IX. EFFECT OF CARBON : NITROGEN RATIOS ON THE GROWTH AND PROTEIN PRODUCTION BY SUBMERGED MYCELIA OF GYMNOPSIS CHRYSONYCES, LEUCOCOPRINUS BRINBAUMII AND LEUCOCOPRINUS CEPAESTIPES UNDER OPTIMUM CONDITIONS.

INTRODUCTION

The effect of different ratios of carbon and nitrogen sources on growth and production of protein by mushroom mycelium has shown their intricate relationship. Reusser et al. (1958a, 1958b) have shown the increase in mycelial growth and crude protein content along with decrease in C/N ratio. They have also found that 4-24% yield of fat as dry wt. could be obtained in Tricholoma mucedum which is dependent on C/N ratio. Litchfield et al. (1963) have demonstrated that yield of morel mushrooms is dependent on the C/N ratio of medium. Falina et al. (1970) have obtained optimum C/N ratio as 10:1 for Boletus variegatus in a nutrient medium. C/N ratio of 15:1 was found optimum for Trametes versicolor (Maslova, 1971). Highest mycelial yield was obtained in Serpula lacrimans when concentration of both sucrose and aspartate was high (Watkinson, 1975). Hong et al. (1981) have found that yield of mycelia of Agaricus bitorquis and Pleurotus ostreatus decrease under lower or higher C/N ratio. At the same C/N ratio, the higher the concentration of glucose and peptone, the more increase in yield is found.

Best carbon and nitrogen sources obtained are maltose and peptone in case of G. chrysomyces glucose and yeast extract for
L. birnbaumii and starch and yeast extract in case of L. cepaestipes respectively. As such, necessary experiments has been done to determine optimum C : N ratio for the test-fungi.

MATERIALS AND METHODS

Test Organism: The mycelial cultures of Gymnopilus chrysosmyces (Berk.). Sacc., Leucocprinus birnbaumii (Corda) Sing. and Leuco-
primus cepaestipes (Sow. ex Fr.) Pat. were used in the study. Cul-
tures were maintained by subculturing in 3% maltexract agar
medium at definite interval of 15 days and maintained at 25°C in
complete darkness. Glucose-asparagine medium of Lilly and Barnett
(1951) was used as liquid basal synthetic medium.

Preparation of inoculum: A small portion of actively growing
mycelium of each test-fungus from agar slants was transferred
separately to a 250 ml. Erlenmeyer flask containing 50 ml. of
basal liquid synthetic medium and incubated on a shaking incubator
(120 r.p.m.) at 30°C (± 0.5°C) for 7 days in complete darkness.
After incubation period, the mycelial mass was aseptically frag-
mented into small pieces in a waring blender. This fragmented mass
was washed several times with sterile distilled water to remove
any trace of medium and then suspended in a phosphate buffer
(pH 5.5) for 24 hours to overcome the shock encountered during
blending. 1 ml. of the mycelial cell suspension was used as
inoculum.

Growth medium: According to the optimum carbon and nitrogen
sources obtained, the basal synthetic medium was modified. Glucose
and asparagine were replaced by maltose and peptone respectively
In case of *G. chrysomyces*, by starch and yeast extract respectively in case of *L. cepaestipes* and asparagine was replaced by yeast extract in case of *L. birnbaumii*. Different C : N ratios taken were 

\[ C_1 N_1, C_1 N_2, C_1 N_3, C_2 N_1, C_2 N_2, C_2 N_3, C_3 N_1, C_3 N_2, C_3 N_3 \]

as \( C_1 = 20 \text{ g}, C_2 = 30 \text{ g}, C_3 = 40 \text{ g} \); \( N_1 = 1 \text{ g}, N_2 = 2 \text{ g}, N_3 = 3 \text{ g/litre} \)

Respective optimum carbon and nitrogen sources for the three test fungi were added accordingly and the pH of the medium was adjusted to 5.5 for *G. chrysomyces* and *L. cepaestipes* and to 6.0 for *L. birnbaumii* with the help of 0.2 M phosphate buffer before sterilization. 50 ml. of different media was dispensed in each of 250 ml. Erlenmeyer flasks, plugged and sterilized at 10 p.s.i. for 20 minutes. Several flasks were taken to have five replicates for each set.

**Growth conditions:** Each set of flasks were inoculated with 1 ml of cell suspension of each test-fungus separately and incubated in a shaking incubator (120 r.p.m.) at 30°C (+ 0.5°C) in complete darkness for 20 days in case of *G. chrysomyces* and for 16 days in case of other two fungi.

**Measurement of growth:** After the incubation period, the medium and mycelium were separated by filtration through a tarred sintered funnel (Jena IG-3). Filtered mycelium was repeatedly washed with sterile distilled water to make it free from adherent medium and dried to constant weight at 60°C. Dry weight of mycelium thus obtained was taken as index of growth.
estimation of protein.

The total nitrogen content of the dried mycelium powder obtained in each treatment was estimated using photoelectric colorimeter (Model AE-11, Tokyo Erma Optical works Ltd., Japan) following the method of Folin and Wu (1919) and method of Vogel (1961). The crude protein value was also calculated on the basis of 16 per cent nitrogen content of protein and consequently a factor of 6.25 was used to convert the nitrogen values to crude protein content. Each complete set of experiment was done in triplicate.

RESULTS AND DISCUSSION

The data obtained during the experiment are given in Table 9 and Text-fig. 9.

The data in Table 9 show that for G. chrysosmyces, C3 N3 is best C : N ratio for growth and protein production by mycelium. When growth is considered, C3 N3 is followed by C2 N3, C3 N2, C2 N2, C1 N3, C3 N1, C2 N1, C1 N2 and C1 N1 and when protein yield is considered, it is followed by C3 N2, C2 N2, C3 N1, C1 N1, C1 N2, C2 N3, C1 N3 and C2 N1 (Text-fig. 9). So growth and protein yield are not proportional to C : N ratio. The test-organism favours high proportion of carbon and nitrogen in the medium for growth.

In case of L. birnbaumi (Table 9) C2 N2, is best ratio for high yield both of mycelium and protein. In growth it is followed by C3 N3, C3 N1, C2 N1, C2 N3, C1 N3, C1 N1, C3 N2 and
Table 9. Data (mean*) showing the effect of different C:N ratios on the growth and production of protein by the mycelia of *G. chrysoscyces*, *L. birnbaumii* and *L. cepsaestipes* at their respective optimum submerged conditions.

<table>
<thead>
<tr>
<th>C:N ratio</th>
<th><em>G. chrysoscyces</em></th>
<th><em>L. birnbaumii</em></th>
<th><em>L. cepsaestipes</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dry wt. of mycelium (g/l)</td>
<td>Protein content (%)</td>
<td>Dry wt. of mycelium (g/l)</td>
</tr>
<tr>
<td>C1N1 (20:1)</td>
<td>4.30 ± 0.17</td>
<td>21.25 ± 0.04</td>
<td>6.49 ± 0.18</td>
</tr>
<tr>
<td>C1N2 (20:2)</td>
<td>5.44 ± 0.17</td>
<td>20.93 ± 0.04</td>
<td>1.03 ± 0.13</td>
</tr>
<tr>
<td>C1N3 (20:3)</td>
<td>6.77 ± 0.15</td>
<td>17.81 ± 0.04</td>
<td>7.64 ± 0.30</td>
</tr>
<tr>
<td>C2N1 (30:1)</td>
<td>5.63 ± 0.19</td>
<td>11.25 ± 0.05</td>
<td>8.22 ± 0.14</td>
</tr>
<tr>
<td>C2N2 (30:2)</td>
<td>6.86 ± 0.15</td>
<td>22.96 ± 0.06</td>
<td>9.84 ± 0.13</td>
</tr>
<tr>
<td>C2N3 (30:3)</td>
<td>8.18 ± 0.22</td>
<td>18.76 ± 0.02</td>
<td>7.94 ± 0.24</td>
</tr>
<tr>
<td>C3N1 (40:1)</td>
<td>6.42 ± 0.16</td>
<td>22.65 ± 0.04</td>
<td>9.52 ± 0.27</td>
</tr>
<tr>
<td>C3N2 (40:2)</td>
<td>8.08 ± 0.21</td>
<td>23.59 ± 0.01</td>
<td>2.87 ± 0.21</td>
</tr>
<tr>
<td>C3N3 (40:3)</td>
<td>9.40 ± 0.20</td>
<td>27.34 ± 0.04</td>
<td>9.57 ± 0.15</td>
</tr>
</tbody>
</table>

* Results are expressed as average of five replicates for Dry wt. and three replicates for protein content.
Text - fig. 9.
C1N2 while in protein content of mycelium C2N2 is followed by C1N2, C3N2, C2N3, C1N3, C3N3, C1N1, C3N1, and C2N1 (Text-fig.9). The results reveal that protein content is best in case of N2 ratios followed by N3 and N1 ratios.

In L. cepaestipes (Table 9) C3N3 ratio has been found to be the best combination for growth and protein production. The growth of mycelium is found to be in decreasing order in the following ratios C3N3, C2N3, C2N2, C3N2, C3N1, C1N3, C1N2, C2N1, and C1N1. Increase in growth is found with increase in nitrogen from N1 to N3. C3N3 is followed, in protein production, by C2N3, C1N3, C2N2, C1N2, C3N1, C2N1, C3N2, and C3N1 (Text-fig.9). It further reveals that high nitrogen ratios yield much protein.

In G. chrysomyces, no gradual increase in mycelium and protein content is found with the increase in C:N ratios. In L. cepaestipes, increase in growth with increase in nitrogen proportion is found irrespective of the amount of carbon. The result does not agree with that of Reusser et al. (1958a, 1958 b) who found the increase in growth and protein content along with the decrease in C:N ratios. Of the nine combinations of C:N ratios taken in the present experiment, C3N3 (highest concentrations) was found to be best in two of the test-fungi. This is similar to the report of Watkins (1975) that greatest mycelial growth of Serpula lacrimans are found when both sucrose and aspartate high in amount.
REFERENCES


