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Effect of nitrogen source on the growth and protein production by the mycelia of some edible mushrooms under submerged culture

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The effect of different nitrogen sources on the growth and protein production by the mycelia of *Gymnopilus chrysomyces, Leucocoprinus burnbaumii* and *Leucocoprinus cepaestipes* under submerged cullure was studied. It was found that the best inorganic nitrogen source for the mycelial growth and protein production of *G. chrysomyces* and *L. birnbaumii* was Di-ammonium monohydrogen phosphate while it was ammonium nitrate for growth and urea for protein production of *L. cepaestipes*. The best organic nitrogen source for growth of. *G. chrysomyces* and *L.cepaestipes* was L-asparagine and for *L. birnbaumii* it was L-arginine, The best nitrogen sources for protein production of *G.chrysomyces, L.cepaestipes* and *L.birnbauaii* were L.leuaiue, L.asparagine and. L-argexinine respectively. The best complex nitrogen sources for the growth and protein production of *G.chrysomyces* , *L. birnbaumii* and *L.cepaestipep* were peptone, yeast-extract and yeast extract respectively.

Key words : Mycelial growth, protein production, nitrogen sources, *Gymmopilus chrysomyces, Leucocoprinus birnbaumii, Leucocoprinus cepaestipes*

INTRODUCTION

Mushrooms are able to utilise different forms of inorganic, organic or complex nitrogen sources though their ability of utilisation differs. Urea has been utilised by *Agaricus campestris* (Humfeld, 1948, Humfeld and Sugihara, 1949, and Sugihara and Humfeld, 1954). Ammonium salts have been found to be favoured by *Tricholoma nudum* (Reusser *et al.* 1958). The utilisation of nitrate has been observed in *Agaricus blazei* (Block *et al.*, 1953) and *Tricholoma nudum* (Reusser *et al.*, 1958). Jennison *et al.* (1955) have studied the nitrogen requirements of 42 species of basidiomycetes, some of which grow well in inorganic sources of nitrogen, while some utilize amino acids. Reusser *et al.* (1958) have observed maximum growth of *T.nudum* in 6 g/litre of ammonium tartrate but maximum protein in 8 g/litre of the salt. Usef and Magid (1967) have shown that organic nitrogen is better than inorganic nitrogen sources to *Pleurotus ostreatus*. Beever(1970) has demonstrated the use of ammonia by *Peniophora sacrata*. Furuta and Okimoto (1970) have reported that *Auricularia mesentica, Pholiota nameko, Lentinus edodes, Flammulina velutips, Agaricus bisporus* and *Pleurotus ostreatus* utilize all nitrogen compounds tested and the most suitable medium for the mushrooms contained 2 g of pepton. Eger (1970) has obtained urea as best N-source for mycelial growth of *Pleurotus sp.* Bukhalo *et al.* (1972) have observed that the different strains of basidiomycetes under study except *A. bisporus* could assimilate nitrate and -ammonium nitrogen and some fungi grow better in a medium with ammonium nitrate. Johri (1972) has worked on *Cyathus helenae* and related species which show a tendency for latent utilization of nitrate nitrogen and better growth in a combination of nitrate and asparagine. Luppi *et al.* (1975) have observed that *Boletus luteus*- mycelium is able to grow with protein nitrogen (egg albumen) as sole N-source. Peptone has been found to be best source for *Phellinus Poria weirii* (Li and Bollen, 1975). Goto and Kawamura (1978) have stated that even physiological races of *Lentinus edodes* differ in nitrogen nutrition. Kitamoto *et al.* (1980) have studied the nitrogen uptake of *Favolus arcularius* in peptone containing medium. Kosaric and Nabuo (1981) have obtained peptone and yeast extract as optimum N-sources for morel mushrooms. Heng *et al.* (1981) have obtained peptone as best N-source for *Agaricus bitorquis* and *Pleurotus ostreatus*.

The present investigation has been taken to see the effect of twenty different nitrogen sources on growth and protein yield by mycelia of *G. chrysomyces, L. birnbaumii* and *L.cepaestipes* in a liquid synthetic medium.

MATERIALS AND METHODS

Test organisms: The cultures of the mushrooms

Gymnopilus chrysomyces (Berk.) Sacc. , *Leucocoprinus birnbaumii* (Corda) Sing. and *Leucocoprinus cepaestipes* (Sow.ex Fr.) Pat. were used in the study. Cultures were maintained by subculturing on 2% malt extract agar slants at definite intervals (15 days) and keeping at 25°C in complete darkness. Glucose asparagine medium of Lilly and Barnett (1951) was used as basal liquid synthetic medium.

Preparation of inoculum : A small portion of actively growing mycelium from mushrooms culture in agar slant was transferred to a 250 ml Erlenmeyer flask containing 50 ml of basal medium and was incubated on a shaking incubator (120 r.p.m.) at 30°C (±.5°C) for 7 days in complete darkness. After incubation period the mycelial mat was aseptically fragmented into small pieces with the help of a waring blender. Fragmented mycelial mass was washed several times with sterile distilled water to remove any trace of medium and suspended in a phosphate buffer medium (pH 5.5) for 24 h to overcome the shock encountered during blending. 1 ml of the mycelial cell suspension was then used as inoculum.

Growth medium : To test the utilization of different nitrogen sources by the test-organisms, the investigation was carried out in glucose- asparagine medium containing each of the different N- sources separately in place of asparagine when asparagine was not used as N-source. The different sources of nitrogen used in the experiment were ammonium nitrate, ammonium sulphate, ammonium chioride, diammonium mono hydrogen phosphate, monoammonium hydrogen phosphate, asparagine, arginine, valine, lysine methionine, phenylalanine, histidine, threonine, tryptophan, leucine, isoleucine,urea, peptone, yeast-extract and casein hydrolysate.The yeast extract was made vitamin-free by treating it with activated charcoal powder. (5 g/litre) before incorporating in the medium .The amount of nitrogen sources used was 2 g/litre. Before sterilization the pH of the medium was adjusted to 5.5 in case of *G.chrysomyces* and *L.cepaestipes* and adjusted to 6.0 in case of *L. birnbaumii*. 50 ml of the medium was dispensed in each flask. Flasks containing medium were sterilized at 15 p.s.i. for 20 minutes. The flasks without any nitrogen source were treated as controls. Five replicates were taken for each set.

Growth conditions : The sterilized flasks set. each with one type of nitrogen source were inoculated separately with 1 ml of inoculum of each mushroom culture and incubated in a shaking incubator at 30°C (± 0.5°C) in complete darkness. The flasks inoculated with *G. chrysomyces* were incubated for 20 days and those with *L.birnbaumii* and *L. cepaestipes* were incubated for 16 days according to their optimum incubation periods.

Measurement of growth : After the incubation period, the medium and mycelium were sepatated by filtration through a tarred sintered funnel (Jena 1G-3). The filtered mycelium was washed repeatedly with sterile distilled water to make it free from adherent medium, dried to constant weight at 60°c and the dry weight of the mycelium was taken as index of growth.

Estimation of protein : The total nitrogen content of the dried mycelium was determined following the colorimetric 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 of protein and consequently a factor of 4.25 was used to convert the nitorgen values of crude protein content. Each complete set was done in triplicate.

RESULTS AND DISCUSSION

The experimental data obtained are given in Tables 1-3.

The data in Table 1 revealed that di-ammonium monohydrogen phosphate is the best inorganic nitrogen source for growth and protein production by the mycelium of *G. chrysomyces*. It is being followed by ammonium sulphate, mono-ammonium dihydrogen phosphate, ammonium chloride, ammonium nitrate and urea. In *L. birnbaumii*, best source of inorganic nitrogen for producing protein-rich mycelium is di-ammonium monohydrogen phosphate. Regarding growth, this source was being followed by mono-ammonium dihydrogen phosphate, ammonium nitrate, urea and ammonium sulphate and in protein production, it was followed by urea, ammonium sulphate, ammonium chloride, mono-ammonium dihydrogen phosphate, ammonium nitrate. For *L. cepaestipes*, best inorganic nitrogen source of growth was ammonium nitrate followed by urea and that for protein production is urea follwed by di-ammonium monohydrogen phosphate. Other sources are not well utilized.

From Table 2, it appeared that for *G. chrysomyces*, asparagine is best amino acid for growth which is being followed by arginine, valine and histidine. In protein production, leucine was best source followed by asparagine, tryptophan and valine. Other sources were

Table 1. Data (mean*) showing the effect of inorganic nitrogen sources on growth and protein production by the mycelia of *G. chrysomyces*, *L. birbaumii* and *L. cepaestipes* at their respective optimum submerged culture

Nitrogen	*G.chrysomyces*		*L. birnbaumii*		*L. cepaestipes*	
	Dry.wt. of mycelium (g/l)	Protein content (%)	Dry.wt. of mycelium (g/l)	Protein content (%)	Dry.wt. of mycelium (g/l)	Protein content (%)
Ammonium nitrate	2.20 ±0.01	18.75 ±0.01	4.02 ±0.10	15.46 ±0.04	4.16 ±0.10	9.27 ±0.01
Ammonium sulphate	2.73 ±0.14	19.37 ±0.04	3.21 ±0.21	21.25 ±0.02	1.46 ±0.13	11.87 ±0.02
Ammonium choloride	2.24 ±0.12	18.90 ±0.21	4.24 ±0.13	19.37 ±0.06	1.31 ±0.04	11.18 ±0.04
Di-ammonium monohydrogen phosphate	3.72 ±0.20	21.05 ±0.04	4.58 ±0.13	23.85 ±0.05	2.62 ±0.02	14.53 ±0.05
Mono-ammonium di-hydrogen phosphate	2.65 ±0.13	18.90 ±0.02	4.30 ±0.21	17.81 ±0.04	2.08 ±0.18	10.47 ±0.02
Urea	0.72 ±0.01	5.62 ±0.04	3.88 ±0.17	23.40 ±0.02	3.90 ±0.13	14.68 ±0.02
Control	1.16 ±0.21	2.06 ±0.01	0.86 ±0.01	2.31 ±0.01	0.80 ±0.13	3.60 ±0.01

* Average of five replicates for dry weight and three replicates for protein yield were taken

Table 2. Data (mean*) showing the effect of animo acids on gowth and production of protein by the mycelia of *G.chrysomyces*, *L. birnbaumii* and *L. cepaestipes* at thier respective optimum submerged culture

Nitrogen sources	*G.chrysomyces*		*L. birnbaumii*		*L. cepaestipes*	
	Dry.wt. of mycelium (g/l)	Protein content (%)	Dry.wt. of mycelium (g/l)	Protein content (%)	Dry.wt. of mycelium (g/l)	Protein content (%)
L-asparagine	5.59 ±0.20	18.74 ±0.01	2.68 ±0.18	14.84 ±0.09	4.19 ±0.17	17.38 ±0.02
L-arginine	4.08 ±0.17	15.94 ±0.04	4.05 ±0.17	16.25 ±0.01	2.51 ±0.01	15.15 ±0.20
L-histidine	3.30±0.17	7.65±0.04	0.83±0.17	10.93±0.01	1.49±0.20	8.61±0.01
L-methionine	1.53±0.10	9.37±0.01	2.67±0.09	11.25±0.04	1.22±0.04	6.56±0.01
L-phenylalanine	2.02 ±0.21	10.16 ±0.04	1.92 ±0.14	13.60 ±0.05	1.42 ±0.22	11.87 ±0.05
L-lysine	1.97±0.18	5.46±0.03	1.09±0.01	10.00±0.01	0.83±0.03	7.34±0.05
L-leucine	2.56±0.12	21.05±0.04	2.43 ±0.07	15.63 ±0.09	1.32 ±0.19	8.60 ±0.05
DL-isoleucine	1.50 ±0.15	15.90 ±0.02	1.99 ±0.10	11.25 ±0.08	2.08 ±0.17	2.34 ±0.03
L-valine	3.50 ±0.15	16.25 ±0.04	2.57 ±0.15	11.56 ±0.03	1.52 ±0.13	9.84 ±0.04
L-tryptophan	0.95 ±0.13	18.40 ±0.02	0.80 ±0.11	16.20±0.05	1.04±0.17	11.87±0.06
Dl-threonine	2.82 ±0.13	14.84 ±0.03	1.64±0.13	8.12±0.05	1.52±0.01	5.62±0.01
Control	1.16±0.21	2.06±0.01	0.86±0.01	2.31±0.01	0.80±0.13	3.60±0.01

* Average of five replicates for dry weight and three replicates for protein yield were taken.

not appreciably utilized. *L. birnbaumii* utilized arginine as best amino acid source for growth,and protein enrichment which was being followed, in growth, by asparagine, methionine and valine and, in protein production, by tryptophan, leucine and asparagine. The test-organism failed to utilize other amino acid sources well. For *L. cepaestipes*, asparagine was best amino acid source followed by arginine. Other sources were not suitable for the test-organism.

Data in Table 3 revealed that among the complex nitrogenous compounds used, peptone was the best source for growth of protein-rich mycelium of *G. chrysomyces* which is being followed by yeast-extract and casein hydrolysate. For *L. birnbaumii*, yeast extract was the best source for growth of mycelium followed by casein hydrolysate and peptone. For protein production, yeast extract and peptone were equally good followed by casein hydrolysate. *L. cepaestipes* utilized yeast extract most followed by peptone and casein hydrolysate.

Table 3. Data (mean*) showing the effect of complex nitrogeneous compounds on growth and production of protein by the mycelia of *G.chrysomyces*, *L. birnbaumii* and *L. cepaestipes* at thier respective optimum submerged culture

Nitrogen	*G.chrysomyces*		*L. birnbaumii*		*L. cepaestipes*	
	Dry.wt. of mycelium (g/l)	Protein content (%)	Dry.wt. of mycelium (g/l)	Protein content (%)	Dry.wt. of mycelium (g/l)	Protein content (%)
Peptone	7.66±0.23	21.25±0.04	5.31±0.21	24.27±0.06	5.27±0.20	13.28±0.04
Casein hydrolysate	6.45±0.23	15.94±0.05	6.24±0.21	16.56±0.05	5.05±0.14	11.87±0.01
Yeast-extract	6.07±0.20	16.40±0.04	8.02±0.10	24.06±0.01	8.04±0.21	18.54±0.05
Control	1.16±0.21	2.06±0.01	0.86±0.01	2.31±0.01	0.80±0.13	3.60±0.01

* Average of five replicates for dry weight and three replicates for protein yield were taken.

In control sets, growth and protein production by mycelia of all the three test-fungi were not appreciable.

So, in overal study, peptone was found to be best utilized nitrogen source for *G. chrysomyces* and yeast extract for *L. birnbaumii* and *L. cepaestipes*. Peptone was best source for *G. chrysomyces*. This is in agreement with the similar findings on other mushrooms (Furuta and Okimoto, 1970; Li and Bollen, 1975; Hong *et al*, 1981; Kosaric and Nabuo, 1981) and does not agree with the observation of slight inhibitory effect of peptone in *Cyathus helenae* and related species (Johri, 1972). Yeast extract was best nitrogen source for the two species of *Leucocoprinus*. This is similar to the report of yeast extract utilization by moral mushrooms (Kosaric and Nabuo, 1981). Among the inorganic nitrogen sources under study, utilization of dibasic ammonium salts by *G. chrysomyces* and *L. birnbaumii* supported the ammonium salts utilization by other mushrooms (Reusser *et al.*, 1958, Bukhalo *et al.*, 1972).

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