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Stimulatory effect of different vitamins on mycelial biomass and protein production of *Pleurotus ostreatus and Volvariella volvacea* grown on agroindustrial wastes

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Vitamins are essential for normal growth and development of fungi (Cochrane, 1958; Fries, 1965). Many fungi require minute amount of vitamins as they suffer from partial deficiency of vitamins and grow slowly in absence of external supply of a particular vitamin, as these fungi ar partial auxoheterotrophs. Most of the vitamins have catalytic functions in the cell and are known to be precursors of coenzymes or constituent parts of enzymes. As such, the deficiency of a vitamin interfares with the activity of an enzyme system and prevents normal metabolic changes accomplished through the agency of that system. With relation to fungal growth and development, only a few chemically pure vitamins have so far been tested. Reports for dificiency are most common for thiamine, mumorous for biotin, infrequent for pyridoxine and rare for inosital, riboflavin, nicotinic acid, pantothenic acid. An attempt was, therefore, made to find out the effect of some vitamins on mycelial growth and protein production of *Pleurotus ostreatus* and *Volvariella volvacea*. Four vitamins viz, thiamine, biotin, pyridoxine and inositol, for which fungi are most frequently dificient, were selected for the present study. Some promoting effect was also observed in decreasing order by biotin, inositol, pyridoxine and thiamine. It was evident that all the vitamins were more or less equaily in improving growth and protein production of the test fungi but at different levels.

Key words : Partial auxoheterotrophs, vitamins, mycelial growth, protein production, *Pleurotus ostreatus*, *Volvariella volvacea*, thiamine, biotin, inositol, pyridoxine

INTRODUCTION

Vitamins are essential for mormal growth and development of fungi (Tandon and Bilgrami, 1957; Cochrane, 1958; Fries, 1961, 1965; Singh, 1963 and Sankhla and Mathur 1967). A number of vitamins have stimulatory effect on reproduction of fungi (Lilly and Barnett, 1951; Cochrane, 1958). Amongst fungi, auxoautotrophic species are independent of external supply of vitamins because of their ability to synthesize the substance from simple foods in amounts adequate for their normal and perhaps optimum development, while auxoheterotrophic forms suffer from complete deficidncids for one or more vitamins and do not grow at all in a vitamin-free medium because of their inability to synthesize any of the required vitamins. There is also an intermediate group which suffers from partial deficiency of vitamins and as such grows slowly in absence of external supply of a particular vitamin, but more rapidly in its presence. This is because of the fact that it is able to synthesize some of the vitamins but not enough for its maximum growth. Most of the vitamins have catalytic functions in the cell and are known to be precursors of coenzymes or constituent parts of enzymes. As such, the deficiency of a vitamin interferes with the activity of an enzyme system and prevents normal metabolic changes accomplished through the agency of that system. With relation to fungal growth and development, only a few chemically pure vitamins have so far been tested. Reports for deficiency are most common for thiamine, numerous for biotin, in frequent for pyridoxine and rare for inositol, riboflavin, nicotinic acid and pantothenic acid. An attempt was, therefore, made to find out the effect of some vitamins on mycelial growth and protein production of *Pleurotus ostreatus* and *Volvariella volvacea.* Four vitamins viz., thiamine, biotin, pyridoxine and

inositol, for which fungi are most frequently deficient, were selected for the study.

MATERIALS AND MEHTODS

For determining the vitamin requirement mycelia of two edible mushrooms, *Pleurotus ostreatus* (Jacq. ex. Fr.) Kummer obtained from Mushroom Research Centre, Chinsurah, Government of West Bengal, and *Volvariella volvacea* (Bull ex.Fr.) Sing. procured from Indian Agricultural Research Institute (Culture No. 2830), New Delhi were maintained on 2% Difco Malt-agar at 27 1°C and used in the present study.

The test fungi were grown on the liquid salt solution-I of Hofsten and Ryden (1975) containing 0.2 % phosphoric acid, 0.2 % ammonium sulphate and 0.2 % magnesium sulphate, 2 % straw dust (40 mesh) was added which served as carbon source. The medium minus the vitamins was prepared in double distilled water and was boiled with activated charcoal (Norit 5.0 g/1) for 5 minutes and then filtered to remove any trace of vitamins. The pH of the media were then adjusted to 6.8 for *Pleurotus ostreatus* and 6.4 for *Volvariella volvacea* with 2 (M) KOH which were optimum.

Stock solutions of thiamine, biotin, inositol and pyridoxine were prepared in double distilled water and were mixed with the basal medium in desired quantities (100 ppm, 10 ppm, 1 ppm, 0.1 ppm 0.01 ppm) separately. 50 ml of the medium was then poured in each 250ml Erlenmeyer flask, plugged and sterilized at 15 lb pressure p.s.i for 20 minutes. One set of flasks with the vitamin-free medium was kept as control. The flasks were inlculated aseptically with very thin and uniform discs of mycelia (5 mm in diameter) of the test fungi from actively growing cultures on vitamin-free malt-agar medium and incubated for 15 days of 27 1°C for *Pleurotus ostreatus* and 35 + 1°C for *Volvariella volvacea*. After the incubation period, the mycelia were harvested by filtering and washing thoroughly over previously dried and weight at 50°C in an oven. Final pH of the filtrates were recirded. Mycelial dry weight was recorded and total protein content of the mycelia was estimated as before.

RESULTS

It was evident from Tables 1 and 2, that among the vitamins tried, thiamine promoted growth of the test fungi most effectively even at the lowest concentration (0.01 ppm). Some promoting effect was also observed in decreasing order by biotin, inositol and pyridoxine. However, the same concentration did not prove optimum for all vitamins. With increase in concentration of vitamins growth increased upto a certain level and then decreased excepting in case of pyridoxine where maximum growth was observer at the highest concentration (100 ppm) used in both test organisms.

Protein production by the test fungi also increased with addition of vitamins. It was maximum with thiamine followed by biotin, inositol and pyrodoxine. Effect of vitamin concentration on protein production followed the same trend as observed in mycelial growth.

DISCUSSION

It was evident that the test fungi were able to synthesize in part the vitamins required for their normal metabolism since some growth did take place in control flask. All the vitamins were more or less equally effective in improving growth and protein prodiction of the test-fungi but at different levels. The present investigation revealed that the test fungi were partial auxoheterotroph for all the vitamins. However, significant increase in growth as well as protein production when externally supplied with thiamine even at the lowest tested concentration proved that the fungi synthesized thiamine in quantity much below the level required for its normal development. Kim *et al.*, (1988) reported that among the various vitamins viz., Thiamine, biotin, pyridoxine, inositol, pantothenic acid, riboflavin, nicotinic acid and p-aminobenzoic acid, thiamine was most suitable for mycelial yield of *Agrocybe aegerita*. Hayes (1972) reproted good mycelium growth on a simple defined medium composed of simple sugar and salt supplemented with vitamin like biotin or thiamine. Das and Purakayastha (1987) studied the effect of different

Table 1 : Effect of different concentrations of vitamins on mycelial growth and protein production of *Pleurotus ostreatus* on rice straw dust (2 %) at optimum pH, temperature and nitrogen source.

Concentratins	Control		Thiamine		Biotin		Inositol		Pyridoxine	
(PPM)	Mycelial* biomass (mg)	Protein* (%)	Mycelial* biomass (mg)	Protein* (%)	Mycelial* biomass (mg)	Protein* (%)	Mycelial* biomass (mg)	Protein* (%)	Mycelial* biomass (mg)	Protein* (%)
0.0	128.7	30.1								
0.01	—	—	138.0	37.0	135.0	33.0	130.0	13.1	128.9	30.3
0.1	—	—	136.0	34.0	136.3	34.0	132.0	32.0	129.7	30.9
1.0	—	—	137.7	36.4	134.5	36.6	129.1	30.5	130.4	31.3
10.0	—	—	130.0	31.1	131.0	31.8	128.9	30.2	131.0	31.8
100.0	—	—	129.5	30.9	130.0	31.1	128.8	30.0	132.0	32
LSD (P=0.05)		0.4	0.3	0.3	0.3	0.2	0.1	0.2	0.1	

* Mean of 5 replicates.

Table 2 : Effect of different concentrations of vitamins on mycelial growth and protein production of *Volvariella volvacea* on rice straw dust (2 %) at optimum pH, temperature and nitrogen source.

Concentratins	Control		Thiamine		Biotin		Inositol		Pyridoxine	
(PPM)	Mycelial* biomass (mg)	Protein* (%)	Mycelial* biomass (mg)	Protein* (%)	Mycelial* biomass (mg)	Protein* (%)	Mycelial* biomass (mg)	Protein* (%)	Mycelial* biomass (mg)	Protein* (%)
0.0	183.3	25.9								
0.01	—	—	186.0	27.3	184.6	26.3	183.8	25.7	183.7	25.7
0.1	—	—	189.6	29.5	185.1	26.8	185.5	27.1	184.0	26.6
1.0	—	—	185.4	27.0	186.5	27.6	184.4	26.3	184.4	26.6
10.0	—	—	184.9	26.3	185.0	26.8	183.7	25.7	185.0	26.8
100.0	—	—	184.0	26.0	184.0	26.0	183.6	25.4	185.6	27.2
LSD (P=0.05)			0.5	0.2	0.4	0.3	0.3	0.2	0.4	0.1

* Mean of 5 replicates.

vitamins on growth of *Volvariella volvacea* and *Coprinus cinereus* and reported that among the six vitamins (viz., ascorbic acid, nicotinic acid, pyridoxine, riboflavin, thiamine and biotin) tested, thiamine was the most favourable.

The stimulatory nature of biotin was also reported by Vir and Grewal (1973) and Chattopadhyay (1976) in different fungi. Among the filamentous fungi total or partial deficiency of inositol was recorded in *Cercospora cruenta* (Jandaik and Kapoor, 1972) and *Fusarium oxysporum* f. *cumini* (Mathur *et al.*, 1964). Among the filamentous fungi pyridoxine-heterotrphy was reported in *Colletotricum capsici* (Mishra and Mahmood,1961) and *C. gloeosporiodes* (Prasad, 1966).

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