

EFFECT OF TEMPERATURE ON GROWTH AND PROTEIN PRODUCTION BY THE MYCELIA OF *GYMNOPILUS CHRYSOMYCES*, *LEUCOCOPRINUS BIRNBAUMII* AND *LEUCOCOPRINUS CEPAESTIPES* UNDER SUBMERGED CONDITION

BY

MITRA HALDER AND N. SAMAJPATI

In the present investigation the effect of temperature on growth and production of protein by the mycelia of *Gymnopilus chrysomyces*, *Leucocoprinus birnbaumii* and *L. cepaestipes* were studied. It was found that 30°C is the optimum for growth and protein production of *G. chrysomyces*, *L. birnbaumii* and *L. cepaestipes*. In case of all the three list fungi it was found that 16 days is the optimum days for growth and protein production after which start declining.

INTRODUCTION

Among the external factors which influence the growth of fungi, temperature plays an extremely important role. It affects almost every function of fungi. For each fungus there is a minimum temperature below which it does not grow, and a maximum one above which the growth ceases. These two temperatures indicate the range. From available literature it has been assumed that 25° or 30° is the most favourable temperatures for mycelial growth of majority of fungus species. Linddegren (1933) has demonstrated that the optimum temperature for the vegetative growth of *Polyporus verisicolor* is 27°C—32°C and that for *Lenzites saepiaria* is 32°C—35°C. Cartwright and Findlay (1934) have shown that most fungi make atleast some growth over 25°C—30°C range of temperature although narrower range is found in case of *Merulius lacrymans*. Fries (1956) has recorded 40°C as optimum temperature for *Coprinus fimetarius*. Jennison *et al* (1955) have reported that *Merulius americanus* grows best at 25°C. Litchfield *et al* (1963) have demonstrated that 25°C is optimum temperature for growth of three species of *Morchella*. Davis *et al* (1965) have shown that *Plectonia occidentalis* yields more glucan polymer at 22°C and 25°C than 28°C.

Nizkovskaya and Milova (1965) have recorded the optimum temperature for growth of member of Thelephoraceae and Polyporaceae to be 26°C and for most of the Agaricaceae it is 20°C. Langvad *et al* (1967) have observed that optimum temperature for mycelial growth of *Merulius lacrymans* (Jacq.) Fr. to be 22°C and they have found no growth above 28°C. Brodziak (1980) has reported 35°C to be optimum temperature for best growth of *Lentinus edodes* and 25°C

TABLE 1. Data (mean*) showing the effect of different temperatures on the mycelial growth (g/l) and yield of protein (%) by the mycelia of *Gymnopilus chrysomyces* at different incubation periods under submerged condition

Tem- pera- ture (°C)	Incubation Period (Days)											
	4			8			12			16		
	Dry Wt. of Mycelium (g/l)	Pro- tein con- tent (%)	Dry Wt. of Mycelium (g/l)	Pro- tein con- tent (%)	Dry Wt. of Mycelium (g/l)	Pro- tein con- tent (%)	Dry Wt. of Mycelium (g/l)	Pro- tein con- tent (%)	Dry Wt. of Mycelium (g/l)	Pro- tein con- tent (%)	Dry Wt. of Mycelium (g/l)	Pro- tein con- tent (%)
20	0.09 ±0.01	10.63 ±0.03	0.20 ±0.02	16.60 ±0.03	0.29 ±0.08	19.00 ±0.04	1.30 ±0.11	20.21 ±0.05	1.61 ±0.08	22.97 ±0.05	1.22 ±0.11	14.78 ±0.04
25	0.48 ±0.02	12.18 ±0.02	1.20 ±0.13	18.70 ±0.02	1.70 ±0.06	18.70 ±0.03	2.34 ±0.08	20.78 ±0.08	3.84 ±0.11	25.15 ±0.03	2.29 ±0.13	20.21 ±0.02
30	0.69 ±0.08	16.60 ±0.04	1.36 ±0.11	18.70 ±0.04	2.78 ±0.08	19.85 ±0.02	3.62 ±0.08	23.50 ±0.04	4.00 ±0.13	28.44 ±0.02	2.46 ±0.08	20.27 ±0.03
35	0.45 ±0.08	16.60 ±0.07	0.94 ±0.11	17.66 ±0.03	1.68 ±0.11	19.00 ±0.03	3.07 ±0.11	20.21 ±0.03	3.62 ±0.08	25.16 ±0.05	2.30 ±0.16	19.08 ±0.02
40	0.40 ±0.07	13.43 ±0.06	0.90 ±0.13	14.78 ±0.05	1.27 ±0.08	16.50 ±0.05	1.57 ±0.09	17.60 ±0.02	1.98 ±0.13	20.78 ±0.04	1.74 ±0.11	18.72 ±0.02

*Averages of five replicates for dry wt. and three replicates for protein yield were taken.

to be best temperature for its maximum mycelial mass. Recently Kosaric and Nabuo (1981) have obtained the optimum temperature range for growth of morel mushroom in cheese whey to be 25°C—28°C. Hong *et al* (1981) have reported that 25°C—30°C is optimum temperature range for mycelial growth of *Agaricus bitorquis* and 25°C for *Pleurotus ostreatus*. Cha *et al* (1981) have found intensive mycelial growth of *Agaricus bisporus* at 30°C.

Necessary experiment has been conducted to find out the optimum temperature for growth of *G. chrysomyces*, *L. birnbaumii* and *L. cepaestipes* and production of protein by the mycelia of the test-fungi.

MATERIALS AND METHODS

Test Organisms

The tissues cultures of the mushrooms *Gymnopilus chrysomyces* (Berk.) Sacc. *Leucocoprinus birnbaumii* (Corda) Sing. and *Leucocoprinus cepaestipes* (Sow. ex Fr.) Pat. were used in the study. Subculturing was done on 3% malt extract agar slants at regular intervals (15 days) and cultures were maintained at 25°C in complete darkness. Glucose-asparagine medium of Lilly and Barnett (1951) was used as the basal synthetic medium.

Preparation of Inoculum : A small portion of actively growing mycelium from mushroom culture of each test-fungus was aseptically transferred to a sterile 250 ml Erlenmyer flask containing 50 ml of basal liquid synthetic medium and was incubated on a shaking incubator (120 r. p. m.) at 30°C ($\pm 0.5^\circ\text{C}$) 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 then washed several times with distilled water to remove any trace of medium and suspended in a phosphate buffer medium (pH 5.5.) for 24 hrs. to overcome the shock encountered during blending. One ml of the mycelial cell suspension was then used as inoculum.

Conditions for Growth : 50 ml of the liquid synthetic glucose-asparagine medium was taken in each of 250 ml Erlenmeyer flasks. The pH of the medium was adjusted to 5.5. by 0.2 M phosphate buffer. Flasks were then plugged and sterilized at 10 p. s. i. for 20 minutes. Sterilized flasks were inoculated with 1 ml of cell suspension of each test-fungus separately and incubated on a shaking incubator (120 r. p. m.) in complete darkness for 24 days. Several flasks were inoculated in order to have five replicates for each treatment for each test-fungus. Inoculated flasks were kept at 20°C, 25°C, 30°C, 35°C, and 40°C ($\pm 0.5^\circ\text{C}$).

Measurment of growth : Every fourth day, five flasks for each treatment were harvested. The medium and mycelium were separated by filtration through a tarred sintered funnel (Jena IG-3). The filtered mycelium was washed

TABLE 2. Data (mean*) showing the effect of different temperatures on the growth (g/l) and yield of protein (%) by the mycelium of *Leucocoprinus birnbaumii* at different incubation period under submerged condition.

Tem- pera- ture (°C)	Incubation Period (Days)											
	4			8			12			16		
	Dry Wt. of Mycelium (g/l)	Pro- tein con- tent (%)	Dry Wt. of Mycelium (g/l)	Pro- tein con- tent (%)	Dry Wt. of Mycelium (g/l)	Pro- tein con- tent (%)	Dry Wt. of Mycelium (g/l)	Pro- tein con- tent (%)	Dry Wt. of Mycelium (g/l)	Pro- tein con- tent (%)	Dry Wt. of Mycelium (g/l)	Pro- tein con- tent (%)
20	0.14 ±0.08	11.25 ±0.02	0.24 ±0.11	15.46 ±0.01	0.44 ±0.17	19.37 ±0.02	0.90 ±0.11	20.78 ±0.02	0.70 ±0.09	20.78 ±0.06	0.64 ±0.11	16.25 ±0.05
25	0.37 ±0.11	17.81 ±0.05	0.92 ±0.13	22.81 ±0.05	1.83 ±0.16	23.75 ±0.05	3.33 ±0.16	23.90 ±0.06	3.08 ±0.11	22.18 ±0.01	1.76 ±0.13	20.78 ±0.04
30	0.57 ±0.13	23.13 ±0.05	1.16 ±0.08	23.59 ±0.01	2.24 ±0.13	23.90 ±0.02	4.26 ±0.13	28.13 ±0.01	3.96 ±0.11	25.15 ±0.02	1.92 ±0.17	24.37 ±0.04
35	0.28 ±0.08	16.56 ±0.01	1.00 ±0.05	17.81 ±0.02	1.16 ±0.13	19.37 ±0.03	1.93 ±0.08	22.34 ±0.01	1.48 ±0.13	21.56 ±0.07	1.00 ±0.13	17.14 ±0.03
40	0.10 ±0.08	16.03 ±0.05	0.17 ±0.11	16.25 ±0.03	0.29 ±0.16	17.18 ±0.05	0.37 ±0.08	21.88 ±0.06	0.36 ±0.09	19.21 ±0.05	0.24 ±0.11	17.93 ±0.05

*Averages of five replicates for dry wt. and three replicates for protein yield were taken.

repeatedly with distilled water to make it free from any trace of adherent medium and dried to constant weight at 60°C in an oven. Dry weight of the mycelium thus obtained was taken as an index of growth.

Estimation of protein: The total nitrogen content of the dried mycelium obtained in each case was determined following the Colorimetric method of Folin and Wu (1919) and Vogel (1961) using Photo Electric Colorimeter (Model AE-11, Tokyo Erma Optical Works Ltd, Japan).

The crude protein value was calculated on the basis of 16 percent nitrogen values to crude protein content. The complete set of experiment was done in triplicate.

RESULTS AND DISCUSSION

The results thus obtained are given in Tables 1-3,

From Table 1 the optimum incubation period and temperature for growth and protein production of *G. chrysomyces* are found to be 20 days and 30°C respectively. In growth, 30°C is followed by 25°C, 35°C, 40°C and 20°C and in protein production it is followed by 35°C, 25°C, 20°C and 40°C.

In case of *L. birnbaumii*, from Table-2, it is evident that 16 days of incubation and 30°C temperature are optimum for its mycelial growth and protein yield. 30°C is followed in growth by 25°C, 35°C, 20°C and 40°C and in protein content, by 25°C, 35°C, 40°C and 20°C.

In case of *L. cepeestipes*, data in Table 3, shows that 16 days of incubation and 30°C temperature are optimum for its growth and protein production. When growth is considered, 30°C is followed by 25°C, 35°C, 20°C and 40°C and in protein production, it is being followed by 35°C, 25°C, 40°C, and 20°C.

In case of all the three test-fungi, at a definite temperature, growth and protein production gradually increase with increase in incubation period, become maximum at their respective optimum incubation and then start declining.

In the present experiment 30°C is optimum temperature for mycelial growth and protein production of all the three test-fungi which supports the finding of cartwright and Findlay (1934) that most fungi make at least some growth over 25°C-30°C range, observation of Hong *et al* (1981) that 25°C-30°C is optimum temperature range for mycelial growth of *Agaricus bitorquis* and the report of Cha *et al* (1981) that 30°C is optimum for *Agaricus bisporus*. A few uncommon reports of optimum temperatures are, 40°C for *Coprinus fimetarius* (Fries, 1956), 20°C for most of Agaricaceae (Nizkovskaya and Milova, 1966) and 22°C for *Merulius lacrymans* (Langvad *et al*, 1967).

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