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Effect of carbon: nitrogen ratios on the growth and protein production by the mycelia of two species of *Leucocoprinus* under submerged conditions

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Best carbon and nitrogen sources are glucose and yeast extract for *Leucocoprinus birnbaumii* and starch and yeast extract in case of *Leucocoprinus cepaestipes* respectively. As such, necessary experiments were done to determine the optimum C:N ratios for the submerged culture of the test-fungi. The experimental data revealed that C_2: N_2 (30:20) was the best ratio for the high yield of mycelia and protein in case of *L. birnbaumii*. In *L.cepaestipes*, C_3:N_3 (30:3) ratio was found to be the best combination for mycelia and protein production.

Key words: C:N ratio, *Leucocoprinus birnbaumii, L. cepaestipes*

INTRODUCTION

The importance of carbon and nitrogen in the nutrition of fungi is well known. The effect of different ratios of carbon and nitrogen sources on growth as well as production of protein, carbohydrate, fat, fibre and different metabolites of mushroom mycelia has shown their intricate relationship.

Increase in mycelial growth and crude protein content along with decrease in C/N ratio have been found in *Tricholoma nudum* (Reusser *et al.,* 1958 a,b). Yield of morel mushrooms has been found to be dependent on the C/N ratio of the medium (Litchfield *et al.,* 1963). This ratio ranges from 5:1 to 25:1. Optimum C/N ratio is found to be 10:1 for mycelial growth of *Boletus variegatus* (Falina and Paskel 1970), 15:1 for *Tremetes suaveolens* (Maslova, 1971). Watkinson (1975) has obtained highest mycelial yield in *Serpula lacrrimans* when both carbon and nitrogen concentrations are high. Hong *et al* (1981) have found that mycelial yield of *Agaricus bitorquis* and *Pleurotus ostreatus* decreases under lower or higher C/N ratio than the optimum ratio. Most stimulatory C/N ratio has been found to be 1:4 for *Volvariella speciosa* (Fasidi and Akwakwa, 1996). In *Pleurotus tuber-regium*, best mycelial growth is found at ratio 24:1 when total dietary fibre (TDF) and chitin content deceased but glucan content increases (Wu *et al.,* 2004). Best mycelial growth with highest cordycepin (a bioactive metabolite) production was found at optimized C/N ratio of 42.0 g/l and 15.8 g/l of glucose and peptone respectively in *Cordyceps militaris* (Mao *et al*, 2005). In *Lepiota procera* optimum C/N ratio is obtained 4:1 followed by 5:1 and 3:1 respectively (Gbobagade, 2006), Gao *et al. have* (2007) obtained optimum C/N ratio of 12:1 in *Paecilomyces lilacinus*.

In the present investigation necessary experiments have been done to determine the optimum C/N ratio for the growth and protein production by the mycelia of the *Leucocoprinus brinbaumii* and *L. cepaestipes*.

MATERIALS AND METHODS

The mycelial cultures of *Leucocoprinus brinbaumii* (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 regular intervals (15 days) and keeping at 25°C in complete darkness. Glucose- asparagines medium of Lilly and Barnett (1951) was used as liquid basal synthetic medium.

Preparation of inoculum

A small portion of actively growing mycelium from mushroom culture in agar slant was transferred to a 250 ml. Erlenmeyer flask containing 50 ml of basal liquid synthetic glucose- asparagine 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 incubation period the mycelial mat was aseptically fragmented into small pieces with the help of a waring blender. Fragmented mycelial mass was washed severeal times with sterile distilled water to remove any trace of medium and suspended in a phosphate buffer medium (*p*H5.5) for 24 hrs to overcome the shock encountered during blending. 1 ml of this mycelial cell suspension was used as inoculum.

Growth medium

According to the optimum carbon and nitrogen sources obtained, the basal synthetic medium, i.e. glucose asparagine medium (Lilly and Barnett, 1951) was modified (Halder and Samajpati, 1999), glucose and asparagine were replaced by starch and yeast extract for *L. cepaestipes*. Asparagine was replaced by yeast extract in case of *L. birnbaumii*. Different C:N ratios taken were C_1,N_1,C_1N_2, $C_1N_3,C_2N_1,C_2N_2,C_2N_3,C_3N_1,C_3N_2,C_3N_3$ as C_1=20g, C_2=30g, C_3=40g; N_1=1g, N_2=2g, N_3=3g/litre. Respective optimum carbon and nitrogen sources for the two test fungi were added accordingly and the pH of the medium was adjusted to 5.5 for *L. cepaestipes* and to 6.0 for *L. brinbaumii* with the help of 0.2M phosphate buffer before sterilization .50 ml of different media was dispensed in each of 250 ml. Erlenmeyer flasks, plugged and sterilized at 121°C for 25 minutes. Five replicates were taken for each set.

Growth conditions

The sterilized flasks sets for two different test-fungi were inoculated with 1ml of cell suspension of two tissue cultures separately and incubated at 30° C (± 0.5°C) in a shaking incubator (120 r.p.m) in complete darkness. According to the optimum incubation period obtained the flasks set were incubated for 16 days.

Measurement of growth

After the optimum incubation period the flasks were harvested. The medium and mycelium were separated by filtration through a tarred sintered funnel (Jena IG-3). The filtered mycelium was washed repeatedly with sterile distilled water to remove any trace of medium. Harvested mycelium was dried to constant weight at 60°C. This dry weight 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 colorimetric method of Folin and Wu (1919) and the method of Vogel (1961). The crude protein value was also calculated on the basis of 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 was done in triplicate.

RESULTS AND DISCUSSION

The experimental data obtained are given in Table 1. The data in the table show that C_2N_2 was the best ratio for mycelial growth and protein yield of *L. birnbaumii*. For mycelial growth, this ratio was followed by C_3N_3,C_3N_1, C_2N_1, C_2N_3, C_1N_3, C_1N_1, C_3N_2, and C_1N_2.For protein yield, C_2N_2 ratio was followed by C_1N_2, C_3N_2,C_2N_3,C_1N_3,C_3N_3,C_1N_1,C_3N_1 and C_2N_1. The results revealed that the protein content was best at N_2 ratios followed by N_3 and N_1 ratios. In *L. cepaestipes* (Table 1), C_3N_3 was best combination for both growth and protein yield. The mycelial

Table. 1 : Data (mean*) showing the effect of different C:N ratio on the growth and production of protein by the mycelia of *L. brimbaumil* and *L. cepaestipes* at their respective optimum submerged conditions.

C:N Ratio	Dry wt. of mycelium(g/1)	Protein Content (%)	Dry wt. of mycelium (g/1)	Protein Content(%)
C_1N_1 (20:1)	6.49 ±0.18	15.93 ±0.04	2.12 ±0.19	17.34 ±0.05
C_1N_2 (20:2)	1.03 ±0.13	23.12 ±0.03	2.98 ±0.18	18.28 ±0.01
C_1N_3 (20:3)	7.64 ±0.30	19.84 ±0.03	3.02 ±0.20	20.47 ±0.04
C_2N_1 (30:1)	8.22 ±0.14	12.12 ±0.01	2.67 ±0.16	14.72 ±0.02
C_2N_2 (30:2)	9.84 ±0.13	25.00 ±0.05	4.09 ±0.22	18.28 ±0.04
C_2N_3 (30:3)	7.94 ±0.24	21.25 ±0.01	4.20 ±0.25	21.25 ±0.01
C_3N_1 (40:1)	9.52 ±0.21	14.37 ±0.02	3.78 ±0.21	12.81 ±0.01
C_3N_2 (40:2)	2.87 ±0.21	22.85 ±0.02	3.91 ±0.21	14.37 ±0.01
C_3N_3 (40:3)	9.57 ±0.15	18.12 ±0.03	4.49 ±0.10	22.65 ±0.06

*Results are expressed as average of five replicatesfor dry wt. and three replicates for protein content

growth was found to be in decreasing order in the following ratios C_3N_3 C_2N_3 C_2N_2 C_3N_2 C_3N_1 C_1N_3 C_1N_2 C_2N_1 and C_1N_1. Increase in growth was found along with increase in nitrogen from N_1 *to* N_3. *For protein production*, C_3N_3 was followed by C_2N_3, C_1N_3 C_2N_2 C_1N_2 C_1N_1 C_2N_1 C_3N_2 and C_3N_1. It further revealed that high nitrogen ratios yielded much protein, irrespective of the amount of carbon. The result does not agree with that of Reusser *et al* (1958) who found increase in growth and protein content with decrease in C/N ratios. Highest concentration C_3N_3 was found best for *L. cepaestipes*. This is similar to the report of Watkins (1975) that highest mycelial growth of *Serpula lacrimans* was found when both sucrose and aspartate are high in amount.

At a given level of nitrogen, mycelial grown of both the test-fungi was changed by change in amount of carbon. In both the cases, high yield was found at C_1N_2 but low at C_3N_2 and C_1N_2. Similar findings were recorded by Gbolagade (2006) that increase or decrease of carbon content caused decrease in mycelial yield of *Lepiota* procera at fixed nitrogen content.

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