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Vitamin B and C content in mycelia of *Collybia diminuta*, *Tricholoma lobayense* and *Oudemansiella canarii* grown in liquid culture

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The vitamin B and C contents in mycelia of *Collybia diminuta*, *Tricholoma lobayense* and *Oudemansiella canarii* grown in liquid media were determined. The data obtained revealed that there was higher vitamin contents in the mycelia of the test fungi obtained from the optimal medium than that of the basal medium. Vit-B-12 was not found in any of the test fungi. Folic acid, and niacin and ascorbic acid were found in all the mycelia.

Key words : Vitamin B, and C, Mycelia, *Collybia diminuta*, *Tricholoma lobayense*, *Oudemansiella canarii*

INTRODUCTION

Mushrooms are reported to be good source of vitamins including thiamine, riboflavin, niacin, biotin and ascorbic acid (Crisan and Sands, 1978). However, there are reports that mushrooms may be heterotrophic or autotrophic in regards to vitamin nutrition.

Duda *et al.* (1980) have studied the level of vitamin contents of *Suillus luteuss*, *Lactarius deliciosus*, *Agaricus bisporus*, *Boletus edulis*, *Tricholoma equestna* and *Leccinium aurantiacum* and have found a range of vitamin contents in tested mushrooms. Thiamine, riboflavin, nicotinic acid, -amide and ascorbates are found to vary in the ranges of 0.144-0.844, 1.82-6.58, 30.1-108.00 and 15.80—153.00 mg/100g dry matter respectively. Wehmeyer *et al.* (1981) have estimated a higher levels of vitamin C, thiamine and riboflavin in *Macrolepiota zeyberi* and *Agaricus brunnescens*. Lasota *et al.* (1983) have estimated the contents of riboflavin, thiamine, niacin, pantothenic acid, vitamin B_6 and folic

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acid in mycelia of *Agaricus campestris*, *A. bisporus*. *Stropharia rugoso-annulata*, *Pleurotus ostreatus* and 14 species of common dried mushroom which are found to be a good source of these vitamins. They have also reported that vitamin B_{12} content varies from 0.41 μg/100g for *Stropharia rugosoannulata* to 8.30 μg/100g for *Boletus edulis*. Ascorbic acid content in *Pleurotus sajor-caju* (Fr.) Singer varies from 11 mg to 16 mg/100 mg fresh weight of mycelium (Sivaprakasam and Kandaswamy 1983). Zakhary *et al.* (1983) have studied the vitamin C content in *Agaricus campestris*, *A. rodmani* and *Collybia* Sp.

In the present investigation an attempt has been made to estimate the contents of vitamins (B and C) in the submerged mycelia of *C. diminuta*, *T. lobayense* and *O. canarii*.

MATERIALS AND METHODS

The tissue culture of *Collybia diminuta* (Berk. & Br.) Sacc., *Tricholoma lobayense* Heim. and *Oudemansiella canarii* (Jungh.) Hohn were used in the present investigation.

For this investigation both basal and optimal media were used for comparative analyses. These were as follows : (i) basal synthetic liquid medium containing : glucose, 30 g ; yeast extract, 2 g ; peptone, 2.5 g ; $MgSO_4.7H_2O$, 0.5 g ; $Ca(NO_3)_2$, 0.5 g ; $(NH_4)_2HPO_4$, 0.25 g ; KH_2PO_4, 0.25 g ; thiamine, 100 μg ; biotin, 50 μg ; folic acid, 100 μg ; inositol, 50 mg ; $FeCl_3$, 10 mg ; CaCl , (0.1M), 5 ml ; $ZnSO_4$, 0.1 mg ; distilled water, 1 litre, pH was adjusted to 5.5 and (ii) optimal synthetic liquid media for maximum growth of the three test fungi, starch (40 g/1) and yeast extract (4 g/1) ; maltose (40 g/1) and yeast extract (4 g/1) ; glucose (40 g/1) and yeast extract (4 g/1) were prepared by adding all other necessary nutrients including vitamins and macro, micro element as same as in the basal medium which was found to best combination for the growth. The pH of the medium of each of the three test fungi viz., *C. diminuta*, *T. lobayense* and *O. canarii* were adjusted to their respective optimal values of 6.5, 6.5 and 6.0 with 0.2M phosphate buffer. An aliquot of 50 ml of each medium was dispensed in each 250 ml Erlenmeyer flask, plugged and sterilized at 10 p.s.i for 20 minutes. The flasks were inoculated separately with 1.0 ml of cell suspension of each test fungus and incubated (stationary) in complete darkness at 30°, 20° and 20°C (+0.5°C) for 15, 20 and 20 days respectively.

The mycelia were harvested by filtration through a tarred sintered funnel (Jena IG—3). The filtered mycelia were washed repeatedly with distilled water to make them free from any adhering media and dried to a constant weight in vaccum oven at 60 C for 24 h, cooled in a desiccator and weighed.

Samples for B-vitamins assay were weighed directly from the desiccator while for vitamin C assay fresh mycelial mats were used. In case of vitamin B_{12} two methods of analysis were followed :

Folic acid content was determined colorimetrically by the method of Hutchings *et al* (1947).

Niacin was determined by a chemical method of Melnick and Field (1940).

Cyanacobolamin was assayed in the mycelial mat by the colorimetric method of Fantes *et al.* (1950) and Boxer and Rickards (1950). Results were read from standard curve plotted using vitamin B_{12} (U.S.P.) as the standard.

Following Diazotized 4-Methoxy-2 Nitroaniline method (A.O.A.C., 1966) the vitamin C was estimated.

RESULTS AND DISCUSSION

The analytical data obtained are given in Tables 1 and 2 show the various vitamin contents (B-vitamins and vitamin C) of mycelia of three test fungi grown under submerged cultural conditions in both basal and optimal synthetic media. In all cases, there was a higher vitamin content in the mycelium of the test fungi obtained from the optimal synthetic medium than that of basal synthetic medium. The mycelia of *Collybia diminuta*, *Tricholoma lobayense* and *Oudemansiella canarii* have been observed to be a fairly good source of folic acid, niacin and ascorbic acid. But none of the test fungi assayed contained Vitamin B_{12} (cyanocobolamin).

Table 1. B-Vitamin composition (dry basis) of the mycelia of *Collybia diminuta*. *Tricholoma lobayense* and *Oudemansiella canarii* grown in submerged culture in basal and optimum synthetic media.*

Test organisms	B-Vitamin composition in μg/g dried mycelium					
	Folic Acid		Niacin		Vitamin B_{12}	
	Basal medium	Optimum medium	Basal med um	Optimum medium	Basal medium	Optimum medium
Collybia diminuta	2.500 ±0.01	8.060 ±0 03	93.037 ±0.08	107.022 ±0.05	Nil	Nil
Tricholoma lobayense	8.050 ±0.03	12.500 ±0.04	589.730 ±0.10	618.120 ±0.09	Nil	Nil
Oudemansiella canarii	6.080 ±0.01	9.500 ±0.02	447.659 ±0.12	458.673 ±0.11	Nil	Nil

* Results are the mean of three replicates

Among three test fungi, *C. diminuta* shows the highest amount in folic acid content in the optimal medium in compared to basal medium which is followed by *T. lobayense* and *O. canarii*. But, individually *T. lobayense* contains maximum amount of folic acid in compared to other test fungi in both basal and optimal media. The values obtained are more or less similar to those reported

Table 2. Vitamin-C content (fresh basis) of the mycelia grown in submerged culture in basal and optimum media.*

Test organisms	Ascorbic acid content in mg/g fresh mat	
	Basal	Optimum
Collybia diminuta	4.700 ±0.13	8.140 ±0.10
Tricholoma lobayense	6.800 ±0.19	7.200 ±0.13
Oudemansiella canarii	4.100 ±0.12	4 600 ±0.13

* Results are the average of three replicates

by some other previous investigators for other fungi. Esselsen and Fellers (1946) have reported 9.8 μg per g fresh weight in *Agaricus bisporus*. However, comparatively lower amount of folic acid content has been determined by Jennison *et al.* (1957). They have found 9.10 μg and 3.30 μg (dry weight basis) respectively in *Lentinus trigrinus* in 1.0% malt extract and optimal synthetic media under submerged conditions. The average folic acid content of *C. diminuta* closely resemble the findings of Atacador *et al.* (1967) on *Volvariella volvacea*.

In *T. lobayense*, maximum amount of niacin (618.12 μg/g dry wt.) is found in the mycelium grown in the optimal medium which is followed by *O. canarii* (458.67 μg/g dry wt.) and *C. diminuta* (107.02 μg g dry wt.). The average values of niacin content of the fungi are slightly higher than the results of other research workers (Duda *et al.*, 1980). Other authors have obtained significantly higher values (Guha and Banerjee, 1973 ; Nag Chaudhuri, 1977) of niacin content.

None of the test organisms estimated chemically by both the methods mentioned above have been found to contain vitamin B_{12}. Few workers, however, have reported the presence of vitamin B_{12} in other mushrooms (Lasota *et al.*, 1983), although in very small amounts.

The fresh mycelium of *C. diminuta*, *T. lobayense* and *O. canarii* have been found to contain fair amount of vitamin C specially under the optimal physiological conditions. The maximum amount of increase in vitamin C content has been found in the optimal conditions of *C. diminuta* (3.44 mg/g fresh weight) which is followed by *O. canarii* (0.50 mg/g fresh weight) and *T lobayense* (0.40 mg/g fresh weight). Individually the lowest content of vitamin C is found in *O. canarii*. The findings of the present investigation are comparatively higher in vitamin C content than those of other previous reports (Esselsen and Fellers, 1946). However, Wehmeyer *et al* (1981) have estimated appreciable amount of vitamin C content in *Macrolepiota zeyheri* and *Agaricus brunnescens*.

From the data of the present investigation, it is evident that vitamin in the mycelium of the test fungi could be increased by altering the composition of the growth media and conditions of growth.

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