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ARTICLE

Examination of the diversity of indoor molds in a Hungarian student hostel

János Varga*, Leila Kerepes, Sándor Kocsubé, Gyöngyi Szigeti, Nikolett Baranyi, Csaba Vágvölgyi

Department of Microbiology, University of Szeged, Szeged, Hungary

ABSTRACT Molds are widely distributed in indoor and outdoor environments. They are common in household dust and can cause allergic symptoms or invasive infections in humans with weak immune system. They can also be harmful through their toxin producing abilities. Our aim was to examine the diversity of indoor molds in a student hostel. Isolation of the samples was carried out using standard dichloran-glycerol media. After purification, the isolates were identified by ITS sequence analysis and morphological traits. Samples were collected from different places of the kitchen, the bathroom and the living room during the summer (in August), and during late autumn (in November). The most frequently identified genera were *Aspergillus, Alternaria, Cladosporium, Penicillium* and *Aureobasidium*. A high diversity was observed at the species level based both on the sampling location and the season. In general, more species were recovered during the summer period than during November. A similar trend was observed when the number of *Aspergillus* species were compared: more species were recovered during summer than in November. High numbers of *Eurotium* and *Aureobasidium* isolates were detectable in the summer sample set, whereas the samples collected in November did not contain any of these species. On the contrary, the frequency of *Alternaria, Cladosporium* and *Penicillium* isolates did not differ significantly between the two sampling periods. Further studies are in progress to examine the diversity of indoor molds in other seasons and in other locations.

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KEY WORDS

indoor fungi
Aspergilli
sequence-based identification

Molds are widely distributed both in indoor and outdoor environments. They are essential components of our ecosystem providing decomposition of many organic substances necessary to plant, animal, and human life. Microscopic fungi are also important biological pollutants in the indoor environment, they are spread generally on building materials, carpets, ceiling tiles, insulations, any surfaces, wallpapers, or in heating, ventilation, and air conditioning systems (Samet and Spengler 2003). Molds are able to grow on any materials, as long as moisture and oxygen are available (Scott 2001). The three main sources of fungal propagula found in indoor air are: outdoor air carried in through doorways and windows; spores carried in on people, pets, or items brought into the home; and fungi that grow and produce spores indoors, usually associated with excess moisture (Nevalainen and Seuri 2005; Khan and Karuppayil 2012). A great deal of concern has arisen recently regarding the potential adverse effects of indoor fungi. The health hazards posed by polluted indoor environments include allergy, infections and toxicity. Many species of fungi are known to cause allergies (Jarvis and Miller 2005, Khan and Karuppayil 2012). These include mainly *Alternaria, Stachybotrys, Penicillium and Aspergillus* species. They can also be harmful through their mycotoxin producing abilities, and as human pathogens (Samson et al. 2010). The most well documented mycotoxins in indoor environments are trichothecenes produced by Fusaria and *Stachybotrys* species, while aflatoxins and ochratoxins are produced by Aspergilli and Penicillia (Robbins et al. 2000).

Our aim was to examine the diversity of indoor molds in a student hostel in Szeged (Hungary) during summer and late autumn in 2009 using morphological and molecular methods.

Materials and Methods

The plate sedimentation method using dichloran 18% glycerol agar (DG-18) plates was used for sampling airborne fungi (Samson et al. 2010). Samples were collected in a student hostel, from different places of the kitchen, the bathroom and the living room during the summer (in August), and during late autumn (in November). After purification, the isolates were identified by their morphological traits. For morphological examinations, the isolates were grown for 7 days as 3-point inoculations on Czapek yeast autolysate agar (CYA), malt extract agar (MEA), and oat meal agar (OA) at 25 °C

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*Corresponding author. E-mail: jvarga@bio.u-szeged.hu

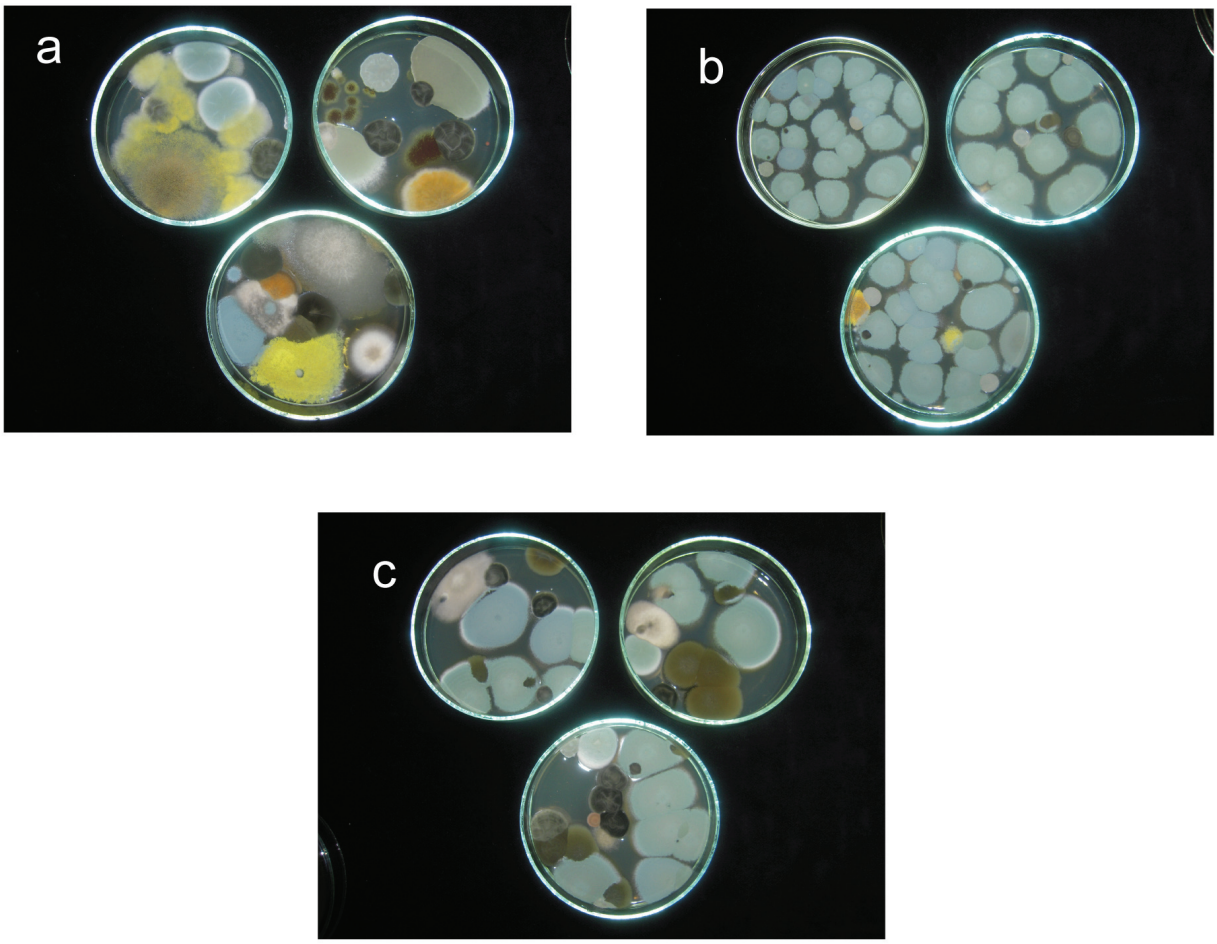

Figure 1. Example of the mycobiota of the living room (a), kitchen (b) and bathroom (c).

(Samson et al. 2010). Morphological identifications were carried out according to the literature (Raper and Fennell 1965; Samson et al. 2010).

The cultures used for the molecular studies were grown on malt peptone (MP) broth using 10% (v/v) of malt extract (Oxoid) and 0.1% (w/v) bacto peptone (Difco), 2 mL of medium in 15 mL tubes. The cultures were incubated at 25°C for 7 days. DNA was extracted from the cells using the Masterpure™ yeast DNA purification kit (Epicentre Biotechnol.) according to the instructions of the manufacturer. Fragments containing the ITS region were amplified using primers ITS1 and ITS4 as described previously (White et al. 1990).

The sequences were edited by BioEdit. Homologous genes have been searched for at the Pubmed (http://www.ncbi.nlm.nih.gov) website using „nucleotide blast" searches (Altschul et al. 1990), and to our own sequence database. Species identification was determined from the lowest expected value of the BLAST output.

Results and Discussion

Indoor environments play important roles in human health. Indoor bacteria or fungi can cause allergic, infectious, toxic or inflammatory diseases called building-related illnesses (Pieckova 2003). Fungi are ubiquitous in distribution and are a serious threat to public health in indoor environments (Samson et al. 2010).

In this study, we examined the distribution of fungi in indoor air in a student hostel in Szeged in August and November 2009. The Petri plate sedimentation method using DG-18 plates were used for sampling airborne fungi. After purification, sequence analysis of the ITS region was carried out to assign the isolates to species. Recently, this region was chosen as the official DNA barcoding region for fungi (Amend et al. 2010). The ITS region of altogether 143 isolates was sequenced. Regarding the distribution of the fungi within the student hostel, significant differences were observed among the different location. In the bathroom *Cladosporium* isolates, black yeasts and *Aspergillus versicolor* dominated,

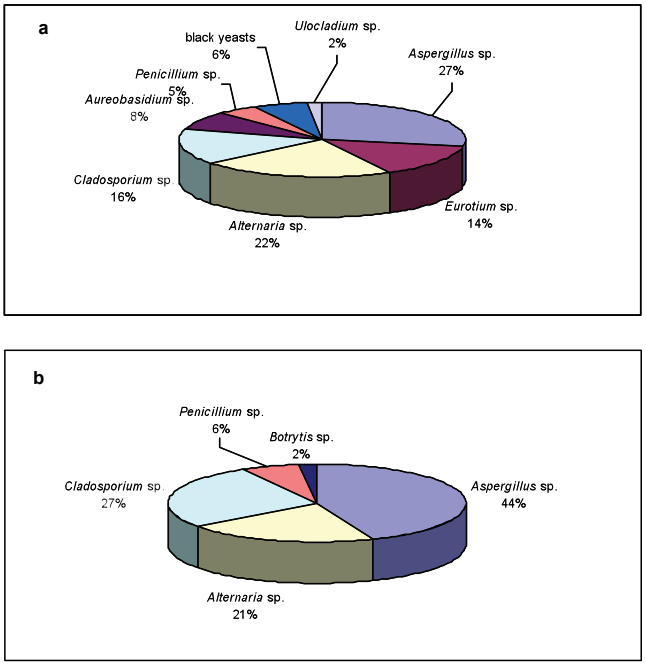

Figure 2. Species diversity of indoor fungi in a student hostel in August (a) and November (b).

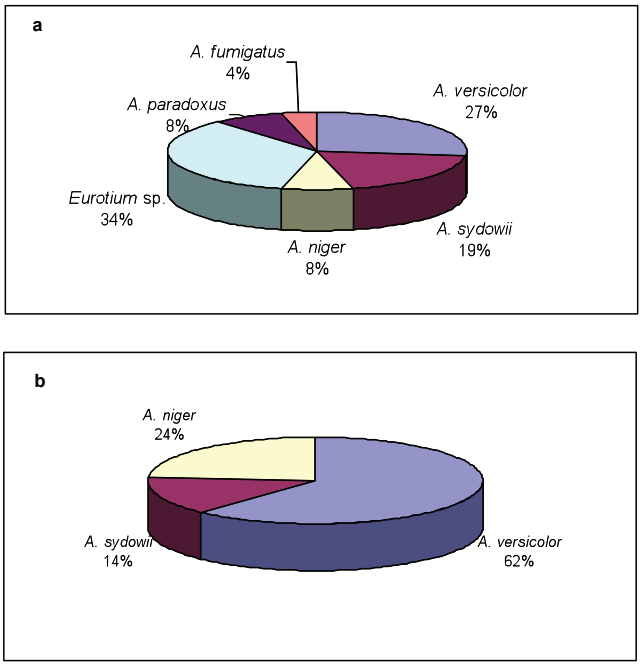

Figure 3. Species distribution of Aspergilli in a student hostel in August (a) and November (b).

while black yeasts could not be detected in other locations. Black yeasts are common in steam baths, dishwashers and bathrooms in Europe (Sudhadham et al. 2008). These species cause chromoblastomycosis and fatal brain infections in East Asia (Matos et al. 2002). The species diversity was generally higher in the living room than in the kitchen (Fig. 1).

The most frequently identified genera were *Aspergillus*, *Alternaria*, *Cladosporium*, *Penicillium* and *Aureobasidium* in both seasons. A high diversity was observed at the species level based both on the sampling location and the season. In general, more species were recovered during the summer period than during November (Fig. 2). A similar trend was observed when the number of *Aspergillus* species were compared: more species were recovered during summer than in November (Fig. 2). On the contrary, the frequency of *Alternaria, Cladosporium* and *Penicillium* isolates did not differ significantly between the two sampling periods. High numbers of *Eurotium* and *Aureobasidium* isolates were detectable in the summer sample set, whereas the samples collected in November did not contain any of these species. Besides, *Aspergillus* species were present at high frequency, and a *Botrytis* isolate was also identified in the second data set. Among the species observed, the conidia of *Alternaria* and *Ulocladium* are well-known as inducing allergic reactions (Samson et al. 2010). Among the *Aspergillus* species, *A. versicolor* and *A. sydowi* are well-known in indoor environments, they are able to produce the carcinogenic mycotoxin sterigmatocystin (Samson et al. 2010), while *A. niger* produces the carcinogenic ochratoxins and fumonisins (Samson et al. 2007; Varga et al. 2011).

Further studies are in progress to examine the diversity of indoor molds in other seasons and in other locations.

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