J. Mycopathol. Res. **39**(2) : 73–76 (2001)

Effect of vitamins on the growth and protein production by the mycelia of three tropical mushrooms under submerged culture

MITRA HALDER (BASU) AND N. SAMAJPATI

Department of Botany, Bangabasi Evening College, Kolkata 700009 and Department of Botany, University of Calcutta, Kolkata 700019

The effect of riboflavin, inositol, thiamine, pyridoxine, ascorbic acid, para-aminobenzoic acid, and biotin on the growth and protein production by the mycelia of *Gymnopilus chrysomyces, Leucocoprinus birnbaumii* and *Leucocoprinus cepaestipes* under submerged culture was studied. The experimental data revealed that all the three mushrooms require riboflavin for growth and protein production. The mushrooms are autotrophic for thiamine and pyridoxine. Other vitamins have negligible effects.

Key words : Growth and protein production, *Gymnopilus chrysomyces, Leucocoprinus birnbaumii, Leucocoprinus cepaestipes,* vitamins

INTRODUCTION

It is evident from the reviews that vitamins have some effect on the nutritional physiology of fungi, although comparatively less work has been done in this aspect on higher fungi. Most of the known vitamins have catalytic functions in cells and are known to be precursor of co-enzymes. As such the deficiency of vitamin prevents normal metabolic activities of the organism (Tanber, 1939). Some fungi are self-sufficient to synthesize vitamins while others are unable to synthesize and variously called vitamin-deficient or heterotrophic with respect to one or more specific vitamin. In earlier works, thiamine deficiency was found to be most common among basidiomycetes (Noecker, 1938 ; Melin and *Nyman*, 1940; Melin and Norkrans, 1942 ; Lindberg, 1944, 1946 ; Fries, 1945). *Polyporus texanus* exhibits almost an absolute deficiency for pantothenic acid (Yusef, 1953). *Poria villantii* requires external supply of riboflavin (Jennison *et al.*, 1955). Biotin requiring fungi generally show thiamine heterotrophy as found in *Lactarius* sp. (Jayko *et al.*, 1962). Lankramar (1969) have found that on *Suillus variegatus*, vitamin B_1 and PABA show slight inhibitory effect as compared to control, vitamin B_2 and B_6 promote growth. Beever (1970) has shown tha *Peniophora sacrata* is autotrophic for thiamine. Reid (1975) has found that thiamine was only vitamin required for growth of *Tremella mesenterica*. Hongo and Hirozumii (1974) have shown that thiamine is essential micronutrient for *Tremella fuciformes*. Li and Bollen (1975) have found that *Phellinus (Poria) weirii* requires thiamine hydrochloride, though not absolutely but give better growth. Jandaik and Kapoor (1976) have shown that *Pleurotus sajor-caju, Podoxis pistillaris* and *Phellorinia inquinans* are partially deficient for thiamine and latter two for biotin as well. Hong *et al.* (1981) have found that thiamine, ca-pantothenate and folic acid are suitable for mycelial growth of *Agaricus bitorquis* and thiamine, folic acid and inositol are best for *Pleurotus ostreatus*. In the present experiment, the effect of seven vitamins on mycelial growth and protein production is evaluated on three mushrooms.

MATERIALS AND METHODS

Test organisms

The myceliyal 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 in 3% malt-extract agar medium at definite interval of 15 days and manitained at 25^{0}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 of each test-fungus from agar slants was transferred separately to 250 ml Erlenmeyer flask containing 50 ml of basal liquid synthetic medium and incubated on a shaking incubator (120 r.p.m.) at 30^{0}C (±0.5^{0}C) for 7 days in complate darkness. After incubation period, the mycelial mass was aseptically fragmented into small-pieces in a waring blender. This fragmented mass was washed several times with sterile distilled water to remove any trace of medium and then suspended in a phosphate buffer (pH 5.5) for 24 to overcome the shock encountered during blending. One ml of the mycelial cell suspension was used as inoculum.

Growth medium

In case of *G. chrysomyces*, maltose-peptone synthetic medium was used having the following composition : Maltose-40 g, Peptone-3 g, $MgSO_4$, $7H_2O$-500 mg, KH_2PO_4-1 g, $FeSO_4$, $ZnSO_4$, $MnSO_4$ $CuSO_4$ and MoO-0.2 mg each and distilled water to make 1000 ml.

For *L. cepaestipes* starch-yeast extract synthetic medium was prepared containing starch- 40 g, yeast extract- 3 g, $MgSO_4$, $7H_2O$- 500 mg, KH_2PO_4- 1 g, $ZnSO_4$, MoO, $CuSO_4$, $CaSO_4$ & $MnSO_4$- 0.2 mg each and distilled water to make the volume to 1000 ml.

After preparation, the removal of any trace of vitamins, if already present in the medium, was done by treating the medium with activated charcoal powder (5 g/litre) and boiling for 10 minutes. Finally filtration was done through a tarred sintered funnel (Jena IG-3).

The experiment was carried out according to the method of Bukholder (1943) for deficiency study of vitamins. A set of complete medium (CM) was prepared containing all the vitamins taken in the experiment. Other sets were prepared in a way that to detect the deficiency of a specific vitamin against the test-fungi by its viability to grow, it was not added in the complete medium. Different vitamins used in the present deficiency study were taken in following concentrations viz. thiamine, pyridoxin, ascorbic acid, para-amino benzoic acid (PABA)-100 mg/1 biotin and riboflavin - 5 µg/l, and inositol - 5 mg/l.. The pH of the different sets containing glucose-yeast extract medium was adjusted to 6.0 and pH of sets containing maltose-peptone and starch-yeast extract medium was adjusted to 5.5 with the help of 0.2 M phosphate buffer before sterilisation, 50 ml of different medium was taken in each of the 250 ml Erlenmeyer flasks, plugged and sterilized at 10 p.s.i. for 20 minutes. Five replicates were taken for each set.

Growth conditions

Each flask was inoculated with 1 ml of cell suspension of each test-fungus separately and incubated in a shaking incubator (120 r.p.m) at 30^{0}C (± O.5^{0}C) in complete darkness for 20 days in case of *G. chrysomyces* and for 16 days in case of *L. birnbaumii* and *L. cepaestipes* according to their respective optimum incubation period.

Measurement of Growth

After incubation period, the medium and mycelium were separated by filtration through a tarred sintered funnel (Jena IG-3). Filtered mycelium was repeatedly washed with distilled water to make free from any trace of adherent medium and dried to constant weight at 60^{0}C. Dry weight of mycelium thus obtained was taken as index for growth.

Measurement of Protein

The total nitrogen content of the dried mycelium was determined following the colorimetric method of Folin and Wu (1919) and the method of Vogel (1961) using photoelectric colorimeter (Model AE-11, Tokyo, Erma Optical Works Ltd. Japan). On the basis of 16 per cent nitrogen content of protein, a factor of 6.25 was used to convert the nitrogen values to crude protein content. Each set of experiment was done in triplicate.

RESULTS AND DISCUSSION

The data in Table 1 show that for all the three test-

fungi, control sets produce moderate quantity of mycelium and protein thereby suggesting that the test-fungi are autotrophic for one or more vitamins.

Table 1 : Data (mean*) showing the effect of vitamins on growth (g/l) and protein production (%) by the mycelia of *G. chrysomyces*, *L. birnbaumii* and *L. cepaestipes* at their respective optimum submerged conditions

	Test—fungi					
	G. chrysomyces		*L. birnbaumii*		*L. cepaestipesi*	
Source of Vitamins	Dry wt. of mycelium (g/l)	Protein content (%)	Dry Wt. of mycelium (g/l)	Protein content (%)	Dry wt. of mycelium (g/l)	Protein content (%)
Complete medium (CM)	5.98 ± 0.10	21.25 ± 0.05	7.50 ± 0.15	19.37 ± 0.05	4.94 ± 0.20	23.70 ± 0.02
CM–Riboflavin	5.54 ± 0.16	28.25 ± 0.04	9.08 ± 0.10	21.25 ± 0.04	6.62 ± 0.15	13.56 ± 0.05
CM–inositol	6.13 ± 0.17	24.22 ± 0.04	7.80 ± 0.12	23.12 ± 0.02	6.24 ± 0.05	15.62 ± 0.03
CM–thiamine	6.73 ± 0.18	24.84 ± 0.02	10.17 ± 0.18	29.68 ± 0.05	6.26 ± 0.10	23.21 ± 0.02
CM–pyridoxine	6.72 ± 0.13	22.50 ± 0.04	7.18 ± 0.09	28.12 ± 0.06	5.26 ± 0.16	21.64 ± 0.03
CM–ascorbie acid	6.65 ± 0.09	21.25 ± 0.03	9.89 ± 0.15	21.25 ± 0.03	8.59 ± 0.09	25.08 ± 0.04
CM–PABA	7.35 ± 0.15	28.68 ± 0.02	8.44 ± 0.16	25.00 ± 0.04	6.69 ± 0.05	16.16 ± 0.05
CM–biotin	6.43 ± 0.17	23.43 ± 0.03	7.64 ± 0.16	19.84 ± 0.03	5.75 ± 0.10	21.64 ± 0.05
Control	6.18 ± 0.13	22.66 ± 0.05	7.88 ± 0.12	18.43 ± 0.04	6.82 ± 0.06	15.28 ± 0.01

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

In case of *G. chrysomyces*, CM-PABA produces maximum mycelium and protein content. It suggests that the organism is autotrophic to synthesize this vitamin and therefore does not require the external supply. Thiamine and pyridoxine-deficient media produce comparatively much mycelia. Riboflavin-free medium is equally good as CM-PABA in producing protein and is followed by CM-thiamine. This indicates the inhibitory effect of the presence of these vitamins to the test-fungi. Production of comparatively lower amount of mycelium in absence of riboflavin and that of protein in absence of ascorbic acid shows the requirement of these two vitamins. The requirement of riboflavin to the test-fungus is in agreement to the similar report of Jennison *et al.* (1955) for *Poria vilantii*. Inhibitory action of PABA has also been found in case of *Suillus variegatus* (Langkramer, 1970).

In case of *L. birnbaumii* thiamine-deficient medium is most favourable suggesting that it does not require external supply of thiamine and is autotrophic to it. Ascorbic acid and riboflavin-free media produce high yield of mycelium and pyridoxine and PABA-free media produce high protein content. So, presence of these vitamins may be slightly inhibitory to the test-fungus. Autotrophy for thiamine is similar to the report of Beever (1970) on *Peniophora sacrata*. Slight inhibitory effect of PABA to the test-fungus supports similar result on *Suillus variegatus* (Langkramer, 1970).

In case of *L. cepaestipes*, ascorbic acid-free medium is found to be most appreciably utilized for growth and protein yield. So, the test-fungus does not require addition of this vitamin. Production of cmparatively lower mycelial content in absence of pyridoxine and biotin and low protein in absence of riboflavin and inositol indicate the requirement of these vitamins. Riboflavin requirement has been found in *Poria villantii* (Jennison *et al.*, 1955).

REFERENCES

Beever, R. E. (1970). Some studies on the nutrition of *Peniophora sacrata* G. H. Cunn. *WZJ. SCI.* **13(1):** 160-165.

Burkholder, P. R. (1943). Vitamin deficiencies in yeasts. *Am. Jour. Botany.* **30:** 206-211.

Folin, O and Wu, H. (1919). Determination of non-protein nitrogen. *J. Biological Chemistry.* **38:** 87-90.

Fries, L. (1945). Uber das wachstum einiger Coprinus-Arten bei verschiedenen wasserstoffionen-konzentrationen. *Arkiv Botan.* **32:** 1-8.

Hong, J., Kap, S. L. and Dong, S. C. (1981). Studies on Basidiomycetes : I. Mycelial growth of *Agaricus bitorquis* and *Pleurotus ostreatus*. *Korean J Mycol.* **9(1):** 19-24.

Hongo, I. and Hirosumii, K. (1974). Experimental culture of *Tremella fuciformis*. *Bull. Coll. Agric. VET. Med. Univ.* **31:** 176-181.

Jandaik, C. L. and Kapoor, J. N. (1976). Studies on vitamin requirements of some edible fungi. *Indian Phytopathology.* **29(3):** 259-261.

Jayko, L. G. ; Baker, T. I. ; Stubblefield, R. D. and Anderson, R. F. (1962). Nutrition and metabolic products of *Lactarius* species. *Canad J. Microbiol.* **8:** 361-371.

Jennison, M. W. Newcomb, M. D. and Henderson, R. (1955).

Physiology of the wood rotting basidiomycetes. I. Growth and nutrition in submerged culture in synthetic media. *Mycologia.* **47:** 275-304.

Lankramer, O. (1969). Effect of some vitamins on the growth of *Suillus variegatus* (Sow. Ex Fr.) O Kuntz. *Ceskka Mycol* **23(1):** 53-60.

Li, C. Y. and Bollen, Y. B. (1975). Growth of *Phellinus (Poria) weirii* on different vitamins and carbon and nitrogen sources. *Am. J. Bot.* **62(8):** 838-841.

Lilly, V. G. and Barnett, H. L. (1951). *Physiology of fungi.* McGraw Hill Book Co., London. pp. 425.

Lindberg, G. (1944). Uber Die Physiologie Ligninabbauender Bodenhymenomyzeten. *Symbolae Botan Upasaliense.* **8(2):** 1-183.

Lindberg, G. (1946). The effect of biotin and thiamine on the growth of *Collybia dryophila* Fr. *Sv. Bot. Tidak.* **40:** 63-69.

Melin, E. and Norkrans, B. (1942). Uber den Einfluss der Pyrimidin-und der Thiazolkomponente des Aneurins auf das Wachstum vou Wurzelpilzen. *Svensk Botanisk Tidskrift.* **36:** 271-286.

Melin, E. and Nyman, B. (1940). Weitere untersuchungen Uber die Wirkung vou Aneurin and Biotin auf das wachstum vou wurzelpilzen. *Arch. Mikrobiol.* **11:** 318-328.

Noecker, N. L. (1938). Vitamin B_1 in the nutrition of four species of wood destroying fungi. *Amer. J. Bot.* **25:** 345-348.

Reid. I. D. (1975). Growth of *Tremella mesenterica* haplonts with various nitrogen sources. *Can. J. Bot.* **53(1):** 73-77.

Tanber, H. (1939). Vitamins as co-enzymes. *J. Chem.* **16:** 11-15.

Vogel, A.I. (1961). *A Textbook of Quantitative Inorganic Analysis,* 3rd. edition. The English Language Book Society and Longman. pp. 783-784.

Yusef, H. M. (1953). The requirements of some Hymenomycetes for essential metabolites. *Bull. Torrey Botan. Club.* **80:** 43-64.

(Accepted for publication August 10, 2001)