XII. Effect of Hormones On The Mycelial Growth And Yield Of Protein By Agaricus trisulphuratus Rhodocybe subaliva And Agrocybe praecox In Submerged Culture

INTRODUCTION

There is evidence that cytokinins, auxins and gibberellins have profound influence on the mycelial growth and protein content of *Agaricus bisporus*, *Coprinus comatus* (Szabo et al. 1972), *Amanita pantherina* (Vötter and Maroti, 1971). Fraser (1953) has, however, reported that utilization of indole-3-acetic acid by *Psalliota hortensis* is affected in the presence of vitamins in the culture medium. It is also known that high concentrations of heteroauxin are toxic to the growth of *Pleurotus corticatus* (Leonian and Lilly, 1937). But low concentrations are neither stimulatory nor inhibitory to the growth of the organism. Bohus (1959) has observed neither promotion nor inhibition of growth in several strains of cultivated mushrooms at 10 ppm heteroauxin. He has also found that 50 ppm concentration is inhibitory for all the test strains. Inhibitory action of IAA on the growth of some mycorrhizal mushrooms including two species of *Amanita* and two species of *Suillus* in liquid medium has been studied by Fortin (1967). The growth of the four species tested has been inhibited,
varying degrees by IAA at 1.75 mg/l concentration. Guha and Banerjee (1974) have studied the effects of kinetin and indole-3-acetic acid on the mycelial growth and protein content of *Agaricus campestris* S₁₂ strain and found maximum stimulation of growth of *A. campestris* at 0.4 ppm IAA but noted inhibition at higher concentrations. They have also reported no significant change in the protein content between different treatments.

In this experiment, an attempt has been made to study the effects of different plant hormones on the mycelial growth and protein content of *Agaricus trisulphuratus*, *Rhodocybe subgilla* and *Agrocybe praecox*.

**MATERIALS AND METHODS**

**Test Organisms**

The tissue cultures of *Agaricus trisulphuratus* Berk, *Rhodocybe subgilla* (Berk & Br.) Pegler and *Agrocybe praecox* (Pers ex Fr.) Fayod were used in the study and were maintained on 3% malt extract agar medium. The Glucose-Asparagine medium of Lilly and Barnett (1951) was used as the basal synthetic liquid medium.

**Preparation of Inoculum**

A small portion of actively growing mycelium from agar slant of each test fungus was aseptically transferred
to an Erlenmeyer flask (250 ml) containing 50 ml of the basal liquid medium and was incubated on a shaking incubator (120 r.p.m.) at 30°C (±0.5°C) for 7 days in complete darkness. After the incubation period, the mycelial mass was aseptically fragmented into small pieces with the help of a Waring blender. The fragmented mycelium was washed several times with distilled water to remove any trace of the medium and then suspended in a phosphate buffer medium (pH 5.5) for 24 hours to overcome the shock encountered during blending. 1.0 ml of the mycelial cell suspension was used as the inoculum.

Medium and Growth Conditions

To study the effect of indole-3-acetic acid, gibberellic acid, α-napthal acetic acid and maleic hydrazide on mycelial growth and protein content, stock solutions of the hormones were prepared separately. Finally, dilution grade of 25 ppm of the hormones was made by adding appropriate volumes of stock solution to the sterilized synthetic liquid medium of each test fungus containing the different nutrients in their respective optimum concentrations. The pH of the medium of *A. trisulphuratus*, *R. subaliva* and *A. praecox* was adjusted to their respective optimum values of 5.5, 5.0 and 5.0 with 0.2M phosphate buffer before sterilization. 50 ml of the medium
was dispensed in each Erlenmeyer flask (250 ml). Media without hormones served as controls while complete media contained all the hormones under study.

Each set of sterilized flasks with one type of hormone source was inoculated separately with 1.0 ml of cell suspension of the mycelium of the three test fungi—A. trisulphuratus, R. subgliva, A. praecox, separately and incubated separately in a shaking incubator (120 r.p.m.) in complete darkness for 16, 20, 16 days respectively at their respective optimum temperatures 25°, 35° and 35°C (±0.5°C). Sufficient number of flasks were inoculated in order to have five replicates for each treatment.

Measurement of Growth

After the respective incubation periods, five flasks were harvested for each test fungus for each treatment. The medium and the mycelium were separated by filtration through a tared sintered funnel (Jena IG-3). The filtered mycelium was washed repeatedly with distilled water to make it free from adherent nutrients and dried to constant weight in a vacuum oven at 60°C for 24 hours, cooled in a desiccator and weighed. The dry weight of mycelium was taken as a measure of cell growth.
Measurement of Protein

Total nitrogen of the dried mycelium was determined by the colorimetric method of Polin and Wu (1919) and Vogel (1961) using a colorimeter (Model-AE-11, Tokyo Erma Optical Works Ltd., Japan). Mycelial protein was assumed to contain 16% nitrogen and consequently a factor of 6.25 was used to convert nitrogen values to crude protein content. The complete set of experiments was done in triplicate.

RESULTS AND DISCUSSION

Four hormones were tested on Agaricus trisulphuratus, Rhodocybe subgliva and Agrocybe praecox with a view to ascertain their effect on the mycelial and protein content of the test-fungi. The results are given in Table 15 and Text-fig.11.

Maximum yields of the three test fungi are obtained in the medium without hormones which serves as control. The data obtained indicate that at a dilution grade of 25 ppm all the four hormones have been found to have an inhibitory effect on both growth and protein content of A. trisulphuratus, R. subgliva and A. praecox. The test fungi exhibit only slight growth in the complete medium containing all the hormones tested.
Table 15. Data (mean)* showing the effect of hormones on the growth and yield of protein by the mycelia of *Agaricus trisulphuratus*, *Rhodocybe subgliva* and *Agrocye praecox* at their respective incubation periods under submerged condition.

<table>
<thead>
<tr>
<th>Hormone source</th>
<th>A. trisulphuratus</th>
<th>R. subgliva</th>
<th>A. praecox</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dry wt. Protein</td>
<td>Dry wt. Protein</td>
<td>Dry wt. Protein</td>
</tr>
<tr>
<td></td>
<td>of mycelium (%)</td>
<td>of mycelium (%)</td>
<td>of mycelium (%)</td>
</tr>
<tr>
<td>Complete</td>
<td>0.59</td>
<td>11.51</td>
<td>1.30</td>
</tr>
<tr>
<td></td>
<td>±0.04</td>
<td>±0.01</td>
<td>±0.08</td>
</tr>
<tr>
<td>Medium</td>
<td>±0.04</td>
<td>±0.01</td>
<td>±0.03</td>
</tr>
<tr>
<td>Maleic hydrazide</td>
<td>10.97</td>
<td>30.75</td>
<td>10.16</td>
</tr>
<tr>
<td></td>
<td>±0.14</td>
<td>±0.03</td>
<td>±0.12</td>
</tr>
<tr>
<td>Maleic hydrazide</td>
<td>±0.10</td>
<td>±0.02</td>
<td>±0.10</td>
</tr>
<tr>
<td>Maleic hydrazide</td>
<td>±0.10</td>
<td>±0.02</td>
<td>±0.10</td>
</tr>
<tr>
<td>+Gibberellic acid</td>
<td>10.08</td>
<td>30.24</td>
<td>9.28</td>
</tr>
<tr>
<td>+Gibberellic acid</td>
<td>±0.12</td>
<td>±0.03</td>
<td>±0.11</td>
</tr>
<tr>
<td>+Indole-3-acetic acid</td>
<td>8.75</td>
<td>28.21</td>
<td>3.95</td>
</tr>
<tr>
<td>+Indole-3-acetic acid</td>
<td>±0.10</td>
<td>±0.02</td>
<td>±0.09</td>
</tr>
<tr>
<td>Control</td>
<td>13.90</td>
<td>31.88</td>
<td>12.84</td>
</tr>
<tr>
<td></td>
<td>±0.12</td>
<td>±0.02</td>
<td>±0.11</td>
</tr>
</tbody>
</table>

* Averages of five replicates for growth and three for protein yield.
Text - fig. 11
Text - fig. 11

PROTEIN CONTENT DRY WT. OF MYCELUM ($/£)

A. TRISULPHURATUS. (16 DAYS) ------
R. SUBGLIVA. (20 DAYS) -----------
A. PRAECOX. (16 DAYS) ----------

1. COMPLETE MEDIUM.
2. + MALEIC HYDRAZIDE.
3. + L- NAPHTHALACETIC ACID.
4. + GIBBERELLIC ACID.
5. + INDOLE-3-ACETIC ACID.
6. CONTROL.

HORMONE SOURCES.

DRY WT. OF MYCELUM (%)
In case of *A. trisulphuratus*, α-naphthalacetic acid markedly inhibits the yields while indole-3-acetic acid shows similar effect on *R. subgliva*. IAA, however, has a comparatively lesser retarding effect on growth and protein yield of *A. praecox*.

In general, it may be concluded that 25 ppm concentration of the hormones tested individually has an inhibitory effect on the growth and yield of protein of all the three test fungi studied.

High concentrations of heteroauxin are known to be toxic to the growth of *Pleurotus corticus* (Leonian and Lilly, 1937). But low concentrations are neither stimulatory nor inhibitory to the growth of the organism. Bohus (1959) has observed neither promotion nor inhibition of growth in several strains of cultivated mushrooms at 10 ppm heteroauxin. He has also found that 50 ppm concentration is inhibitory for all the test strains.

The growth of some mycorrhizal mushrooms including two species of *Amanita* and two species of *Suillus* in liquid medium has been inhibited, to varying degrees by IAA in 1.75 mg/l concentration (Fortin, 1967). Guha and Banerjee (1974) have studied the effects of kinetin and indole-3-acetic acid on the mycelial growth and protein content of *Agaricus campestris* strain
and found inhibition of growth at concentrations higher than 0.4 ppm IAA. They have reported no significant change in the protein content between different treatments.

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