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


Sledovala sa ciliostatická aktívita termostabilných (100°C/10 min.) a chloroformom extrahovateľných metabolitov 63 kmeňov vláknitých húb, rastúcich na tekutom médiu, na riasinkách priedušnice jednodňových kurčiat in vitro. Ciliostatické metabolismy 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.


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 B₁, 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 expressive 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 extractable 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 (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 extract (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 Na₂SO₄ without water and then the chloroform was evaporated in a water bath.

**The determination of the aflatoxin B₁ production ability by strains of Aspergillus flavus**

The *Aspergillus flavus*-strains were investigated for the aflatoxin B₁-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 Earlt’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% NaHCO₃ 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 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% CO₂.

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 B₁ producing ability and 1 strains of *Aspergillus niger* group from cotton, and *Paecilomyces 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, Paecilomyces 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

<table>
<thead>
<tr>
<th>Fungi a</th>
<th>Proto coled number of the strains</th>
<th>Time (in hours)</th>
<th>Number of strains</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>24</td>
<td>48</td>
</tr>
<tr>
<td>Absidia sp.</td>
<td>So/86</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Acremonium sp.</td>
<td>So/71,76,83,88</td>
<td>+</td>
<td>+</td>
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<tr>
<td>A. candidus</td>
<td>S/163</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>A. flavus</td>
<td>C/131</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>A. flavus</td>
<td>C/92,215</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>A. fumigatus</td>
<td>F/19,38,40,43,53</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>A. fumigatus</td>
<td>F/18</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>A. glaucus group</td>
<td>So/77,89,F/29,31</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>A. glaucus group</td>
<td>So/85</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>A. glaucus group</td>
<td>F/21</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>A. niger group</td>
<td>C/114,132</td>
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</tr>
<tr>
<td>A. niger group</td>
<td>C/207</td>
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</tr>
<tr>
<td>A. niger group</td>
<td>C/124</td>
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</tr>
<tr>
<td>A. niger group</td>
<td>C/112</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>A. niger group</td>
<td>C/91,133,F/208</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>A. ochraceus</td>
<td>C/93</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>A. ochraceus</td>
<td>C/100</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>A. terreus</td>
<td>C/97,104,122,216</td>
<td>+</td>
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<tr>
<td>A. terreus</td>
<td>C/110,111</td>
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<td>+</td>
</tr>
<tr>
<td>Fusarium sp.</td>
<td>F/2</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Fusarium sp.</td>
<td>C/210,346</td>
<td>+</td>
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<tr>
<td>Mucor sp.</td>
<td>C/125</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Nigrospora sp.</td>
<td>S/150</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Paeilomyces variotii</td>
<td>C/119,F/46,49,52</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Paeilomyces variotii</td>
<td>F/54</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Penicillium sp.</td>
<td>So/67,75,F/7,15,42,51</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Penicillium sp.</td>
<td>F/64</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Penicillium sp.</td>
<td>F/48</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Stachybotrys sp.</td>
<td>F/24</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Trichoderma sp.</td>
<td>So/80,F/33,57,58,59</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Trichoderma sp.</td>
<td>So/72,87</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Strains with ciliostatic activity % 4.7 7.9 3.1 19.0 100 34.9

Note a: A ... Aspergillus
b: aflatoxin B1 producing strains
the ciliary movement was not affected by metabolites of the other 41 strains and in the reference media (Table 1).


Ochratoxin A and zearalenon (Palmgren et al. 1983), secalonic acid D with acute inflammatory 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 activities of some mycotoxins on one-day-old chickens tracheal organ cultures in vitro, especially by sterigmatocystin, trichothecens, aflatoxin B₁ and B₂ were in the previous studies described (Jesenská et Bernát – in 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 textile 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 inways 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.

**References**


References:


