PENETRATION OF OVICIDAL FUNGI THROUGH ALTERED EGG-SHELLS OF ASCARIS SUUM

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Abstract. Ovicial activity was studied in microscopic fungi penetrating into and destroying eggs of geohelminths surviving in the soil. The character of penetration was compared in eggs obtained mechanically with those obtained by means of a chemical process. A total of 13 strains of ovicial fungi of 4 species were studied. The shells of eggs obtained by the chemical process were so altered that under the same conditions they were attacked and penetrated by the fungi much more than the eggs obtained by a mechanical way. The advantages and disadvantages of these two methods of eggs isolation are discussed.

One of the basic characteristics of the ovicial fungi is their ability to destroy the eggs of geohelminths. The rate of their efficacy is termed ovicial activity.

The quantitative determination of the ovicial activity is necessary for an exact comparison of the efficacy of ovicial fungi in a certain time period (Lýsek and Chalupová 1976). Until now, the eggs of Ascaris suum obtained by mechanical pressing from uteri of dissected females have been used for this purpose. This method is very laborious and time-consuming, since a large number of eggs are needed for the experiments. Stromberg et al. (1977) described a simple and rapid method for the isolation of A. suum eggs based on a chemical decomposition of shells of uteri by treatment with a solution of NaOH.

The aim of this paper was to compare the character of penetration of ovicial fungi into the eggs obtained by these two methods. Similar problems were dealt with in a previous paper characterizing the penetration of ovicial fungi through A. lumbricoides eggs exposed to higher temperature and UV-irradiation (Lýsek and Bačovský 1979).

MATERIAL AND METHODS

Strains of fungi. Two groups of strains were used in the experiments: a) 5 strains from the collection of ovicial fungi of the Department of Biology, Faculty of Medicine, Palacký University, Olomouc: Paecilomyces marquandii—strains No. 5 and 21, Botrytis fimicola—strain No. 17, Humicola fusariosa—strain No. 53, and Verticillium chlamydothorium—strain No. 68. (For more detail about these strains see Passantová and Lýsek 1982.) b) 7 mutants of strain No. 9 of Verticillium chlamydosporium from the same collection and initial strain No. 9 (repeatedly). The mutants were induced by UV-irradiation (Chalupová and Lenhart 1984) and differed from the initial strain in the rate of proteolytic activity. “Positive mutants 15, 18 and 33 with increased proteolytic activity and “negative mutants” 4, 12, 50 and 73 with decreased proteolytic activity were used.

Preparation of Ascaris suum eggs. Method A. The eggs were obtained by mechanical pressing from uteri of dissected worms. They were sterilized on the surface by 10 % hydrogen peroxide before use (Lýsek et al. 1976). Method B. Uteri of A. suum females were obtained by dissection. They were put in 2 % solution of NaOH and left there for 24 h. After the decomposition of uterine walls the eggs were repeatedly decanted by sterile distilled water to remove the remaining NaOH. The whole process was performed in a sterile medium so that additional surface sterilization was unnecessary.

Quantitative determination of ovicial activity. The method after Chalupová and Lýsek (1979) was used. Water suspension of spores of fungi and eggs in the ratio 1 : 40 (5,000 spores and 200,000 eggs per 1 ml) was placed into 4 tubes containing 1 ml each. For a better stirring of the contents,
capillaries were put in the tubes and the tubes were placed for 168 h in a revolving cultivator. After the termination of the experiment, the number of attacked eggs inside and outside the mycelium clusters was assessed in two tubes, always 100 eggs inside and 100 eggs outside the clusters. The ovicidal activity was expressed by the percentage of attacked eggs (mean number of eggs in the two tubes).

RESULTS

Table 1 shows the percentage of attacked eggs inside and outside the clusters of mycelium in two tubes and a mean value of ovicidal activity of each strain obtained by the two methods. The values obtained by method B were higher in all cases. The differences in the percentage of attacked eggs were tested by a variance analysis (Table 2) and they were demonstrated to be significant (p < 0.05).

Table 2. Effect of the method of egg isolation on the percentage of their attack

<table>
<thead>
<tr>
<th>Source of variability</th>
<th>Sum of squares</th>
<th>Degrees of freedom</th>
<th>Mean square</th>
<th>Test criterion $F$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effect of the factor</td>
<td>806.18</td>
<td>1</td>
<td>806.18</td>
<td>23.72*</td>
</tr>
<tr>
<td>Residual</td>
<td>883.69</td>
<td>26</td>
<td>33.99</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1689.87</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$T_{2(0.05.26)} = 4.22$

DISCUSSION

The described and standardized evaluation of ovicidal activity of fungi was carried out with eggs obtained mechanically, not affected by any chemical treating. Particularly the 3rd type of ovicidal activity (Lysák 1978) was used, when the eggs were penetrated and gradually destructed by the fungus. In this most aggressive type of ovicidal activity a decisive role is played by the barrier of egg-shells which are enzymatically and mechanically destructed by the fungus (Chalupová and Lenhart 1984).

The undamaged and fully developed fertilized egg is surrounded by three shells — lipid, chitinprotein and protein layers. When the eggs are mechanically pressed from the uterus and sterilized on the surface by $H_2O_2$ solution, their protein layer may often be partly or completely separated. The other layers remain undamaged. If the eggs are released by the decomposition of uterine walls by NaOH solution, not only the outer protein layer (visible in a light microscope), but evidently also some protein components in the superficial layers of the chitinprotein layer may be damaged after 24 h. The chitinprotein layer is usually the most important barrier against the penetration of hyphae if the eggs were obtained by a mechanical method. If this shell is partly destructed by NaOH solution, the fungi may more easily penetrate through it.

The method using chemical treatment for the isolation of eggs is more simple and less laborious, but the egg-shells are so altered that the fungus penetrates more rapidly and destructs a higher percentage of eggs. Consequently, this method cannot be used for the demonstration of ovicidal activity of fungi, because it does not correspond to the conditions in free nature.
ПРОНИКАНИЕ ОВИЦИДНЫХ ГРИБОВ ЧЕРЕЗ ИЗМЕНЕННЫЕ
ОБОЛОЧКИ ЯЙЦ Ascaris suum

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Резюме. Изучали овцидную активность микроскопических грибов, проникающих в переживающие в почве яйца геогельминтов и уничтожающих эти яйца. Характер проникания сравнивали у яиц, полученных механическим путем и у яиц, полученных при помощи химической обработки. В объеме изучали 13 штаммов овцидных грибов, относящихся к 4 видам. Оболочки яиц, полученных при помощи химического процесса, были настолько изменены, что при одинаковых условиях, грибы проникали в эти яйца в гораздо более высокой степени, чем в яйца, полученные механическим путем. Обсуждаются преимущества и недостатки обоих методов.

REFERENCES


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