dialysed (cut-off 6000–8000, Spectroapor® Industries Inc., Los Angeles, USA) against 25% PBS for 6 h at 4 °C, then dialysed again overnight against double-distilled water. The dialysed materials were lyophilized using a freeze-drier system (Model 5, Labconco, New York, USA). Aqueous extracts were filter sterilized using 0.22-µm millipore filter. Sterility was tested on blood agar, then 50% glycerin was added to each antigen extract and they were sent to King Khalid Hospital, King Saud University for skin testing. Part of these batches was stored at –20 °C until use for protein estimation and electrophoresis analysis.

Protein determination

Protein content of extracts was estimated using a COBAS-BIO Machine.

Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE)

SDS–PAGE was used to determine the molecular weight of fungal antigen extracts according to Laemmli [17].

Sample preparation

Fungal allergen extracts of each species, *Cladosporium* spp., *Ulocladium* spp., *Aspergillus* spp., *Penicillium* spp. and *Alternaria* spp., were mixed with the sample buffer (reagent E) at a dilution that depended on the protein concentration of each fungal species. Samples (extracts) were heated at 90 °C for 3 min in a water bath.

Experimental procedure

Fifty microlitres of the molecular weight marker and 100 µl the fungal antigen extracts were loaded carefully onto the wells using a micro-syringe (100-µl capacity). The upper reservoir was filled with a running buffer. The electrophoresis apparatus (Protean TM 11, Bio-Rad, Hercules, CA, USA) was connected to the power supply (ATTO, mini-power, Rockville, MD, USA) for 14 h at 30 mA constant current.

Staining of gels

After electrophoresis, gels were stained with a solution of 0.1% (w/v) Coomassie blue (Coomassie Brilliant Blue R-250, Bio-Rad) dissolved in methanol/acetic acid/distilled water (45/10/45 volumes) at room temperature with continued shaking for 60 min. Gels were then destained by washing several times with 40% methanol containing 10% acetic acid, until the appearance of the bands against a clear background. The destained gels were then photographed and scanned to determine different protein bands using a KB 2202 Laser Densitometer and LKB 2220 recording integrator.

Results

The genera identified for the whole year are presented. Figure 1 shows the mean percentage of various airborne components in Riyadh. The genera were classified as 'major' or 'minor' depending upon their frequency of appearance and numbers in the air sample [18].

The SDS–PAGE analysis of fungal extracts gave a range of bands (Figs 2 and 3; Table 1). The figures show that *Alternaria* extracts gave a number of bands, ranging from 13 to 79 kDa. *Ulocladium* extracts gave three bands, ranging from 22 to 27 kDa. *Penicillium* extracts gave a wide range of about 15 bands, ranging from 13 to 77 kDa. *Aspergillus* extracts resolved into nine bands. These bands ranging from 34 to 79 kDa. *Cladosporium* extracts gave four bands from 34 to 79 kDa.

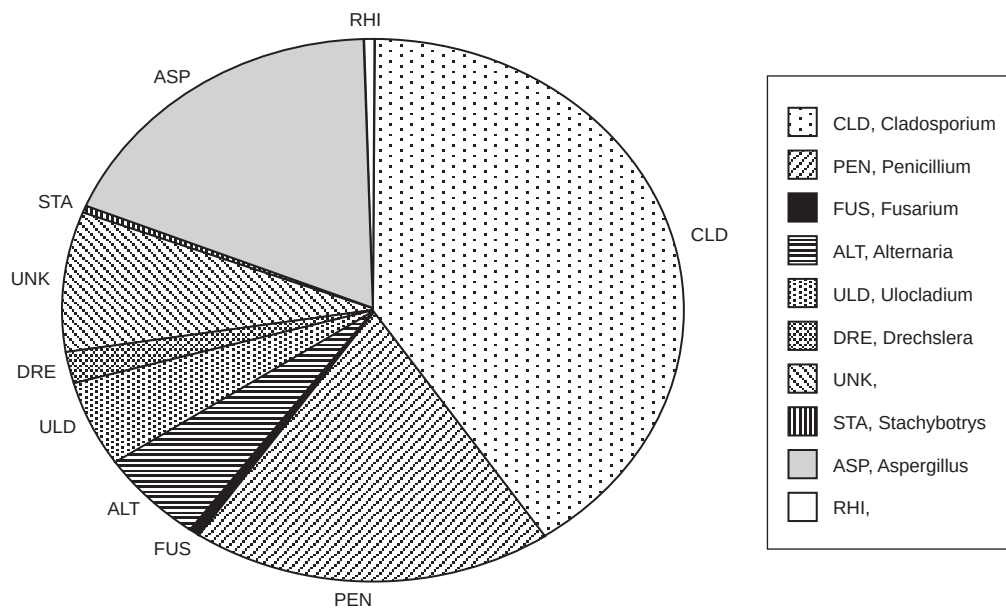
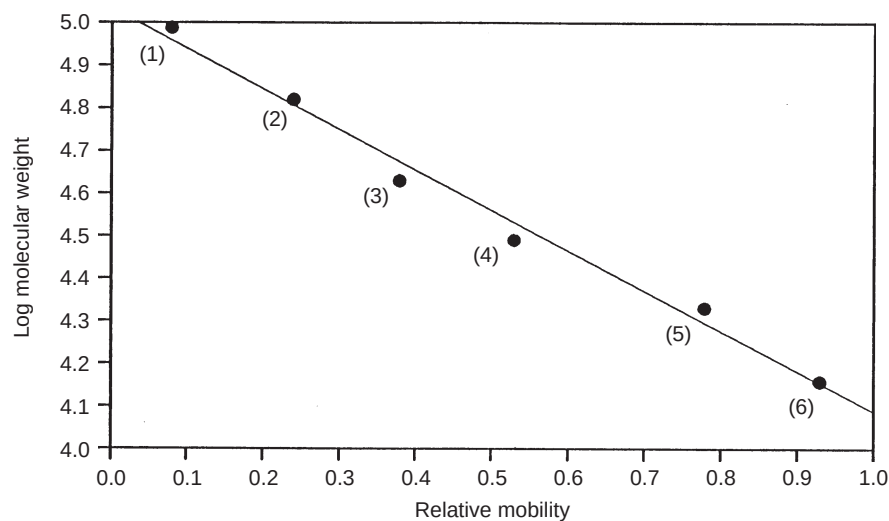


Figure 1. Mean percentage of various airborne components of the two sites in Riyadh: CLD, *Cladosporium*; PEN, *Penicillium*; FUS, *Fusarium*; ALT, *Alternaria*; ULD, *Ulocladium*; DRE, *Drechslera*; UNK, unknown and damaged spores; STA, *Stachybotrys*; ASP, *Aspergillus*; RHI, *Rhizopus*.



Standard curve of six Molecular Weight marker; (1) Phosphorylase b 92,500; (2) Bovine serum albumin (BSA) 67,200; (3) Ovalbumin 42,000; (4) Carbonic anhydrase 31,000; (5) Soybean trypsin inhibitor 21,500; (6) Lysozyme 14,400

Figure 2. Standard curve of six molecular weight markers showing log molecular weight against relative mobility, used for estimating the molecular weight of local fungal extracts: (1) phosphorylase b 92 500; (2) bovine serum albumin 67 200; (3) ovalbumin 42 000; (4) carbonic anhydrase 31 000; (5) soybean trypsin inhibitor 21 500; and (6) lysozyme 14 400.

SDS-PAGE patterns of fungal extracts demonstrated differences in the intensity and numbers of bands. Some bands were faint, others were dark, several of these bands were in the same molecular weight range, e.g. *Alternaria* and *Ulocladium* had almost two common bands at 77 kDa, likewise *Aspergillus* and *Penicillium* had bands in the range of 26 kDa.

Protein content

The results of the protein determination of the fungal extracts are presented in Table 2. The table shows that all extracts contained protein ranging from 0.9 mg ml^{-1} (*Cladosporium* extracts) to 2.1 mg ml^{-1} for *Aspergillus* extract. Though the extracts were standardized on weight per

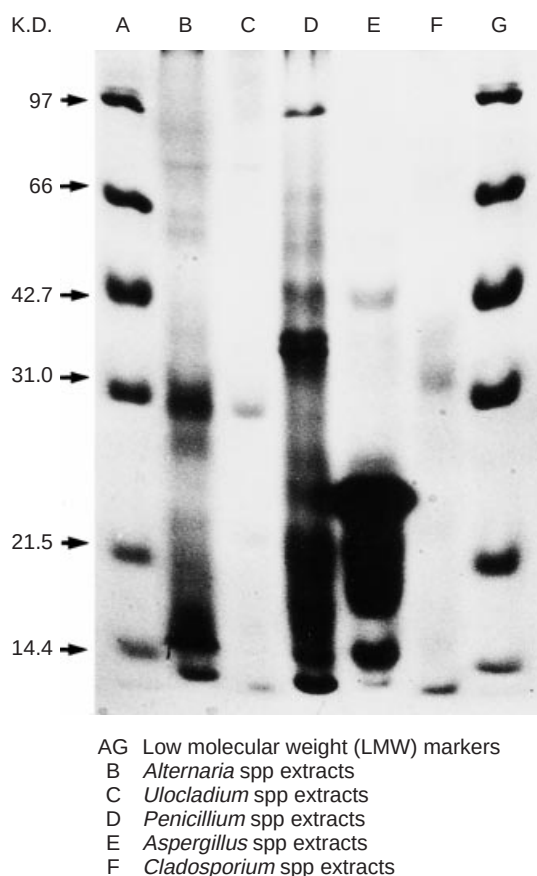


Figure 3. SDS-PAGE of four airborne fungi: lanes A and G, low molecular weight markers; lane B, *Alternaria* spp. extracts; lane C, *Ulocladium* spp. extracts; lane D, *Penicillium* spp. extracts; lane E, *Aspergillus* spp. extracts; and lane F, *Cladosporium* spp. extracts.

Table 1. Represents molecular weight range of local fungal extracts isolated from Riyadh atmosphere using SDS-PAGE techniques

Genus	Molecular weight range (kDa)
<i>Alternaria</i>	13–79
<i>Ulocladium</i>	22–77
<i>Penicillium</i>	13–77
<i>Aspergillus</i>	12–64
<i>Cladosporium</i>	34–79

These molecular weights were estimated from the log molecular weight vs. relative mobility as shown in Fig. 2.

volume (w/v) ratio, protein content showed some correlation in its content particularly, *Ulocladium*, *Drechslera* and *Stachybotrys* extracts.

Skin prick test

Clinical tests were conducted by qualified technicians under the supervision of physicians at King Khalid University Hospital, King Saud University, Riyadh. The fungal extracts prepared were

Table 2. Protein content for airborne fungal extracts isolated from Riyadh atmosphere using COBAS-BIO technique

Genus	Protein content (mg ml ⁻¹)
<i>Cladosporium</i>	0.9
<i>Penicillium</i>	1.7
<i>Aspergillus</i>	2.1
<i>Alternaria</i>	1.9
<i>Ulocladium</i>	1.2
<i>Drechslera</i>	1.3
<i>Stachybotrys</i>	1.3

tested in 100 consecutive patients attending the above clinic. Thirteen patients presenting symptoms of bronchial asthma or allergic rhinitis or both, reacted positively with varying sizes of wheal and erythema. One patient with broncho-pulmonary aspergillosis reacted to *Aspergillus* only. All 13 patients showed multiple reactions with commercial antigens tested along with local antigens (Table 3). The allergenic implications and/or effects of these positive results obtained will be interpreted and managed by the clinicians. However the result showed that antigens from local strains, prepared locally, gave a 13% positive result which should be further examined to obtain the clinical relevance and importance of local allergens for the benefit of allergy sufferers in the Kingdom.

Discussion

The present study is part of a comprehensive aerobiological survey of fungal spores in the Saudi environment [18–20]. Studies on fungi in relation to respiratory allergy, and perhaps, some other form of allergy, such as skin rashes, have been conducted world-wide using techniques ranging from the gravity slide of Durham to modern volumetric air samplers. It is surprising that certain types of fungi are isolated from all over the world and under all climatic conditions. We have just shown the abundance of certain fungi in the air and their concentration level. Undoubtedly, the five genera studied are major biological components of our environment. However, there are a number of minor components which may escape or be overlooked in relation to respiratory allergy in humans, yet they may be potent allergens, or they may play some other role in human diseases [21].

The sensitivity and reactivity of individuals vary from place to place, person to person and from age to age. An important factor in the elicitation of allergic symptoms is the antigenic

Table 3. Immediate skin test reaction to local fungal allergen in 100 consecutive patients attending the allergy clinic at King Khalid University Hospital

No.	Presenting complaint	Sex	Reaction to fungal allergens	Reaction to commercial allergens
1	Asthma	male	<i>Alternaria</i> 3 mm <i>Ulocladium</i> 3 mm	cat fur 5 mm wool 3 mm cotton flock 3 mm grass mix 6 mm
2	Asthma	male	<i>Ulocladium</i> 3 mm	cockroach 5 mm cotton 3 mm
3	Rhinitis	female	<i>Aspergillus</i> ? mm	cockroach 6 mm cotton 6 mm cat fur 8 mm
4	Asthma and rhinitis	male	<i>Aspergillus niger</i> 4 mm	<i>D. farinae</i> ^a 3 mm cockroach 3 mm <i>Chenopodium album</i> 6 mm
5	Rhinitis	male	<i>Alternaria</i> (a) 5 mm <i>Alternaria</i> (b) 4 mm <i>Ulocladium</i> (a) 8 mm <i>perenne</i> 5 mm <i>Ulocladium</i> (b) 5 mm	<i>Lolium</i> 6 mm <i>Poa praetense</i> 6 mm
6	Asthma	female	<i>Aspergillus</i> mix 3 mm	None
7	Asthma and rhinitis	female	<i>Aspergillus niger</i> 3 mm	<i>D. farinae</i> 6 mm cockroach 3 mm cotton 4 mm cat fur 8 mm <i>Chenopodium album</i> 5 mm
8	Asthma and rhinitis	male	<i>Alternaria</i> 6 mm <i>Ulocladium</i> 7 mm	<i>D. farinae</i> 5 mm
9	Asthma and rhinitis	female	<i>Alternaria</i> 3 mm <i>Aspergillus</i> n. 3 mm	cockroach 3 mm cat fur 9 mm
10	Rhinitis	male	<i>Cladosporium</i> mix 4 mm	cotton 6 mm cat fur 7 mm wool 5 mm grass mix 4 mm <i>Chenopodium album</i> 5 mm
11	Rhinitis	male	<i>Alternaria</i> 3 mm	cockroach 5 mm cotton 3 mm cat fur 10 mm wool 4 mm <i>Chenopodium album</i> 4 mm
12	Asthma and rhinitis	male	<i>Aspergillus</i> mix 4 mm	H.D. ^b mix 3 mm cockroach 7 mm cat fur 8 mm grass mix 4 mm
13	Rhinitis	male	<i>Cladosporium</i> 3 mm	H.D. mix 3 mm <i>D. farinae</i> 3 mm cotton 3 mm <i>Candida</i> 3 mm
14	Bronchopulmonary aspergillosis	male	<i>Aspergillus</i> mix 5 mm	None

^aDermatophagoides; ^bH.D. House dust.

component or foreign protein. Species-specific antigenicity for some inhalant allergens is known. Cross-reactivity among species and genera is also common. In addition, protein nitrogen content and biological activity of these antigens have been shown to be poorly correlated. Another factor is the mode of testing, one extract can give up to 5% positive reaction in skin testing, while the same extract can give up to 40% positive reaction in bronchial challenge or in inhalation tests. Thus by simple skin testing it is difficult to reveal the exact relationship or direct clinical importance of airborne organisms to individual's allergic manifestations. Nevertheless, it does indicate an IgE-mediated response to allergen(s) tested.

It appears that aeromycological investigation and acceptance of its role in allergy, compared to other allergenic components, such as house-dust mite, animal dander and pollen, have been slow in spite of advancements in equipment and technique. For the diagnosis of respiratory allergic diseases, there are a number of issues which need serious attention [22]. For example, multiple reactivities by the majority of allergens, the presence of various allergens in the air or in the

patient's surroundings, use of standardized and characterized antigen for both *in vitro* and *in vivo* diagnosis, etc.

Another problem in this field is the lack of good quality extracts and the lower success rate of desensitization (hyposensitization). Species isolated from the patient's environment can therefore be a useful source for the diagnosis of allergenic diseases.

The major part of this study concerns environmental analysis of allergy-producing agents or simply biological pollutants. However, investigation of the possible role of fungi in respiratory problems, such as bronchial asthma and allergenic rhinitis was our main objective. We have demonstrated this role by showing a total of 13% of patients to be positive to our local allergens. The study will help clinicians to adopt preventive measures and or choose a relevant antigen for diagnostic purposes. With basic information now emerging, further investigation with emphasis on purification and standardization of antigenic fractions are required in order to have a comprehensive and clear picture of the role of various fungi (spores) in allergic diseases in Saudi Arabia.

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