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Calcutta University, Calcutta 700 019, India

Effect of different carbon sources on growth and protein production by the mycelia of some edible mushrooms under submerged culture.

MITRA HALDER (BASU) AND N. SAMAJPATI
Department of Botany, Calcutta University, Calcutta 700019

The effect of different carbon sources on the growth and protein production by the mycelia of *Gymnopilus chrysomyces, Leucocoprinus birnbaumii* and *Leucocoprinus cepaestipes* under submerged conditions was studied. It was found that the best carbon source for the mycelial growth of *G. chrysomyces* was maltose, for *L. birnbaumii* was glucose and maltose and for *L. cespaestipes* it was starch.

Key words: Mycelial growth, protein production, carbon sources, *Gymnopilus chrysomyces, Leucocoprinus birnbaumii, Leucocoprinus cespaestipes*

INTRODUCTION

Carbon is known as one of the essential elements required by fungi for their nutrition and as source of energy. Some fungi utilize chemically complex carbon containing compounds while others are selective in their requirements of simple ones. Xylose utilization has been reported (Herrick 1940, Nord and Vitucci, 1947). Cellobiose utilization has been shown in *Tricholoma sp.* (Norkans, 1950). Block *et al.* (1953) have studied the carbon nutrition of *Agaricus blazei* mycelium. Jennison *et al.* (1955) have reported that all of the 42 wood-retting basidiomyceteous fungi under his study favour glucose as carbon source in the medium.Reusser *et al.* (1958) have noticed the xylose utilization by mycelium of *Tricholoma nudum*.Litchfield *et al.* (1963) have demonstrated that three species of *Morchella* grow well in media containing glucose, lactose, or maltose. Usef and Magid (1967) have reported that *Pleurotus ostreatus* grows best in dextrin but not in sodium acetate or citrate. Moore (1969) has studied the effect of 120 carbon compounds on *Coprinus lagopus* in liquid medium and found that only acetate, fructose, glucose, maltose mannitol, xylose and polymers cellulose and starch support growth as sole carbon sources.Furuta and Yoichiro (1970) have observed that mannose, galactose, maltose, mannitol, dextrin and starch are best carbon sources for the mushrooms *Auricularia mesentica, Pholiota nameko, Lentinus edodes, Flammulina velutipes Agaricus bisporus* and *Pleurotus ostreatus*. Guha and Banerjee (1971) have observed the effect of different carbon compounds on growth and protein yield of *A. campestris*. Johri (1972) has shown that *Cyathus helenae* utilizes glucose and maltose most, fructoses, sucrose and starch also utilized but mannitol could not be utilized . Bukalo *et al.* (1972) have obtained starch as best carbon - source for some of the basidiomycetes studied and maltose for others . Maslova (1973) has found that out of 22 carbon compounds , hexoses and disaccharides are the best utulizable sources for six species. Li and Bollen (1975) have reported that *Phellinus (Poria) weirii* grows best in glucose, xylose, maltose or fructose containing liquid media , Chakraborty and Mallick (1979) have recorded best growth of *Volvariella diplasia* and *V. esculenta* in starch and cellulose . Brodziak (1980) has found glucose , mannose , maltose and starch as optimun carbon sources for *Lentinus edodes* . Good carbon sources for increasing mycelial growth have been obtained namely glucose and starch for *Agaricus bitorques* and glucose , fructose and starch for *Pleurotus ostreatus* (Hong *et al.*, 1981) .

The present investigation was undertaken to determine

*Present address : Department of Botany, Bangabasi Evening College, Calcutta 700 009

the effect of differnt carbon sources on the growth and production of protein by the mycelia of *G. chrysomyces*, *L. birnbaumii* and *L. cepaestipes*.

MATERIALS AND METHODS

Test organism

The tissue cultures of *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 regular intervals (15 days) and keeping 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 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 several times with sterile distilled water to remove any trace of medium and suspended in a phosphate buffer medium (*p*H 5.5) for 24 hrs to overcome the shock encountered during blending. 1 ml of this mycelial cell suspension was then used as inoculum.

Growth medium

To test the utilization of different carbon sources, at first the standard glucose - asparagine medium (Lilly and Barnett 1951) was prepared without adding any carbon source. This set was taken as control. In other sets medium was prepared containing each of different carbon compounds separetely in place of glucose. The different sources of carbon compounds used were D(+) glucose, D(+) xylose, D(-) fructose, mannitol, sorbitol, maltose, sucrose, starch, cellulose, sodium acetate and sodium citrate. The amount of carbon sources used was 30 g/l. The *p*H of the medium was adjusted to 5.5 in case of *G. chrysomyces* and *L. cepaestipes* and adjusted to 6.0 for *L. birnbaumii* using 0.2 M phosphate buffer before sterilization. 50 ml of the medium was dispensed in each of 250 ml Erlenmeyer flasks, plugged and sterilized at 121°C for 15 minutes. For each carbon source treatment flasks were taken in five replicates.

Growth conditions

The sterilized flasks set for three different test-fungi were inoculated with 1 ml of cell suspension of three 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 for *G. chrysomyces* were incubated for 20 days and those for the other two test - fungi were incubated for 16 days.

Measurement of growth

After the respective optimum periods, 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 4.25 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. It appears from the experimental data (Table 1) that in *G.chrysomyces*, maltose is most utilizable carbon source which is followed, in growth, by starch, sorbitol and glucose. Other sources are not so suitable. In protein production, maltose is followed by fructose.

L. birnbaumii shows best utilization of glucose for its mycelial growth which is followed by sucrose and fructose (Table 1). Maltose, producing maximum protein, is followed by glucose, fructose, xylose, mannitol, sucrose, sorbitol and cellulose. But comparing glucose and maltose, it is found that maltose produces maximum protein but very low amount of mycelium where as glucose is best carbon source in mycelium production and 2nd best in protein production. So, glucose is said

Table 1 : Data (mean*) showing the utilization of carbohydrates and protein production by the mycelia of *G.chrysomyces, L.birnbaumii* and *L. cepaestipes* grown submerged in respective optimum media

Carbon sources	Test - fungi					
	G.chrysomyces		*L.birnbaumii*		*L. cepaestipes*	
	Dry wt. of my - celium (g/l)	Protein content (%)	Dry wt. of my- celium. (g/l)	Protein content (%)	Dry wt. of my - celium (g/l)	Protein content (%)
D (+) glucose.	3.00 ± 0.07	14.84 ± 0.05	5.10 ± 0.20	29.68 ± 0.05	5.91 ± 0.20	17.34 ± 0.04
D (+) xylose	1.38 ± 0.16	12.81 ± 0.01	2.18 ± 0.08	28.12 ± 0.01	2.61 ± 0.17	14.54 ± 0.04
D (-) fructose	2.89 ± 0.19	21.62 ± 0.02	4.33 ± 0.16	29.08 ± 0.01	5.24 ± 0.10	21.75 ± 0.02
Maltose	5.89 ± 0.20	22.18 ± 0.03	2.85 ± 0.03	29.80 ± 0.03	3.47 ± 0.17	24.22 ± 0.01
Sucrose	2.42 ± 0.22	14.78 ± 0.03	4.36 ± 0.17	27.12 ± 0.02	1.35 ± 0.13	18.60 ± 0.05
Mannitol	1.20 ± 0.11	13.43 ± 0.04	2.00 ± 0.15	28.00 ± 0.03	0.88 ± 0.06	21.25 ± 0.02
Sorbitol	3.84 ± 0.12	12.50 ± 0.04	2.24 ± 0.11	23.75 ± 0.04	0.85 ± 0.06	18.60 ± 0.03
Starch	4.29 ± 0.21	8.60 ± 0.05	2.62 ± 0.19	19.37 ± 0.06	7.67 ± 0.11	24.22 ± 0.01
Cellulose	1.60 ± 0.18	12.25 ± 0.05	1.59 ± 0.04	23.75 ± 0.04	1.43 ± 0.09	22.97 ± 0.01
Sodium acetate	0.10 ± 0.05	2.00 ± 0.01	0.06 ± 0.02	0.31 ± 0.01	0.08 ± 0.01	3.40 ± 0.02
Sodium citrate	0.17 ± 0.02	2.80 ± 0.02	0.02 ± 0.01	0.16 ± 0.01	0.09 ± 0.02	3.60 ± 0.01
Control	0.45 ± 0.17	2.62 ± 0.01	0.57 ± 0.05	9.25 ± 0.04	0.47 ± 0.10	2.81 ± 0.02

* Mean data of five and three replicates for dry weight and protein content, respectively.

to be optimum carbon source for the test - fungus.

In *L.cepaestipes,* starch is favoured most (Table 1). Next to starch, glucose and fructose support growth. In production of protein, starch and maltose are equally good. Cellulose, fructose and mannitol are also utilizable. So starch can be said to be the optimum carbon source for the test - fungus.

It is evident from literature that glucose, maltose and starch are optimum carbon sources for a number of basidiomycetes (Litchfield *et al.* , 1963; Moore, 1969; Bukhalo *et al*; 1972; Li and Bollen, 1975; Chakraborty and Mallick, 1979; Brodziak, 1980) and the present investigation is in agreement with the above observations. The test - organisms did not utilize sodium acetate or sodium citrate. It supports the report of Usef and Magid (1967) that *Pleurotus ostreatus* grows best in dextrin but not in Na - acetate or citrate, and the report of Maslova (1973) that some fungal species (under his study) utilize all the C - compounds (under investigation) except salts of organic acids.

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