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PRELIMINARY INVESTIGATION OF ALLERGENIC FUNGI IN INDOOR ENVIRONMENT

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Abstract

Over the years, there has been a growing interest in better characterizing the features of fungi present indoors, in analyzing the influence that they have as a risk factor for respiratory problems, and in determining how different levels of fungal exposure at home influence the risk of developing or aggravating these troubles.

In this study was performed a preliminary identification of different fungi isolated in a private house in order to detect potential allergens that can trigger human respiratory problems.

Samples were collected from the indoor wall surfaces of different rooms (kitchen, bedroom, bathroom) in a private house where a man had a severe respiratory crisis. An initial identification of fungi was carried out based on the morphological features of colonies and their microscopic examination.

Among fungal genera, *Aspergillus* was the most commonly isolated, with a frequency of 38.56%, followed by *Penicillium* 29.32%, *Alternaria* 20.78% and *Cladosporium* 11.34%. These fungal genera are very common allergens causing allergic reactions but also other illnesses, such as pulmonary diseases and/or sinusitis or depression especially in sensitive individuals.

Further future molecular studies will follow this preliminary identification based on morphological features and allow to detect fungi at species level, in order to assess the link between these fungi and the allergic condition reported and also to discuss their connection to the allergic reaction registered.

Keywords: *allergenic fungi, indoor environment, morphological identification*

Introduction

Fungi are present in both outdoor and indoor environments and are widely distributed in air, soil, water, decaying vegetation and all places with enough moisture [1, 2].

The abundance of fungi in different environments, combined with the small size and easy dispersion of their propagules, favors high concentrations of fungal spores in the air in both indoor and outdoor environments [3, 4]. Indoor and outdoor fungal exposure levels are associated with the development of allergic respiratory diseases [5]. Humans continuously inhale spores and fragments of allergenic moulds. Exposure to fungal allergens in outdoor and indoor environments might result in respiratory diseases in sensitive individuals. However, indoor exposure to mould allergens has not received due attention [6, 7].

The sensitization to specific airborne fungi has been shown to be a risk factor for severe respiratory diseases in children and adults. Over the years, there has been a growing interest in better characterizing the features of fungi present indoors, in analyzing the influence that they have as a risk factor for respiratory problems, and in determining how different levels of fungal exposure at home influence the risk of developing or aggravating these troubles [8-11].

It is interesting to know the distribution of fungi inside homes, because many key factors changed constantly, including life-style habits, the addition of air conditioning and air heating units and the construction of buildings with new materials and ventilation systems. In addition, other external factors, such as increased pollution, may also have contributed to changes in the prevalence and distribution of indoor fungi [12]. Many fungal species have been described as the cause of allergic disease and their concentration is species-specific [7, 13]. Mold allergies are common, they mainly target the respiratory tract and present as allergic rhinitis and/or bronchial asthma. Molds include a large group of different allergens that induce all types of allergic reactions [14]. Allergic diseases are one of the major health problems of the 21st century. An increase in the incidence of allergic rhinitis and asthma cases has been observed, especially in developed countries. [14].

Humid indoor environments may be colonized by allergenic filamentous microfungi (moulds), *Aspergillus* spp., *Penicillium* spp., *Cladosporium* spp., and *Alternaria* spp. in particular. Their development is favoured by high humidity levels and a lack of access to light and ventilation. In the home environment, we meet a high content of these fungi allergens, which are a source of allergies throughout the year. Apart from asthma and allergic rhinitis, patients allergic to moulds may suffer from allergic bronchial-pulmonary aspergillosis, allergic alveolitis, chronic allergic sinusitis, and even allergic dermatitis [6, 7, 14-16].

Further, it has long been recognized that the qualitative determination of the fungi recovered from environmental samples is more useful than determining the concentrations of fungi. For this reason, concerns about health effects of fungi in different environments have been increasing for some years [7, 17, 18].

So, this study aimed to perform a preliminary identification of different fungi isolated in a private house in order to detect potential allergens that can trigger human respiratory problems.

Methods

Sampling and plating conditions

A total of 25 samples were collected from the indoor wall surfaces of different rooms (kitchen, bedroom, bathroom) in a private house.

Samples were collected from walls by delicately scraping off material using a scalpel into sterile tubes and rubbing using sterile swabs surfaces. All materials were stored in laboratory controlled conditions until used.

Sample swabs and scraped materials were dissolved in sterile Ringer's solution and the resuspended solutions were subsequently inoculated on Plate Count Agar (PCA) plates, supplemented with chloramphenicol (100 mg/L), and then incubated at 30°C, in dark, for 7 days. Colonies showing different morphology and appearance were few times transferred to sterile PCA plates to obtain pure fungal cultures.

Identification of fungi

All pure fungal cultures were checked daily in terms of colony growth. The identification of fungi was carried out based on the morphological features of colonies and their microscopic examination. Identification of isolated fungi was done using various mycological references according to the criteria already reported in literature [12, 19, 20]. Preliminary identification of the isolated fungi was performed at genus level.

Results

Isolation on PCA media allowed to obtain a number of 60 pure fungal cultures originated from the investigated house indoor environment.

After macroscopic and microscopic observations of these pure cultures, based on their morphological features, the four genera considered to be the most relevant for allergies, namely *Aspergillus*, *Penicillium, Alternaria* and *Cladosporium*, were identified.

Among fungal genera, *Aspergillus* was the most commonly isolated, with a frequency of 38.56%, followed by *Penicillium* 29.32%, Alternaria 20.78% and *Cladosporium* 11.34% (Fig.1).

All these results are in concordance with what is well known; fungal genera, obtained in this work, are among the most frequent genera encountered indoors and/or outdoors [7, 12, 21]. Besides, fungi from these genera are very common allergens causing allergic reactions but also other illnesses, such as pulmonary diseases and/or sinusitis or depression especially in sensitive individuals [22, 23]. These disorders appear and develop as a consequence of being in contact with the fungi or by inhalation the fungal spores or hyphae.

Discussion

Fungi are ubiquitous allergens and are the leading cause of various human diseases [24].

Our results, reporting the isolation and identification of *Aspergillus* and *Penicillium* fungal genera as being the most abbondant genera discovered on the indoor house walls, are in agreement with earlier studies [21, 25-27].

Penicillium spp. are common wherever organic material is available and some can even grow under conditions with very little water. *Aspergillus* and *Penicillium* are also the most common airborne allergenic fungi, along with *Cladosporium* and *Alternaria*. In addition, *Aspergillus* and *Penicillium* exposure in damp homes is a risk factor for asthma [28].

Indoors, fungal spores usually are present in low concentrations unless sources of indoor growth are present. Lacking such growth, the indoor microbiome depends largely on dispersal from the outdoors [29-31].

The most common taxa found in surveys of contaminated indoor environments include *Penicillium*, *Aspergillus*, *Ascospores*, *Alternaria*, *Periconia*, *Basidiospores*, *Stachybotrys* and *Wallemia* [27].

Fungi are well adapted to varying moisture conditions. The types of fungal species present often change over time, reflecting changes in moisture as the building dries and becomes damp again. Room types most likely to have elevated spore counts include (in descending order) laundry rooms, bathrooms, basements, and bedrooms, with the lowest counts being found in living rooms, kitchens, and family/television rooms [32].

Generally, fungal species of *Aspergillus*, *Alternaria* and others are harmless fungi but in sensitive individuals with impaired immune system they become a clinical problem. In these persons fungi can be responsible of an array of disorders such as external infections, allergies but also life-treathening systemic mycoses [14, 33]. Several studies have been reported that fungi are sensitizers and aggravate allergic asthma [34-37].

They also produce volatile organic compounds and mycotoxins which can cause severe health problems like liver and/or kidney damage, neurological disorders or one of the most frightening disease the cancer. Moreover, fungal mycotoxins posses different types of toxicity starting from acute to chronic, mutagenic and teratogenic [26, 36].

Therefore, identifying and subsequently eliminating the molds surely remains one of the best way to control them and their mycotoxins and consequently remove the negative effects that they may have on human health.

This study represents a preliminary investigation to identify allergenic fungi isolated in indoors of a private house where a man had a severe respiratory

crisis. This initial identification of fungi found in the indoor house environment, based on morphological features, will be followed by future molecular studies. Additional molecular and/or volatomic approaches using various genes as markers will allow, as demonstrated by previous research, [30, 38, 39] to detect indoor fungi at species level, in order to assess the link between them and the allergic condition reported and to discuss their connection to the allergic reaction registered.

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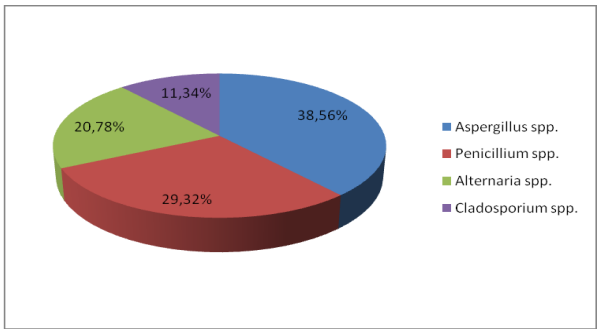

Figure 1: Distribution of fungal genera isolated from indoor walls of a private house.