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The effect of the heat-stable and chloroform-extractable secondary metabolites of filamentous fungi on the respiratory tract cilia movement of one-day-old chickens in vitro

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The ciliostatic activity of the heat– stable (100°C/10 min.) and chloroform-extractable metabolites of 63 strains of filamentous fungi – growing on the liquid medium – on tracheal cilia of one-day-old chickens in vitro was evaluated. Twenty two (34.9%) from the investigated strains produced ciliostatic metabolites, 4.7%, 7.9%, 3.1%, resp. 19.0% of the strains stopped the movement of cilia after 24, 48, 72, resp. 144 hours. The results are discussed in connection with chronic bronchitis of people working with moulded materials or living in moulded dwellings.

Key words: Fungi, cilia, trachea, chickens, metabolites, bronchitis

Piecková E. a Jesenská Z. (1994): Účinok termostabilných a chloroformom extrahovatelných sekundárnych metabolitov vláknitých húb na pohyb riasiniek respiračného traktu jednodňových kurčiat in vitro. – Czech Mycol. 47: 215–222

Sledovala sa ciliostatická aktivita termostabilných (100°C/10 min.) a chloroformom extrahovatelných metabolitov 63 kmeňov vláknitých húb, rastúcich na tekutom médiu, na riasinkách priedušnice jednodňových kurčiat in vitro. Ciliostatické metabolity produkovalo 22 (34.9%) z vyšetrených kmeňov, 4.7%, 7.9%, 3.1% a 19.0% kmeňov zastavovalo pohyb riasiniek po 24, 48, 72 a 144 hodinách. Výsledky sa diskutujú v súvislosti s chronickými bronchitídami u ľudí, ktorí pracujú s plesnivými materiálmi, alebo ktorí žijú v plesnivých bytoch.

Children and adults living in the moist and moulded dwellings have health problems with the illnesses of respiratory tract (Platt et al. 1989, Summerbell et al. 1992). Chronic bronchitis is a part of the pathologic processes of some workers of textil factories (Awad-el-Karim et Onsa 1987, Engelberg et al. 1985, Holt 1987, Jaroš 1989, Kawamoto et al. 1987, Kennedy et al. 1987, Lu et al. 1987, Zuskin et al. 1991), in agriculture (Zejda et Dosman 1991) and in other employments. It is considered about the connection between filamentous micromycetes and the "sick building syndrome" or "building related illnesses" (Mishra et al. 1992) – the nonspecific respiratory complaints of uncertain aetiology.

There are many different germs of filamentous fungi or toxic metabolites of filamentous fungi in the working and home environment, intracellular mycotoxins in fungal propagules or extracellular mycotoxins in contaminated plant detritus and

dust. Aflatoxin B_1, ochratoxin A, zearalenone, secalonic acid D and deoxynivalenol were detected in the working and some trichothecenes in the dwelling environment (Croft et al. 1986, Hendry et Cole 1993, Jesenská 1993, Pasanen et al. 1993). It was proved that some mycotoxins have expresive ciliostatic effect on the cilia of the tracheal epithel of the one-day-old chickens in vitro (Jesenská et Bernát – in press, Nair et al. 1970, Cardeilhac et al. 1972).

The aim of our work was to study the ciliostatic effect of the secondary metabolites of the strains of the filamentous fungi, isolated from the samples of cotton, flax, straw and sorghum on the cilia of the one-day-old chickens in tracheal organ cultures in vitro. In this part of our work we studied the ciliostatic activity of the heat-stable (100°C/10 min.) metabolites extract able by chloroform of the isolated strains growing on the liquid medium with saccharose and yeast extract.

MATERIAL AND METHODS

Strains of fungi and their heat-stable and chloroform-extractable metabolites

Sixty three strains of filamentous fungi were isolated from the samples of flax (26 strains), cotton (22), straw (2) and sorghum straw (13) (Table).

The isolated strains were growing 14 days on slant Sabouraud agar (Sabouraud agar IMUNA Co., Šarišské Michaľany, Slovak Republic) in tubes at 25°C.

The culture of each strain growing in 3 tubes was scratched into 200 ml of the liquid medium with yeast extraxt (2%) and saccharose (10%) in 500 ml Erlenmayer flasks and cultivated as a stationary culture at 25°C. After 10 day of growing of the culture the flasks' contents were heated 10 minutes at 100°C. Each fluid was extracted twice by 200 ml of chloroform, the united extract was dried by Na_2SO_4 without water and then the chloroform was evaporated in a water bath.

The determination of the aflatoxin B_1 production ability by strains of *Aspergillus flavus*

The *Aspergillus flavus*-strains were investigated for the aflatoxin B_1-producing–ability by the method of Arca et al. (1988) on liquid medium with 2% yeast extract and 20% saccharose, pH 5.5.

Culture medium for tracheal rings of one-day-old chickens

The minimal essential medium according to Eagle, containing Eartl's salts – E-MEM (The Center for Sera and Vaccination Co. – ÚSOL, Prague, Czech Republic).

Added to the medium were: 1% of the 3% solution of glutamine, 2.5% of the 7.5% $NaHCO_3$ solution with phenol red (ÚSOL, Prague), 10% of fetal serum,

100 μg of streptomycin and 100 U. of penicillium/ml medium and the heat-stable a chloroform-extractable extracts from the investigated strains (40 μg/ml dissolved in dimethylsulfoxid – DMSO). Reference media in the same way with DMSO, whose concentration in the medium was 1%, as well as control with pure medium were prepared.

Tracheal rings of one-day-old chickens

One-day-old chickens (State Research & Productional Company, Častá hatchery, Slovak Republic) were killed by decapitation. Further treatment and testing of tracheal rings was similar to that reported by Nair et al. (1970), with the following modifications: tracheas were taken within 3 minutes of decapitation and twice washed in E-MEM. After that they were cut with a scalpel into thin slices. 20 – 30 tracheal rings were placed on a Petri dish (diameter – 60 mm) with 2 ml of culture medium.

The cultivation of tracheal rings was carried in an incubator at 37°C, and the atmosphere was enriched with 5% CO_2.

Test evaluation

Ciliary movement was observed on 5 – 7 tracheal rings using a microscope after 0, 24, 48, 72 and 144 hours (Histology Jenamed, Carl Zeiss Jena, magnification 250 times).

Movement of cilia was evaluated as "+", or "–" when the cilia did not move.

RESULTS AND DISCUSSION

Twenty two (34.9%) strains from the 63 investigated strains produced heat-stable and chloroform-extractable metabolites with ciliostatic activity against tracheal cilia of the one-day-old chickens in vitro:

3 (4.7%) strains stopped the movement of cilia already after 24 hours, there were strains of *Aspergillus niger* group (2 strains from cotton and 1 strain from flax),

5 (7.9%) strains after 48 hours, there were 2 *Aspergillus flavus* strains with aflatoxin B_1 producing ability and 1 strains of *Aspergillus niger* group from cotton, and *Pæcilomyces variotii* and *Penicillium* sp. from flax,

2 (3.1%) strains after 72 hours – *Aspergillus glaucus* group from flax and *Aspergillus ochraceus* from cotton

and 12 (19.0%) strains after 144 hours of activity – there were the strains from straw (*Aspergillus candidus*), from flax (*Aspergillus fumigatus, Pæcilomyces variotii*), from sorghum (*Aspergillus glaucus* group, *Trichoderma* sp.) and from cotton (*Aspergillus niger* group, *Aspergillus terreus, Fusarium* sp. – 2 strains),

Tab. 1 The effect of the heat-stable and chloroform extractable secondary metabolites of fungi isolated from the samples of flax (F), straw (S), cotton (C) and sorghum (So) on the respiratory tract cilia movement of one-day-old chickens in vitro

Fungi[a]	Protocoled number of the strains	Time (in hours) Movement of cilia				Number of strains	
		24	48	72	144	Σ	+
Absidia sp.	So/86	+	+	+	+	1	0
Acremonium sp.	So/71,76,83,88	+	+	+	+	4	0
A. candidus	S/163	+	+	+	–	1	1
A. flavus	C/131	+	+	+	+		
	C/92[b], 215[b]	+	–	–	–	3	2
A. fumigatus	F/19,38,40,43,53	+	+	+	+		
	F/18	+	+	+	–	6	1
A. glaucus group	So/77,89;F/29,31	+	+	+	+		
	So/85	+	+	+	–	6	2
	F/21	+	+	–	–		
A. nidulans	C/114, l32	+	+	+	+	2	0
A. niger group	C/207	+	+	+	+		
	C/124	+	+	+	–		
	C/112	+	–	–	–	6	5
	C/91,133;F/208	–	–	–	–		
A. ochraceus	C/93	+	+	+	+		
	C/100	+	+	–	–	2	1
A. terreus	C/97,104,122,216	+	+	+	+		
	C/110,111	+	+	+	–	6	2
Fusarium sp.	F/2	+	+	+	+		
	C/210,346	+	+	+	–	3	2
Mucor sp.	C/125	+	+	+	+	1	0
Nigrospora sp.	S/150	+	+	+	+	1	0
Pæcilomyces varioti	C/119;F/46,49,52	+	+	+	+		
	F/54	+	+	+	–	6	2
	F/48	+	–	–	–		
Penicillium sp.	So/67,75;F/7,15,42,						
	51	+	+	+	+		
	F/64	+	–	–	–	7	1
Stachybotrys sp.	F/24	+	+	+	+	1	0
Trichoderma sp.	So/80;F/33,57,58,59	+	+	+	+		
	So/72,87	+	+	+	–	7	2
Strains with ciliostatic activity		3	5	2	12	63	22
	%	4.7	7.9	3.1	19.0	100	34.9

Note [a] : A ... Aspergillus

[b] : aflatoxin B_1 producing strains

the ciliary movement was not affected by metabolites of the other 41 strains and in the reference media (Table 1).

Various mycotoxins – citreoviridin, cyclopiazonic acid, luteoskyrin, penitren A., sterigmatocystin, verruculogen, viomellein, xanthomegnin – can be stored intracellularly in filamentous micromycetes (Filtenborg et al. 1983). Stachybotryotoxins are concentrated in airborne fungal propagules and are not present as free aerosols in the air (Pasanen et al. 1993). Spores and mycelium of toxigenic strains of *Aspergillus flavus* and *Aspergillus parasiticus* are able to contain aflatoxin B_1 (Hesseltine et al. 1966, Shih and Marth 1975). Detritus from plant material is an important vehicle for inhalation of extracellular mycotoxins (Burg et al. 1981, 1982, Burg and Shotwell 1984, Sorenson et al. 1981). In vitro aflatoxin destroys cultured mice embryonic lung cells (Šabad et al. 1976) and affected function of tracheal cells (Cardeilhac et al. 1972, Cardeilhac and Nair 1974). Aflatoxins are carcinogenic for lung cells (Autrup et al. 1979, Wang et Cerutti 1979).

Ochratoxin A and zearalenon (Palmgren et al. 1983), secalonic acid D with acute inflamatory activity in bronchi (Ehrlich et al. 1982, Sorenson et al. 1982) and deoxynivalenol (Young et Fulcher 1984) were in dust from cereal grains detected.

Ciliostatic activies of some mycotoxins on one-day-old chickens tracheal organ cultures in vitro, especially by sterigmatocystin, trichothecens, aflatoxin B_1 and B_2 were in the previous studies described (Jesenská et Bernát – ín press, Nair et al. 1970, Cardeilhac et al. 1972). We continued in the study of ciliostatic activity of secondary metabolites of the strains of filamentous fungi, isolated mainly from substrates of the textil factories – flax and cotton– and from straw and sorghum. Our attention was concentrated on the heat-stable and chloroform extractable metabolites. We found in our study that 34.9% of the investigated strains were in vitro able to produce metabolites with ciliostatic activity against tracheal cilia of one-day-old chickens in vitro. The ciliostatic activity of the metabolites of the filamentous fungi need to be further studied because the movement of cilia is one of the most important defense function of the airway and is a biological barrier between man and his environment (Etievant 1992). The destroyed ciliary movement in airways by mycotoxins may be the first step of the giving rise to bronchitis, chronic bronchitis and chronic respiratory tract illnesses of man in moulded working environment or dwellings. Much research is need to clarify this problem.

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