In vitro toxicity of indoor moulds from Slovak dwellings

E. Piecková & Z. Kolláriková
Slovak Medical University in Bratislava, Slovakia

Abstract

In vitro toxicity of complex mixtures of endo- and exometabolites of representative indoor, and related outdoor, fungal isolates from 80 dwellings in Slovakia – mouldy and control ones – has been evaluated by a bioassay with 1-d-old chicks' tracheal organ cultures. Micromycetes, mostly *Aspergillus versicolor* (which is able to synthetize a mycotoxin sterigmatocystin), *A. flavus* (non-aflatoxigenic), other aspergilli, penicillia (potential sources of wide mycotoxins´ spectrum), produced secondary metabolites that ceased ciliary beating in the tracheal epithelium of the organ cultures within 24 hrs of the activity, i.e. in the sense of the method used, they belong to strong toxicants. Nineteen of 55 mould isolates tested so far also produced extrolites without toxic effects detectable by this method. It has been proven that toxin production in fungi depends not only on the species but may vary between every single isolate as well. The most important outcome of the study is that microscopic filamentous fungi present in the dwelling indoor environment under Slovak (Central European) building/housing conditions might produce compounds with a strong potential to damage the upper airways of occupants, mainly children.

Keywords: dwellings, indoor moulds, mycotoxins, respiratory toxicity, tracheal organ cultures, ciliary beating.

1 Introduction

Currently, a general approach to the study of the mechanism of indoor fungal effects on human beings is becoming more urgent. Such an approach includes the immunosuppressive influence of beta-glucans from fungal cell walls as well as toxic and irritative effects of secondary metabolites – mycotoxins and/or volatile organic compounds (VOCs) [1, 2].

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Low molecular organic compounds, namely alcohols, aldehydes, ketones, aromatic compounds, amines, terpenes, chlorinated hydrocarbons and sulphuric compounds, typically cause not only a mouldy odour but also an inflammation of the airways of sensitive people. *Aspergillus* sp., *A. versicolor, Cladosporium* sp. and *Penicillium* sp. are strong producers of the VOCs. Such effects are associated with invisible moulds usually growing under wallpaper, carpets or mattresses [3, 4].

Although many occupational pulmonary mycotoxicoses as adverse effects of inhaled organic dust contaminated by microbial toxins have been reported [5], to date there have been no objective proofs of real clinical diseases born from mycotoxins, especially produced by *Aspergillus* sp., *Penicillium* sp., *Fusarium* sp., *Trichoderma* sp. and *Stachybotrys chartarum* in the indoor environments. A study was carried out on some cases of respiratory diseases in inhabitants of dwellings in Chicago and Cleveland, heavily contaminated with *S. chartarum, Memnoniella echinata* and *A. versicolor* [5–7]. Particular fungal isolates were toxic *in vitro: S. chartarum* produced cytotoxic and immunosuppressive macrocyclic trichothecenes having caused also inflammation and haemorrhagies in the respiratory tract and intestines of laboratory animals [7, 8], *M. echinata* griseofulvins [7] and *A. versicolor* produced carcinogenic sterigmatocystin [6]. The *S. chartarum* isolates from moist schools and dwellings in Denmark produced trichothecenous mycotoxin trichodermol when cultivated onto cardboard [9] and vinyl ceiling [6]. Mutagenic and foetotoxic mycotoxins alternariol and its monomethylether adversely affecting mice were detected in cellulose tiles overgrown with *Alternaria alternata*. This isolate was also able to grow on cardboard [10]. The metabolite synthesis of fungi depends on the quality of construction materials [11, 12]. In our previous experiments on tracheal organ cultures of 1-d-old chicks, varying ciliostatic activity was found in chloroform extracts of biomass from building materials (mineral wool, plasterboard, cardboard) inoculated with pure isolates of some moulds of indoor origin (*Penicillium chrysogenum, P. palitans, Trichoderma viride, Stachybotrys* sp. and *A. versicolor*). Generally, extracts from growth on materials composed of finely divided cellulose were more active than those from growth on mineral wool [13]. The only data available on the ciliostatic activity of indoor mould metabolites involves sterigmatocystin, from *A. versicolor*, which is among the most active metabolites examined [14]. Viable spores of *A. versicolor* and *Penicillium* sp. were isolated from different plasters after 40 d – 3 months i.e. until the end of the experiment [15].

Evaluation of the *in vitro* toxicity of indoor fungal isolates from mouldy dwellings over the whole of Slovakia, collected for 3 years, expressed as the tracheal ciliostatic activity of their secondary metabolites was the aim of our present study.

2 Materials and methods

In vitro toxicity of complex mixtures of endo- and exometabolites of representative (chosen from the most frequent or potentially toxic, fig. 1) indoor,

and related outdoor, fungal isolates (55 in total) from 80 dwellings in Slovakia – mouldy and control ones – has been evaluated by a bioassay with 1-d-old chicks' tracheal organ cultures. The mixtures were screened for some mycotoxins (sterigmatocystin, aflatoxins, patulin, trichothecenes) by thin-layer chromatography [16]. The bioassay with organ culture developed at our lab was employed: the effect of 20 mikrog/ml of chloroform extracts of fungal extrolites after stationary cultivation on the liquid medium with 2% yeast extract and 10% sucrose at 25 °C 10 d on the cilia movement in chick tracheal cultures *in vitro* after incubation in the minimal essential medium according to Eagle enriched with the Earl's salts at 37 °C in the atmosphere enriched with 5% CO_2 [17–19]. The adverse effect of toxicants tested was expressed as the ability to cease the beating of epithelial cilia in 24, 48, or 72 hrs' of co-incubation and observed microscopically.

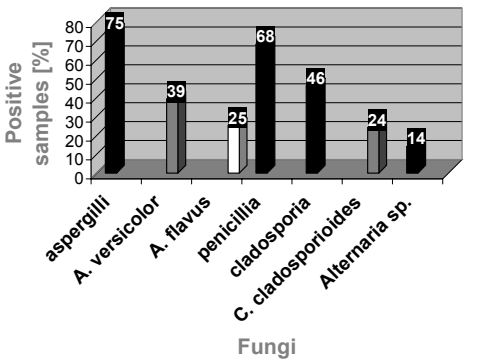

Figure 1: Relative incidence of indoor moulds in Slovak dwellings.

3 Results and discussion

Micromycetes, mostly *Aspergillus versicolor* (which is able to produce a mycotoxin sterigmatocystin), *A. flavus* (non-aflatoxigenic), other aspergilli, penicillia (potential producers of wide mycotoxins' spectra), airborne or stuck to the indoor surfaces, synthetized secondary metabolites – endo- and/or exocellular – broke down ciliary beating in the tracheal epithelium of the organ cultures within 24 h of the activity, i.e. in the sense of the method used, they belong to strong toxicants. Nineteen of the 55 mould isolates tested so far also produced extrolites without toxic effects detectable by this method. It has been proven that toxin production in fungi depends not only on the species but may vary between every single isolate as well (e.g. recently, taxonomic definitions of species/strains also undergo re-evaluation because of such characteristics). Data are shown in table 1.

To determine a causal relationship between mycotoxins in the indoor environment and particular human health disorders, it is necessary to quantify mycotoxin concentration in damp and healthy buildings, to estimate the minimal

Table 1: *In vitro* tracheal ciliostatic activity of indoor fungal metabolites.

ID	Origin	Mould	Endometabolites			Exometabolites		
			24h	48h	72h	24h	48h	72h
142	PD, living room, S	*A. versicolor*	+	-	-	-	-	-
142a	ditto	*PNC* sp.	+	+	+	+	-	-
139	ditto	*PNC* sp.	+	+	+	+	+	+
159	PD, sleeping room, S	*C. cladosporioides*	+	+	+	+	-	-
149	PD, balcony, S	*C. cladosporioides*	+	+	-	+	+	-
154	ditto	*Alternaria s*p.	+	+	+	+	+	+
146	PD, living room, S	*Alternaria* sp.	+	-	-	+	+	-
197	BA, window's wall, S	*PNC* sp.	+	+	-	-	-	-
206	BA, room corner, S	*PNC* sp.	-	-	-	+	+	+
199	BA, window wall, S	*A. versicolor*	-	-	-	-	-	-
182	BA, sleeping room, window, S	*PNC* sp.	+	+	+	+	+	+
187	BA, window wall, S	*C. sphaerospermum*	+	+	+	+	+	+
189	BA, ledge, S	*C. sphaerospermum*	-	-	-	-	-	-
C8	KE, S	*A. niger*	+	+	+	+	+	+
C4	KE, S	*Paec. variotii*	-	-	-	-	-	-
185	BA, sleeping room, window, S	*PNC* sp.	-	-	-	+	-	-
C3	KE, S	*A versicolor*	-	-	-	-	-	-
C7	KE, S	*A. ochraceus*	+	-	-	-	-	-
C6	KE, S	*PNC* sp.	+	-	-	-	-	-
C2	KE, S	*C. sphaerospermum*	-	-	-	-	-	-
184	BA, sleeping room, window, S	*PNC* sp.	-	-	-	-	-	-

Table 1: Continued.

214	BA, corridor, S	*Alternaria* sp.	-	-	-	-	-	-
222	BA, kids´ room, S	*PNC* sp.	-	-	-	-	-	-
236	BA, living room, A	*C. sphaerospermum*	-	-	-	-	-	-
234	ditto	*A. versicolor*	-	-	-	-	-	-
212	BA, corridor, S	*A. versicolor*	-	-	-	-	-	-
235	BA, living room, A	*A. versicolor*	-	-	-	-	-	-
241	ditto	*A. versicolor*	+	-	-	-	-	-
217	BA, corridor, S	*A. versicolor*	-	-	-	-	-	-
136	BA,	*Tritirachium* sp.	-	-	-	-	-	-
159	BA,	*Tritirachium* sp.	-	-	-	-	-	-
150	BA,	*A. flavus*	-	-	-	-	-	-
160	BA,	*Alternaria* sp.	-	-	-	-	-	-
155	BA,	*A. flavus*	-	-	-	-	-	-
118	BA, S	*PNC* sp.	-	-	-	-	-	-
123	BA, S	*A. restrictus*	-	-	-	-	-	-
122	BA, S	*A. candidus*	-	-	-	-	-	-
230	Rusovce, kids´ room, window, S	*A. flavus*	+	+	-	-	-	-
238	BA, kitchen, S	*PNC* sp.	-	-	-	-	-	-
229	Rusovce, kids´ room, window, S	*A. flavus*	-	-	-	-	-	-
195	KE, S	*A. flavus*	-	-	-	-	-	-
204	Rusovce, kids´ room, S	*PNC* sp.	+	-	-	-	-	-
262	PP, living room, S	*A. nidulans*	-	-	-	-	-	-
568	BA, kitchen, A	*A. versicolor*	-	-	-	+	+	-
583	BA, kids´ room, A	*A. versicolor*	-	-	-	-	-	-
557	BA, outdoor	*A. versicolor*	-	-	-	-	-	-
601	LC, kitchen, A	*A. versicolor*	-	-	-	-	-	-

Table 1: Continued.

620	PP, wardrobe, A	*A. versicolor*	-	-	-	-	-	-
297	PP, kitchen, S	*A. versicolor*	-	-	-	-	-	-
180	KE, S	*A. versicolor*	-	-	-	-	-	-
392	KE, sleeping room, S	*A. versicolor*	-	-	-	-	-	-
531	BA, A	*A. versicolor*	-	-	-	-	-	-
495	BA, A	*A. versicolor*	-	-	-	-	-	-
536	BA, kids´ room, S	*A. versicolor*	-	-	-	-	-	-

Notes: + – ciliary beating observed, - – ciliary beating ceased, *A. – Aspergillus, C. – Cladosporium, PNC – Penicillium, Paec. – Paecilomyces*, ID – number of the fungal isolate deposited in the lab-collection, A – air, S – surface, BA – Bratislava, KE – Košice, LC – Lučenec, PD – Prievidza, PP – Poprad.

concentration of mycotoxin able to cause clinical symptoms in comparison with *in vitro* models, to choose optimal animal or other biological models for studying mycotoxin pathogenicity and pathophysiology and to characterize short- and long-term health damage (not only biomarkers) in people under such an influence [2, 14, 20].

4 Conclusion

Micromycetes present in the dwelling indoor environment under particular studied building/housing conditions might produce toxic metabolites with ciliostatic activity, i.e. be able to damage seriously, even irreversibly, the self-cleaning of the upper airways´ epithelium. The effect may contribute to the ill health of occupants of the damp mouldy dwellings, starting with respiratory disorders and, probably, finishing with general intoxication of the macroorganism (human body) via lung tissue during the long-lasting exposure.

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