XVI. CONTENTS OF B-VITAMINS AND VITAMIN C IN THE MYCELIA OF GYMNOPILUS CHRYSOMYCES, LEUCOCOPRINUS BIRNBAUMII AND LEUCOCOPRINUS CEPÆSTIPES GROWN IN BASAL AND OPTIMUM MEDIA UNDER SUBMERGED CONDITIONS.

INTRODUCTION

Mushrooms appear to be good sources of several vitamins. Several authors have worked on the vitamin content of mushrooms. Orton and his co-workers (1922) have found Agaricus campestris to be good source of water-soluble vitamin-B. Anderson and Fellers (1942) have concluded that A. campestris sporophores are excellent source of nicotinic acid, riboflavin and pantothenic acid, fair source of vitamin B, C and K. Brunell (1943) has obtained 57 mg of niacin per 100 g of A. bisporus mycelium. Esselen and Fellers (1946) have noticed the presence of folic acid in A. bisporus. Cream variety of A. campestris contains more riboflavin and niacin than white variety as has been shown by Humfeld and Sugihara (1949). Block et al. (1953) have recorded the presence of 146 mg/g of niacin (dry wt. basis) in Agaricus blazei. Information of vitamin C content of higher fungi has been observed by Giaconmini (1955) and Gilbert and Robinson (1957). Agaricus sylvatica (50 mg/100 g wet wt.) and Boletus edulis (36 mg/100 g wet wt.) are two of the best sources whereas Russula libecea (6 mg/100 g wet wt.) contains very low amount of ascorbic acid. Jennison et al. (1957) have done quantitative analysis of seven B-group vitamins of some wood-rotting basidiomycetes of which white rots contain more vitamins. Watt and Merril (1963) have obtained 43.8 mg of niacin
and 31.3 mg of ascorbic acid per 100 g dry mycelium of A. bisporus. Litchfield et al. (1964) have estimated vitamins including niacin and folic acid in Morchella hortensis. Of 38 strains under study, Pleurotus ostreatus is best producer of vitamin B12 (Shivrina et al., 1965). 55.7 mg of niacin and 81.9 mg of ascorbic acid have been obtained per 100 g dry mycelium of A. bisporus (Altamura et al., 1967). 4.7 mg, 11.9 mg, 1.0 mg, 91.9 mg of niacin per 100 g dry mycelium of Auricularia polytricha, Lentinus edodes, Volvariella volvacea (canned) and V. volvacea (fresh) respectively have been estimated by Food and Agricultural Organization (1972). Zerova et al. (1972) have studied the thiamine, pyridoxine, nicotinic acid and biotin content of four edible boletic mushrooms. Thiamine and riboflavin content of several mushrooms has been estimated (Shivrina and Koryakina, 1966; Fedorova and Milova, 1974; Karosene, 1977, 1978, 1979, 1980). Reported values for the vitamin contents of mushrooms have been summarized by Morgan (1968) and Crisan and Sands (1978).

In the present study, three B-vitamins (niacin, folic acid and vitamin B12) and vitamin C (i.e. ascorbic acid) contents have been assayed in the mycelia of Gymnopilus chrysomyces, L. birnbaumii and L. cepaestipes grown in basal medium and in their respective optimum media under submerged conditions.

MATERIALS AND METHODS

Test organisms: Tissue cultures of Gymnopilus chrysomyces Berk. (Sacc.), Leucocoprinus birnbaumii (Corda) Sing. and Leucocoprinus cepaestipes (Sow. ex Fr.) Pat. were used for study. Cultures were
maintained in 3% malt-extract agar medium at 25°C in complete darkness.

Cultural procedures: Two types of media, basal and optimum, were prepared for each test-fungus. Glucose-asparagine medium (Lilly and Barnett, 1951) was used as basal synthetic medium.

Maltose-peptone, glucose-yeast extract and starch-yeast extract optimum media were prepared for *G. chrysoscyces*, *L. bimbaumii* and *L. cepaestipes* respectively. The carbon and nitrogen sources and other nutrients were added in concentrations as determined in the previous physiological studies. pH of the basal medium, maltose-peptone and starch-yeast extract media were adjusted to 5.5 and that of glucose-yeast extract medium was adjusted to 6.0 with the help of 0.2 M phosphate buffer. 50 ml of each medium was distributed in each of 250 ml Erlenmeyer flasks, plugged and sterilized at 10 p.s.i. for 20 minutes. Each sterilized flask was then inoculated separately with 1 ml cell suspension of each test-fungus and incubated in a shaking incubator (120 r.p.m.) at 30°C (+ 0.5°C) in complete darkness. Flasks set for *G. chrysoscyces* were incubated for 20 days and those for the other two test-fungi were incubated for 16 days according to their respective optimum incubations.

After incubation, the mycelium of each test-fungus was harvested separately by filtration through a tared sintered funnel (Jena IG-3). Filtered mycelium was washed repeatedly with distilled water to make it free from adherent medium and dried to constant weight at 60°C. Dry mycelium was used for analytical studies.
Analytical procedures:

A. Estimation of niacin content: In this estimation, the method of Melnick and Field (1940) was followed:

500 mg of dry finely powdered mycelium of each test-fungus was taken separately. 5 ml of conc. HCl (sp.Gr.of about 1.8) was added in graduated test tube containing mycelium powder and this was followed by addition of distilled water until the total volume reached 15 ml. Acidity was approximately 4 N. Test tube was immersed in boiling water bath and hydrolysis occurred for 40 minutes with occasional stirring. Sample was then cooled to room temperature and the volume restored to original 15 ml. 10 ml absolute alcohol was then added to the solution. This suspension was taken in a 150 ml Erlenmeyer flask and 200 mg charcoal powder was added to it, mixture shaken and filtered at room temperature. An aliquot of filtrate, 8.33 ml was pipetted into a graduated tube, one drop of phenolphthalein was added and neutralized in cold to pH 7.0 with 10% NaOH. Final volume was made upto 10 ml. 3 ml aliquots of the test solution (already containing alcohol 1 part to 2 parts water and equivalent to 1/10th of original sample) were used for the tests. To the first sample, 7 ml of alcoholic buffer solution of pH 10 (made up of water, H₃PO₄, NaOH and absolute alcohol) was added. To the second aliquot, 6 ml Cyanogen bromide(CNBr) (0.5 M) and 1 ml aniline (4%) was added and to the third aliquot, 10 μg of nicotinic acid was added along with the CNBr and aniline. Second aliquot was used for the chemical reaction and maximum total photometric density was measured. From the first aliquot,
photometric density of the color remaining after charcoal decolorization was measured. By difference that resulting from chemical reaction alone was obtained. This value was converted into ug of nicotinic acid by use of K-value obtained from third aliquot. The photometric density was measured at 420 nm by using a colorimeter (Model AE-ll, Tokyo Erma Optical Works Ltd, Japan). Calculation was done by using the formula:

\[ \text{Niacin in } \mu g(Y) = 10 \times \frac{C-(B-A)}{0.229} \]

B. Estimation of folic acid: Pteroyl glutamic acid or folic acid was estimated by measuring the aromatic amine colorimetrically by method of Hutchings et al (1947).

2 g finely powdered dry mycelium of each test-fungus was taken separately in each of 100 ml Erlenmeyer flask for analysis. To this flask, 80 ml distilled water, 10 ml 5.0 N HCl and 1.0 ml 0.5% gelatin solution was added. Volume was then made up to 100 ml. 4 ml of this unreduced solution was used for determining the free amine. The remainder of the solution was transferred to a 250 ml flask and reduced by adding 500 mg of zinc dust and shaking the flask intermittently for 10 minutes. Then the zinc dust was filtered off. The 4 ml aliquot of this reduced sample was diluted to 6.6 ml with distilled water and to this sample were added, with thorough mixing, 0.4 ml of 5.0 N HCl and 1 ml of NaNO₂ solution. After 3 minutes, 1 ml of ammonium sulfamate solution was added and decomposition of nitrous acid was allowed to proceed for 2 minutes. At the end of this period, 1 ml of N-(1-naphthyl) ethylenediamine solution was added. After 10 minutes this sample was used for de-
termining amine. Photoelectric colorimeter (Model AE-ll, Tokyo Erma Optical Works Ltd, Japan) was used to measure the colour at 550 μ. A response curve was constructed using Para-amino benzoic acid (PABA) as standard. The difference between the amine obtained after reduction and that present in unreduced solution gave the combined amine present. This value was multiplied by the factor (mol. wt. of folic acid/mol. wt. of PABA) giving micrograms of folic acid in diluted sample.

C. estimation of vitamin B12 (cyanocobalamin): A quantitative colorimetric method of Fantes et al (1950) was followed. One g dry finely powdered mycelium of each test-fungus was hydrolyzed separately with 10 N HCl at 100°C for 18 hours. The hydrolysate was filtered with 1 N HCl and volume was made up to 10 ml. Taking the hydrolysate in a separating funnel, 10 ml of n-octyl alcohol was added to it and shaken for 3 hours. Thus the organic acid was converted to ester. One ml of the alcohol layer was diluted with up to seven volumes of light petroleum and washed with a mixture of methanol; N HCl (4:1). This Octyl alcohol-petroleum solution was used for colorimetric determination with 604 filter in photoelectric colorimeter (Model AE-ll, Tokyo Erma Optical Works Ltd, Japan).

D. estimation of vitamin C (ascorbic acid): The method of A.O.A.C. (1966) has been followed for the purpose.

The method consists of the followings:

1) Extraction: 40 g of fresh harvested mycelium of each test-fungus was taken separately in each of 100 ml Erlenmeyer flasks. 40 ml of 6% metaphosphoric acid (in 0.005 M EDTA) was added and allowed to
stand for 30 minutes with occasional swirling. It was then diluted
to 80 ml with distilled water, mixed and filtered.

ii) Colour development: A colour reagent was prepared, immediately
before use, by pipetting 2 ml of 0.2% 4-methoxy-2-nitroaniline and
2 ml of 0.2% NaNO₂ solution into a 125 ml flask, swirling until the
orange colour disappeared and adding 75 ml of alcohol to the solu-
tion.

Ascorbic acid standard stock was prepared by weighing accu-
rately 100 mg ascorbic acid into a 250 ml Erlenmeyer flask, dissolv-
ing and diluting to volume with 3% metaphosphoric acid solution
(in 0.0025 M EDTA.) Ascorbic acid standard I and standard II were
prepared by diluting 3 ml and 5 ml of the standard stock solution
respectively to 100 ml with 3% metaphosphoric acid solution (in
0.0025 M EDTA). Standard I and Standard II contained 15 μg and 25
μg of ascorbic acid respectively.

The following sets were prepared for colour development.
a) Sample (U) - To 6.5 ml of colour reagent taken in a colorimeter
tube, 1 ml sample extract was added and mixed.

b) Standards (S₁ and S₂): 6.5 ml of colour reagent was pipetted
in each of two colorimeter tubes. 1 ml of standard I was added to
one tube (S₁) and 1 ml of Standard II to the other (S₂).

Both the sets a and b were allowed to stand for 15 minutes,
2.5 ml of 10% NaOH was added and centrifuged.

c) Reagent blank: In a colorimeter tube, 6.5 ml of colour reagent
and 1 ml of 3% metaphosphoric acid (in 0.0025 M EDTA) were mixed
and 2.5 ml of NaOH was added to it.
d) Blank (BS): 1 ml of glacial acetic acid, 1 ml of 10%(w/v) aqueous H₂SO₄, 2 ml of 0.2% aqueous NaN₃ were taken in an Erlenmeyer flask, 75 ml of 95% ethyl alcohol was mixed. 6.5 ml of this solution was added with 1 ml sample extract and 2.5 ml of NaOH in a colorimeter tube and mixed.

iii) Photometric determination: This was done by using 570 filter in a colorimeter (Model AE-11, Tokyo Erma Optical Works Ltd, Japan). The concentration of ascorbic acid in the sample was calculated as:

\[ C = 15 + \frac{10(Au-AS₁)}{(AS₂ - AS₁)} \times \frac{1}{1000 Cu} \]

Where C = concentration of ascorbic acid in the sample in mg/g.

\[ Au = \text{Absorbance of the sample tube.} \]

\[ AS₁ = \text{" } S₁ \text{ tube.} \]

\[ AS₂ = \text{" } S₂ \text{ tube.} \]

\[ Cu = \text{Concentration of the sample in the final dilution in g/ml.} \]

RESULTS AND DISCUSSION

The data obtained during the experimental period are given in Table-19.
Table 19. Data (mean*) showing the content of B-vitamins (mg/100 g dry wt.) and vitamin C (mg/100 g fresh wt.) of the mycelia of G. chrysosmyces, L. birnbaumii and L. cepaestipes grown in basal and optimum media under submerged conditions.

<table>
<thead>
<tr>
<th>Vitamin contents</th>
<th>Test - fungi</th>
<th>G. chrysosmyces</th>
<th>L. birnbaumii</th>
<th>L. cepaestipes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mycelium from OM</td>
<td>Mycelium from BM</td>
<td>Mycelium from OM</td>
<td>Mycelium from BM</td>
</tr>
<tr>
<td>(B-vitamins)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Niacin (mg/100 g dry weight).</td>
<td>8.00 ±0.02</td>
<td>4.90 ±0.01</td>
<td>7.16 ±0.05</td>
<td>6.02 ±0.04</td>
</tr>
<tr>
<td>Folic acid (mg/100 g dry weight).</td>
<td>6.01 ±0.03</td>
<td>2.40 ±0.04</td>
<td>5.21 ±0.05</td>
<td>3.40 ±0.01</td>
</tr>
<tr>
<td>Vitamin B&lt;sub&gt;12&lt;/sub&gt;</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Vitamin C</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ascorbic acid (mg/100 g fresh weight).</td>
<td>11.00 ±0.01</td>
<td>5.60 ±0.03</td>
<td>5.50 ±0.05</td>
<td>4.30 ±0.02</td>
</tr>
</tbody>
</table>

* Averages of three separate determinations under identical conditions.

The data in Table 19 show that none of the test-organisms contain vitamin B<sub>12</sub>. Reports of vitamin B<sub>12</sub> in other mushrooms are rare. Contents of other vitamins (under study) are more in mycelia from optimum media (OM) than in mycelia from basal media (BM).

If the vitamin contents of mycelia from OM are considered, it is found that ascorbic acid content of L. birnbaumii is low. Similar report has been obtained in Russula libacea (Gilbert and...
Robinson, 1957). The ascorbic acid contents of other two test-fungi are not very low. Niacin content of the test-fungi is not very high whereas folic acid content is fairly good in comparison to others already reported (Crisan and Sands, 1978).

REFERENCES


Quantitative analysis on seven B group vitamins of 17 species of wood-rotting Basidiomycetes in synthetic and malt-extract media. Appl. microbiol. 5: 87-95.


