Nutrition Requirements of *Pleurotus flabellatus*

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The mycelium of *Pleurotus flabellatus* was grown in a synthetic medium to obtain accurate information on its nutritional requirements. Among various carbon sources tried, the organism was found to utilize hexose sugars more readily than other sugars. Ammonium citrate was found to be the best source of nitrogen. The yield of dry matter increased as the concentration of nitrogen was increased up to a certain stage beyond which there was no increase in the yield, but the crude protein content of the mycelium increased. Detailed studies on the effect of varying the concentrations of other major nutrients, i.e., potassium, phosphorus, calcium, and magnesium, on the growth and crude protein content of the mycelium were also carried out. Optimal pH range was fairly broad, lying between 4.5 to 7.5.

The possibility of growing mycelium of *Agaricus campestris* in submerged culture by an aeration and agitation method was first demonstrated by Humfeld (7). Block (5) has suggested that such a process would offer a new and revolutionary method of food production. Detailed investigations were carried out on the nutritional requirements of *A. campestris* by Humfeld and Sugihara (8, 9), who grew the fungus on a chemically defined medium containing suitable sources of carbohydrate and nitrogen with some mineral salts. Whitaker (17) also studied in detail the growth requirements of some mushrooms. The medium defined of Humfeld and Sugihara (8) was later tried by others (10) for the submerged culture of a few species of *Psalliota* and *Boletus*, and satisfactory results were achieved. In 1954, Sugihara and Humfeld (15) tried to grow selected stock of various species of mushrooms and tested their ability for growth in liquid medium. Mycelium, like the fruiting body, has been shown to be valuable nutritionally as a source of amino acids and B complex vitamins (4, 8). Eddy (6) mentioned that higher fungi grown in submerged culture were capable of producing materials with good nutritive value. They can, therefore, serve as a protein supplement for human beings or as an animal feed. Mushrooms should be considered as one of the world’s greatest natural resources, since they have the ability to transform nutritionally valueless substances into high fat and protein foods (13).

No literature seems to be available on the submerged propagation of *Pleurotus flabellatus* (Berk. & Br.) Sacc. Since this species of *Pleurotus* is eaten by the people in Mysore State (India), a detailed investigation was undertaken to obtain accurate information on its nutritional requirements, with the idea that it might be grown on a cheap, natural medium on a large scale for food.

**MATERIALS AND METHODS**

*P. flabellatus* grows on dead and decaying wooden logs under natural conditions. This mushroom is cooked like a vegetable and is eaten by the people in Mysore. The culture of *P. flabellatus* was obtained from the tissue of the sporophore. By use of standard aseptic methods, small pieces of sporophore tissue were placed on potato-dextrose-agar slants (potato infusion from 200 g of potatoes; dextrose, 20 g/liter; agar, 20 g/liter) and incubated at room temperature (21 to 35°C). Subsequent subculturing produced a pure culture of mushroom mycelium which was maintained on potato-dextrose-agar.

Inoculum was produced in 500-ml Erlenmeyer flasks containing 50 ml of potato-dextrose-broth. Bits of mycelium planted on this medium were allowed to grow at room temperature (21 to 35°C) under stationary conditions for 15 days. The mycelium was then washed with sterile distilled water and transferred to 250-ml glass-stoppered flasks containing 50 ml of sterile distilled water and glass beads. The mycelium was broken up by vigorous shaking, and the resultant suspension was used as inoculum—about 4 ml per flask. Flasks were incubated on a rotary shaker having an eccentricity of 1 in and were operated at 200 rev/min until the mycelium had attained its maximum growth with complete utilization of substrate (approximately 8 days).

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The basal medium constituents per liter were as follows: KH₂PO₄, 1 g; MgSO₄·7H₂O, 0.5 g; CaCl₂·2H₂O, 0.35 g; MnSO₄·H₂O, 3 mg; ZnSO₄·7H₂O, 3 mg; FeSO₄·7H₂O, 3 mg; Na₂MoO₄, 3 mg. A mixture of vitamins containing thiamine, niacin, riboflavin, pantothenic acid, p-aminobenzoic acid, each at the rate of 100 µg/liter, and cyanocobalamin, biotin, pyridoxine, and folic acid, each at the rate of 50 µg/liter, were added.

The basal medium plus 50 g of glucose was used for the study of nitrogen utilization. Nitrogenous compounds were added at the rate of 0.424 g of nitrogen per liter (11). Other nutrients were added to the medium as shown (Tables 1–4).

To obtain a clear indication of the effect of increasing the concentration of each major element, i.e., phosphorus, potassium, magnesium, and calcium, on the yield and the crude protein content of the mycelium, some experiments were conducted in which the nutrition was uniform except for the element under study. The quantities of major elements supplied per liter of the basal medium were as follows: phosphorus, 0.22 g; potassium, 0.287 g; magnesium, 0.049 g; and calcium, 0.098 g. Phosphorus was added in the basal medium at four different levels (varying from 0 to 0.33 g/liter) and was supplied in the form of potassium dihydrogen phosphate. A complete reduction of phosphorus in treatment 1 (Table 5) was achieved by not adding potassium dihydrogen phosphate, and substituting for it sufficient potassium citrate to maintain the level of potassium. In treatment 2, in which the phosphorus content was reduced to 0.11 g/liter, 0.5 g of potassium dihydrogen phosphate per liter was added, and it was supplemented with potassium citrate so as to supply an equivalent quantity of potassium. In treatment 3, since 0.22 g of phosphorus was the basal dose required, no manipulation was needed. In treatment 4, a higher dose of phosphorus was added by supplementing the medium with ammonium phosphate and adjusting the nitrogen content of the medium with ammonium citrate.

Potassium was added in different concentrations (varying from 0 to 0.42 g/liter) in the basal medium (Table 5). The other nutrient elements were kept at a uniform level in all the treatments. Potassium was supplied in the form of potassium dihydrogen phosphate. A complete reduction of potassium in treatment 1 (Table 5) was achieved by withholding potassium dihydrogen phosphate and substituting for it sufficient ammonium phosphate and ammonium citrate as to maintain the same level of both phosphorus and nitrogen. In treatment 2, in which the potassium content was reduced to 0.14 g/liter of the medium, only 0.5 g of potassium dihydrogen phosphate was added, and it was supplemented again with ammonium phosphate and ammonium citrate, so as to supply an equivalent quantity of phosphorus and nitrogen. In treatment 3, since 0.28 g of potassium was the basal dose required, no manipulation was needed. In treatment 4, a higher dose of potassium was added by supplementing the medium with potassium citrate. Calcium and magnesium were added to the basal medium in the form of calcium chloride and magnesium sulfate, respectively.

Initial pH was adjusted in all the experiments to 5.5 with lactic acid and sodium hydroxide.

Analytical methods. The mycelial weights were obtained by filtering the culture fluid containing the mycelial pellets through a fine sieve, washing the mycelium with distilled water, and drying it in an oven at 60 C for 15 hr before weighing. The total nitrogen in the mycelium was estimated by the micro-Kjeldahl method (1), and the formula, protein = total nitrogen × 6.25, was used to calculate crude protein.

Reducing sugars were determined colorimetrically with the alkaline copper reagent of Somogyi (16) and the arsenomolybdate reagent of Nelson (12).

All the data represent averages of four flasks.

RESULTS

Among various carbohydrates tried (Table 1), the mycelium of P. flabellatus grew fast and gave maximal yield of dry matter when D(-)+mannose, D-fructose, or D-glucose was used. The sugar alcohols like glycerol and mannitol were not utilized by the mycelium. Other carbon sources, i.e., starch (soluble), maltose, and sucrose, gave

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### TABLE 1. Effect of different carbon sources on yield in a synthetic medium

| Treatment no. | Carbon source (15 g/liter) | Yield of mycelium (g/liter) | Crude protein content (% dry wt) |
|---------------|----------------------------|----------------------------|---------------------------------|
| 1             | D-Glucose                  | 8.0                        | 32.5                             |
| 2             | D-Fructose                 | 8.3                        | 32.5                             |
| 3             | D(-)+Mannose               | 9.4                        | 28.1                             |
| 4             | Maltose                    | 6.0                        | 37.0                             |
| 5             | Sucrose                    | 2.0                        | 38.0                             |
| 6             | Starch                     | 7.0                        | 26.0                             |

* Medium: basal medium plus (per liter) 2.456 g of ammonium citrate; initial pH 5.5; incubation time, 8 days.

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### TABLE 2. Effect of different concentrations of carbon source (glucose) on yield of the mycelium

| Treatment no. | Glucose (g/liter) | Yield of the mycelium (g/liter) | Crude protein content (% dry wt) |
|---------------|------------------|---------------------------------|---------------------------------|
| 1             | 10               | 4.17                            | 25.0                             |
| 2             | 20               | 6.8                             | 31.25                            |
| 3             | 30               | 8.13                            | 19.875                           |
| 4             | 40               | 10.20                           | 19.45                            |
| 5             | 50               | 13.00                           | 19.40                            |
| 6             | 60               | 11.90                           | 12.30                            |

* Medium: basal medium plus (per liter) 2.456 g of ammonium citrate; initial pH 5.5; incubation time, 8 days.
TABLE 3. Effect of different nitrogen sources on yield and protein content of mycelium in a synthetic medium

| Treatment no. | Nitrogen source (0.42% g/liter) | Sugar utilized (%) | Yield of mycelium (g/liter) | Yield of mycelium (g/100 g of sugar used) | Crude protein content (% dry wt) | Final pH | Efficiency (g of protein/100 g of sugar) |
|---------------|--------------------------------|-------------------|-----------------------------|------------------------------------------|---------------------------------|---------|--------------------------------------|
| 1             | Asparagine                      | 62                | 9                           | 29                                       | 25.2                            | 4.6     | 7.3                                  |
| 2             | Ammonium citrate                | 88                | 13.0                        | 29.6                                     | 19.45                           | 5.6     | 5.7                                  |
| 3             | Ammonium tartrate               | 20                | 5.4                         | 25                                        | 50                              | 13.5    | 6.5                                  |
| 4             | Ammonium sulfate                | 8                 | 2.0                         | 13                                        | 10.5                            | 3.2     | 4.1                                  |
| 5             | Potassium nitrate               | 6                 | 1.0                         | Traces of growth                         | 12.5                            | 3.4     | 4.0                                  |

* M: Medium; basal medium plus (per liter) 50 g of glucose; initial pH 5.5; incubation time, 8 days.

TABLE 4. Effect of ammonium citrate concentration on yield and protein content of *P. flabellatus* mycelium

| Treatment no. | Ammonium citrate (g/liter) | C:N ratio | Final pH | Yield of mycelium (g/liter) | Crude protein content (% dry wt) |
|---------------|-----------------------------|-----------|---------|-----------------------------|---------------------------------|
| 1             | 0.25                        | 466.2     | 5.8     | 5.75                        | 8.12                            |
| 2             | 0.50                        | 233.6     | 5.7     | 7.25                        | 8.12                            |
| 3             | 1.0                         | 117.4     | 5.7     | 10.36                       | 8.12                            |
| 4             | 2.0                         | 61.07     | 5.6     | 12.87                       | 19.00                           |
| 5             | 3.0                         | 39.9      | 4.1     | 12.5                        | 20.00                           |
| 6             | 4.0                         | 30.2      | 4.0     | 10.5                        | 22.70                           |
| 7             | 5.0                         | 24.4      | 4.0     | 7.8                         | 31.25                           |
| 8             | 6.0                         | 20.5      | 4.8     | 5.5                         | 32.88                           |
| 9             | 8.0                         | 15.6      | 4.9     | 3.75                        | 39.20                           |
| 10            | 10.0                        | 12.8      | 5.0     | 3.25                        | 35.00                           |

* M: Medium; basal medium plus (per liter) 50 g of glucose; initial pH 5.5; incubation time, 8 days.

Omission of phosphorus from the medium reduced the yield to 0.25 g of mycelium per liter of the medium, and the crude protein content was 5%. Maximal yield of the mycelium was obtained when phosphorus content was 0.22 g/liter. Higher concentration of phosphorus did not significantly improve yield, nor was there any increase in the crude protein concentration of the mycelium. Similarly, in the case of potassium, calcium, and magnesium, mycelial growth was slow and the protein content was low when these elements were omitted from the medium. Again, higher concentration of these elements did not improve the crude protein content of the mycelium nor was there any increase in the yield.

No growth occurred after 8 days at an initial pH of 3.0 (Table 6). Highest yields were obtained when the initial pH was 5.5. However, growth was observed when the initial pH was between 4.0 and 9.0 and the corresponding final pH was 3.9 to 6.0.
**TABLE 5. Growth of *P. flabelatus* in a synthetic medium with added salts**

| Treatment no. | Phosphorus (g/liter) | Yield of mycelium (g/liter) | Crude protein content (% dry wt.) | Potassium (g/liter) | Yield of mycelium (g/liter) | Crude protein content (% dry wt.) | Calcium (g/liter) | Yield of mycelium (g/liter) | Crude protein content (% dry wt.) | Magnesium (g/liter) | Yield of mycelium (g/liter) | Crude protein content (% dry wt.) |
|---------------|----------------------|-----------------------------|----------------------------------|---------------------|-----------------------------|----------------------------------|----------------|-----------------------------|----------------------------------|----------------|-----------------------------|----------------------------------|
| 1             | 0.0                  | 0.25                        | 5.0                              | 0.0                 | 1.5                         | 8.0                              | 0.0           | 1.07                        | 10.0                             | 0.0           | 2.0                         | 10.0                             |
| 2             | 0.11                 | 10.75                       | 18.25                            | 0.14                | 10.8                        | 19.0                             | 0.049         | 10.4                        | 19.0                             | 0.0245        | 11.4                        | 19.25                            |
| 3             | 0.22                 | 13.00                       | 19.45                            | 0.28                | 13.0                        | 19.45                            | 0.098         | 13.0                        | 19.45                            | 0.049         | 13.0                        | 19.4                             |
| 4             | 0.33                 | 13.20                       | 19.40                            | 0.42                | 13.0                        | 19.45                            | 0.147         | 13.0                        | 19.45                            | 0.073         | 12.8                        | 19.45                            |

* Medium: basal medium plus (per liter) 2.456 g of ammonium citrate and 50 g of glucose; initial pH 5.5; incubation time, 8 days.

**TABLE 6. Effect of pH on yield of the mycelium**

| Treatment no. | Initial pH (before sterilization) | Final pH | Yield of mycelium (g/liter) | Crude protein content (% dry wt.) |
|---------------|----------------------------------|----------|-----------------------------|----------------------------------|
| 1             | 3.5                              | 3.8      | Trace                       | 12.0                             |
| 2             | 4.0                              | 3.9      | 7.15                        | 12.0                             |
| 3             | 4.5                              | 5.0      | 10.0                        | 19.30                            |
| 4             | 5.0                              | 5.5      | 11.5                        | 19.37                            |
| 5             | 5.5                              | 5.6      | 13.0                        | 19.45                            |
| 6             | 6.0                              | 5.5      | 10.8                        | 18.75                            |
| 7             | 6.5                              | 5.8      | 9.95                        | 18.75                            |
| 8             | 7.0                              | 5.9      | 8.78                        | 18.70                            |
| 9             | 7.5                              | 5.8      | 8.6                         | 18.70                            |
| 10            | 8.0                              | 6.0      | 8.3                         | 17.50                            |
| 11            | 8.5                              | 6.0      | 6.96                        | 17.50                            |
| 12            | 9.0                              | 6.0      | 5.06                        | 17.00                            |

* Medium: basal medium plus (per liter) 50 g of glucose and 2.456 g of ammonium citrate; initial pH adjusted to values shown, with lactic acid and sodium hydroxide solution; incubation time, 8 days.

**DISCUSSION**

The results obtained (Table 1) by growing the mycelium of *P. flabelatus* on D(+)mannose, D-glucose, and D-fructose are in agreement with previous reports (2, 14) on other fungi. Mannitol and glycerol were not utilized by the mycelium at all, which compares favorably with nutritional requirements of *Morchella esculenta* studied by Brock (3). Xylose and arabinose, normally present in wood, were not utilized, even though *P. flabelatus* normally grows on dead or decaying wood.

It has been observed that, when ammonium salts of inorganic acids were used as sole source of nitrogen, the final pH of the medium went down to 3.0. Probably there was an accumulation of inorganic acids in the medium which are strongly dissociated as compared to organic acids. Consequently, poor growth, of mycelium occurred. The results obtained for urea do not confirm the earlier report on *Psalliota campestris* (8).

*P. flabelatus* forms firm mycelial pellets which can be easily recovered from the culture fluid by filtration. However, the pellets are not fibrous or tough and hence are palatable.

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