

## The effect of chloroform extracts of micromycete biomass on the movement of tracheal cilia in one-day old chickens *in vitro*

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The ciliostatic effect of metabolites from mycelia and spore biomass of 185 micromycete strains extractable with chloroform on tracheal epithel cilia was investigated in 1-d old chickens *in vitro*. The strains were isolated from cotton or flax. Extracts of 54 strains (29 %) displayed ciliostatic activity: 16 (9 %), 6 (3 %), and 32 (17 %) strains stopped the movement of cilia after 24, 48, and 72 hours, respectively. There may be relationships between these results and respiratory tract illnesses in people living in mouldy dwellings, working with mouldy materials, or with sick building syndrome.

**Key words:** Micromycete, biomass, chloroform extract, tracheal cilia.

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Sledoval sa ciliostatický účinok chloroformom extrahovateľných metabolitov z biomasy mycélia a spór 185 kmeňov mikromycét na tracheálnom epiteli jednodňových kurčiat *in vitro*. Mikromycéty boli izolované z bavlny a ľanu. Ciliostatickú aktivitu mali extrakty 54 kmeňov (29 %): 16 (9 %), 6 (3 %) a 32 (17 %) extraktov zastavilo pohyb cilií po 24, 48, resp. 72 h. Možno uvažovať o vzťahu medzi týmito výsledkami a ochoreniami dýchacích ciest u ľudí žijúcich v plesnivých bytoch, pracujúcich s plesnivými materiálmi, resp. trpiacimi tzv. sick building syndrome.

There are many micromycete particles, such as intracellular secondary metabolites, or dust contaminated with extracellular mycotoxins in the air of working and indoor environments. Aflatoxin B<sub>1</sub>, ochratoxin A, zearalenone, secalonin acid D and deoxynivalenol were detected in the working environment. Some trichothecenes were found in the atmosphere of dwellings and offices (Hendry and Cole 1993, Jesenská 1993, Jesenská et al. 1990, Pasanen et al. 1993, Verhoeff et al. 1994).

Tracheal and bronchial illnesses affect people, especially children, living in damp and mouldy dwellings to a higher degree (Smoragiewucz et al. 1993). People working in air-conditioned offices may suffer from sick building syndrome – nonspecific respiratory complaints of uncertain aetiology (Jaakkola et al. 1994, Marasm et al. 1994, Mishra et al. 1991). Increased morbidity from chronic bronchitis in textile and agricultural workers is also known (Jaroš 1989, Summerbell et al. 1992, Zejda

and Dosman 1991, Zuskin et al. 1991). The negative influence of micromycetes and their secondary metabolites, mainly mycotoxins, on respiratory organs in connection with the mentioned illnesses is well-known.

The aim of our work was to contribute to the explanation of the possible aetiology of the above illnesses referring to our former results (Jesenská and Bernát 1994, Piecková and Jesenská 1994, 1995). We studied the ciliostatic effect of chloroform extracts from micromycete mycelia and spores on tracheal cilia in a model system of 1-day old chicken organ cultures. Micromycete strains were isolated from cotton and flax, cultivated stationary on a liquid medium with sucrose and yeast extract during 10 days.

#### MATERIAL AND METHODS

Biomass extracts of micromycetes. 185 strains of filamentous fungi were isolated from samples of cotton and flax. The isolated strains were cultivated on slant Sabouraud agar (IMUNA, Co., Šarišské Michaľany, Slovakia) at 25 °C during 14 days. The culture of each strain growing in 3 tubes was scratched into 200 ml of a liquid medium with yeast extract (2 %) and sucrose (10 %) in 500 ml Erlenmayer flasks and stationary cultivated at 25 °C during 10 days. Biomass of each culture was extracted twice by 200 ml of chloroform after filtration of the cultivation medium. The united extract was dried with Na<sub>2</sub>SO<sub>4</sub> without water and evaporated in a water bath.

The ability of isolated strains of *Aspergillus flavus* to produce aflatoxin B<sub>1</sub> and G<sub>1</sub> was investigated by their cultivation on liquid medium with 20 % sucrose and 2 % yeast extract, at pH 5.5 and 25 °C during 14 days (Abarca et al. 1988).

Cultivation medium for organ cultures, tracheal organ cultures of 1-day old chickens and test evaluation were described in our previous studies (Piecková and Jesenská 1994, 1995).

#### RESULTS

The biomass of 54 (29 %) out of 185 investigated micromycete strains contained chloroform-extractable secondary metabolites with ciliostatic activity against tracheal cilia of the 1-day old chickens *in vitro*:

sixteen strains (9 %) stopped the movement of cilia already after 24 hours, these were strains of *Aspergillus flavus* (2 strains, 1 of them produced aflatoxins *in vitro*), *A. glaucus* group (2 strains), *A. nidulans* (1), *A. terreus* (1), *Fusarium* sp. (7) and *Penicillium* sp. (3);

six strains (3 %), namely *A. flavus* (1 strain), *Fusarium* sp. (1) and *Penicillium* sp. (4), stopped the ciliary movement after 48 hours;

thirty-two strains (17 %), namely *A. flavus* (4 strains, 3 of them produced aflatoxins *in vitro*), *A. fumigatus* (2), *A. glaucus* group (3), *A. nidulans* (6), *A. niger* group (1), *A. ochraceus* (2), *A. terreus* (3), *A. versicolor* (3), *Cladosporium* sp. (1), *Fusarium* sp. (1), and *Penicillium* sp. (6), stopped the ciliary movement after 72 hours.

The ciliary movement was not affected by metabolites of the other 131 studied micromycete strains and in the reference media within the experiment (Table 1).

#### DISCUSSION

Cells of microscopic filamentous fungi may contain various mycotoxins, e. g. citreoviridin, cyclopiazonic acid, luteoskyrin, penitrem A, stachybotryotoxins, sterigmatocystin, verruculogen, viomellein, xantomegnin, etc. (Filtenborg et al. 1983, Pasanen et al. 1993). Airborne fungal particules can become a transfer vehicle of mycotoxins to the human organism (Burg and Shotwell 1984). Aflatoxin B<sub>1</sub> can be stored in spores and the mycelium of toxinogenic strains of *Aspergillus flavus* and *A. parasiticus* (Shih and Marth 1975). It is known that aflatoxins negatively affect the function of tracheal cells of hamsters, rabbits and monkeys *in vitro* (Coulombe et al. 1986, Wilson et al. 1990), and are carcinogenic for lung cells (Autrup et al. 1979). Aflatoxin B<sub>1</sub> transforms the metabolism of tracheal epithelia by its binding to the cell DNA (Daniels et al. 1993).

Ciliostatic activities of some mycotoxins on 1-d old chickens tracheal organ cultures *in vitro* were described in the previous studies (Jesenská and Bernát 1994). Ciliostatic activities of chloroform-extractable and heat-stable metabolites of some micromycetes isolated from cotton, flax, straw and sorghum were published by us, too (Piecková and Jesenská 1994, 1995). In this part of our study we have been concentrating on the problem of ciliostatic activities of chloroform extracts of micromycete biomass *in vitro* in this part of our study. It was found that 29 % of the investigated strains were able to produce metabolites which stopped the movement of tracheal cilia under conditions of the described model system in 24, 48, and 72 hours.

Destroyed ciliary movement in the airways may be the first step in the development of human chronic respiratory illnesses with major health losses and they need to be studied further.

**Table 1.** The effect of chloroform extracts of micromycete biomass isolated from flax and cotton on the movement of tracheal cilia in 1-day old chickens *in vitro*

Micromycetes	Number of strains	Time [h]			Number of strains total	+
		24	48	72		
Movement of cilia						
<i>Acremonium</i> sp.	1	+	+	+	1	0
<i>Alternaria</i> sp.	7	+	+	+	7	0
<i>Aspergillus candidus</i>	1	+	+	+	1	0
<i>A. flavus</i>	19 (5*)	+	+	+	26	7
	4 (3*)	+	+	-		
	1	+	-	-		
	2 (1*)	-	-	-		
<i>A. fumigatus</i>	9	+	+	+	11	2
	2	+	+	-		
<i>A. glaucus</i> group	12	+	+	+	17	5
	3	+	+	-		
	2	-	-	-		
<i>A. niger</i> group	9	+	+	+	10	1
	1	+	+	-		
<i>A. nidulans</i>	2	+	+	+	9	7
	6	+	+	-		
	1	-	-	-		
<i>A. ochraceus</i>	12	+	+	+	14	2
	2	+	+	-		
<i>A. terreus</i>	8	+	+	+	12	4
	3	+	+	-		
	1	-	-	-		
<i>A. versicolor</i>	7	+	+	+	10	3
	3	+	+	-		
<i>A. wentii</i>	1	+	+	+	1	0
<i>Cladosporium</i> sp.	11	+	+	+	12	1
	1	+	+	-		
<i>Fusarium</i> sp.	15	+	+	+	24	9
	1	+	+	-		
	1	+	-	-		
	7	-	-	-		

**Table 1.** The effect of chloroform extracts of micromycete biomass isolated from flax and cotton on the movement of tracheal cilia in 1-day old chickens *in vitro* (Continued).

Micromycetes	Number of strains	Time [h]			Number of strains	
		24	48	72	total	+
Movement of cilia						
<i>Gliocladium</i> sp.	1	+	+	+	1	0
<i>Penicillium</i> sp.	14	+	+	+		
	6	+	+	-		
	4	+	-	-	27	13
	3	-	-	-		
<i>Sporotrichum</i> sp.	1	+	+	+	1	0
<i>Torula</i> sp.	1	+	+	+	1	0
Strains with ciliostatic activity	%	16	6	32	185	54
		9	3	17	100	29

Note: \* aflatoxin B<sub>1</sub> and G<sub>1</sub> producing strain of *Aspergillus flavus*

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