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Protein and amino acids contents in mycelial biomass of *Tricholoma lobayense*, *Tricholoma gigangium* and *Tricholoma crassum*

MAITREYI BANERJEE, SNIGDHA MAITRA AND N. SAMAJPATI
Department of Botany, Calcutta University, Calcutta-700 019

The protein and amino acids content in mycelial biomass of *Tricholoma labayense*, *T. gigantium* and *T. crassum* were evaluated. The protein content in mycelial biomass produced in cheese whey is higher than that of the mycilial biomass produced in optimal synthetic media. The selphur amino acids are not present in appreciable amount.

Key words : Protein content, amino acids contente, myclial biomass, *Tricholoma* spp.

INTRODUCTION

Today, it is well-known that mushroom is very favourable and essential dietary item because of its high protein content and nutritive value. It is a rich source of essential amino acids required for human nutrition. The crude protein content of mushroom may vary from as low as 4-9% in species of *Auricularia* to as high as 44% for *Agaricus* on dry weight basis. Analyses of *Agaricus bisporus* strains alone showed a range of 24-44% crude protein (Crisan and Sands, 1978).

Block *et al.* (1953) reported that 17 amino-acids were present in *Agaricus blazei* similar to that of *A. campestris* with the absence of proline and serine. Jennison *et al.* (1958) noticed that the protein of *Polyporus palustris* contained the same number and similar amounts of amino acids, when it was grown in basal synthetic and malt extract media. The similar finding was also observed by Reusser *et al.* (1958) on *Tricholoma nudum*. *T. nudum* contained all essential amino acids (Bell *et al.*, 1958). All essential amino acids were also present in *Tricholoma flavovirens* (Motskus, 1977 a,b). Nagchaudhuri and Samajpati (1979) estimated free and bound amino acids in mycelium of *Tricholoma gigantium* Masse.

In this present investigation, the crude protein and amino acid content of mycelia of *Tricholoma lobayense* Heim, *Tricholoma gigantium* Masse and *Tricholoma crassum* (Berk.) Sacc. were estimated to ascertain the nutritive value of the test-fungi.

MATERIALS AND METHODS

Test organisms

The tissue cultures of *T. lobayense* Heim, *T. gigantium* Masse. and *T. crassum* (Berk.) Sacc. were used in this experiment and the cultures were maintained on potato-dextrose-agar slant.

Growth media and conditions

For growth experiments, two types of culture media-optimal synthetic medium and cheese whey medium (as such) were chosen to obtain mycelium.

(1) Synthetic media optimal - Glucose – asparagine, Glucose-yeast-extract media were prepared respectively for *T. lobayense*, *T. crassum* and *T. gigantium* to get the maximum growth and protein yield. All other nutrients including vitamins were added in their respective optimal concentration.

(2) Cheese whey medium - Cheese whey was prepared from cow's milk and tonned milk of Haringhata Milk Dairy, West Bengal. The pH of the two media were adjusted to their respective optimal value. 30 ml of medium were dispensed in each 150 ml Erlenmeyer flask and flasks were plugged and sterilized at 15 p.s.i for 15 minutes. The media were inoculated separately with 1.0 ml inoculum of each of the three test-fungi. The flasks were incubated in a shaking incubator (120 r.p.m) in complete darkness at 35°C for 16, 20 days in synthetic media and 16 days in cheese whey.

After the incubation period, the mycelia were harvested and washed to remove adherent medium. These were dried in a vaccum oven at 60°C for 24 hours, cooled in a desiccator and weighed.

Total nitrogen of the dry mycelia obtained from two media were determined by colorimetric method of Folin-Wu (1919) and Vogel (1961). The factor 4.38 was used to calculate the crude protein assuming that mycelial protein contains 22.8% nitrogen.

Amino acids in dry purified mycelial protein of each of the three test-fungi grown on synthetic optimal media and cheese whey medium, were estimated qualitatively and quantitatively by chromatographic and colorimetric methods of Berner *et al.*, (1969) and Giri *et al.*, (1952).

RESULTS AND DISCUSSION

The results of this experiment are given in Tables 1 and 2.

The average crude protein content of dry mycelial powder of *T. labayense*, *T. gigantium* and *T. crassum* was found to be 31.20%, 28.07% and 32.58% respectively in optimal Synthetic media and 39.32%. 30.23% and 35.51% respectively in cheese whey medium.

Table 1. Crude protein content of dry mycelia* grown in two media

Test organism	Mycelial protein % (dry wt. basis)	
	Optimal synthetic medium	Cheese whey
Tricholoma lobayense	31.20±0.02	39.32±0.04
Tricholoma gigantium	28.07±0.02	30.23±0.01
Tricholoma crassum	32.58±0.03	35.51±0.02

*Values are averages of triplicate determinations.

Table 2. Comparison of the Amino-acid content of dry mycelial protein of *Tricholoma lobayense*, *Tricholoma gigantium* and *Tricholoma crassum* grown in synthetic media optimal and cheese whey

Amino-acid	Amino-acid content in g/100g of protein					
	T. lobayense		*T. gigantium*		*T. crassum*	
	Optimal medium	Cheese whey	Optimal medium	Cheese whey	Optimal medium	Cheese whey
Lysine	2.04	3.54	2.04	3.54	5.79	5.79
Leucine	-	-	-	-	-	-
Isoleucine	-	-	5.72	6.28	5.08	6.83
Alanine	-	-	-	-	7.91	5.79
Glutamic acid	2.04	3.54	-	-	-	-
Aspartic acid	4.04	5.08	-	-	-	-
Valine	3.54	4.92	2.54	3.04	7.91	9.83
Histidine	5.79	7.12	6.83	7.40	5.08	9.83
Threonine	4.58	4.58	2.04	2.79	4.58	7.91
Cystine	7.40	7.91	2.04	1.54	-	-
Phenylalanine	-	-	1.54	4.04	6.28	6.83
Tyrosine	4.58	5.79	5.50	7.12	6.81	7.40
Tryptophan	2.28	2.75	-	-	-	-
Proline	2.54	4.04	2.04	2.04	1.54	2.04
Unknown	5.08	6.28	-	-	-	-

Humfeld and Sugihara (1949) reported that white variety of *Agaricus campestris* contained 35.5% protein while brown variety contained 45.3%. Almost similar results were obtained by Crisan and Sands (1978). Jennison *et al.* (1957) estimated the protein content of *Lentinus trigrinus* to be 21.7% and 31.6% in malt extract and optimal synthetic media respectively. Similar results were reported by some other workers. (Bano *et al.*, 1971; Bell *et al.*, 1958; Motskus, 1977a,b).

The data in Table 2 showed the amount of amino acids present per 100g of protein in the mycelia of three test-fungi grown under optimal and cheese whey media. The results showed that mycelia of all the fungi were good source of essential amino-acids.

In case of *T. lobayense* eleven amino acids were isolated of which six were essential amino-acids (lysine, valine, threonine, cystine, tyrosine and tryptophan). Histidine, valine, threonine, cystine and tyrosine were present in quite considerable amount. The mycelia of

cheese whey medium contained higher amount of amino acids than optimal medium. Glutamic acid and aspartic acid were present only in this fungus. An unknown amino acid was detected which was present in considerable amount in *T. lobayense*. Further works are necessary to identify this one. The amount of cystine was highest followed by histidine, tyrosine and aspartic acid. Lysine, valine, histidine, threonine, tyrosine and proline were present in mycelia of all the three mushrooms under study.

In *T. gigantium*, seven essential amino acids were present out of nine found (lysine, isoleucine, valine, threonine, cystine, phenylalanine and tyrosine). Histidine was present in highes amount (7.40 g/100g) follwed by tyrosine and isoleucine. All the amino acids were present in higher amount in mycelia of cheese whey medium excepting cystine and proline.

Six essential amino acids were present in mycelium of *T. crassum* (lysine, isoleucine, valine, threonine, phenylalanine and tyrosine). Alanine was present only in this fungus. Valine, histidine, threonine and tyrosine were present in appreciably high quantity and others were also in greater amount in comparion to the other two test-fungi. Histidine and threonine content were found to be double in mycelia of cheese whey medium than that of optimal synthetic medium. Two essential amino acids-methionine and leucine were absent in mycelia of all the three test-fungi.

Chromatographic analysis of alkaline hydrolysate revealed tryptophan to be present in *T. lobayense* while it was absent in the other two test-fungi.

The average results obtained were comparable to the findings of Bano *et al.* (1971) which revealed that *Volvariella diplasia* lacked leucine and methionine. The high content of histidine - 7.12, 7.40 and 9.83% in all the test-fungi was also similar to that of *Termitomyces* sp. (6.5%) as reported by Bano *et al.* (1964).

The mycelia of all the three studied fungi, however, showed little divergence from the report of Bell *et al.* (1958), which states that *Tricholoma nudum* contained all the essential amino acids. The present results showed quite similarity with the amino acid content of *Tricholoma flavovirens* (Motskus 1977 a,b). A slightly lower value of amino acid percentage was reported by Wang *et al.* (1985) in *Calvatia gigantia*, *Hericium erinaceous* and *Agaricus zisperus*.

From the present findings, it can be concluded that mycelia of the test-fungi are very good sources of protein containing a number of essential amino acids, but the amount might have varied according to variation in species and growth media.

REFERENCES

Bano, Z., Riaz, A. and Srivastava, H. C. (1964). Amino-acids of edible mushrooms *Lepiota* sp. and *Termitomyces* sp. *Ind. J. Chem.*, **2** : 380-381.

Bano, Z., Srinivasan, K. S. and Snigh, N. S. (1971). Essential amino-acid composition of the protein form a mushroom (*Volvariella diplasia*). *J. Food Sci. Tech.*, **8** : 180-181.

Bell, J. M., Erfle, J. D., Spencer, J. F. T. and Reusser, F. (1958). *Tricholoma nudum* as a source of amino-acids, vitamins and unidentified factors. *Canad. J. Animal Sci.*, **38** : 122-128.

Berner, M., Niederwiser, A. and Pataki, G. (1969). In : *Thin-layer chromatography* (Ed. E. Stahl). George

Allen and Unwin Ltd., London : 730-736.

Block, S. S., Stearns, T. W., Stephens, R. L. and McCandless, R. F. (1953). Mushroom mycelium, experiments with submerged culture. *J. Agric. Food Chem.*, **1** : 890-893.

Crisan, E. V. and Sands, A. (1978). *Nutritional value*. In : "*The Biology and Cultivation of Edible Mushroom*". (Eds. Chang, S. T. and Hayes, W. A.). Academic Press Inc. (London) Ltd. : 145-168.

Folin, O. and Wu, H. (1919) Determination of non-protein nitrogen. *J. Biol. Chem.*, **38** : 81.

Giri, K. V., Radhakrishnan, A. N. and Vaidyanathan, C. S. (1952). Some factors incluencing the quantitative determination of amino-acids separated by circular paper chromatography. *Anal. Chem.*, **24** : 1677-1678.

Humfeld, H. and Sugihara, T. F. (1949). Mushroom mycelium production by submerged propagation. *Food Technol.*, **3** : 355-356.

Jennison, M. W., Richberg, C. G. and Krikszeus, A. E. (1957). Physiology of wood-rotting Basidiomycetes II. Nutritive composition of mycelium grown in submerged culture. *Appl. Microbiol.*, **5** : 87-95.

Motskus, A. V. (1977a). Biochemical studies of Agaricales mushroom 3. Concentration of protein substances in the fruit bodies of *Tricholoma flavovires* and amino-acid in the hydrolysates. *Liet. Tsr. Mokslu. Akad. Darb. Ser C Biol. Mokslai*, **1** : 119-124.

Motskus, A. V. (1977b). Biochemical analysis of mushrooms of the Order Agaricales : 4. The concentration of protein substances and amino-acids in the hydrolysate of fruiting bodies from the wooly milk-cap. *Liet. Tsr. Mokslu. Akad. Darb. Ser C. Biol. Mokslai*, **2** : 113-118.

Nagchaudhuri (nee Aditya), M. and Samajpati, N. (1979). Free amino-acid pool and amino-acids in mycelial protein of *Tricholoma gigantium* Masse. In : "*Advances in Mycology and Plant Pathology*". Proceedings of the National Symposium held at Calcutta (Eds. Chattopadhyay, S. B. and Samajpati, N.). Oxford and IBH Publishing Co., pp. 104-110.

Reusser, F., Spencer, J. F. T. and Sallans, H. R. (1958). *Tricholoma nudum* as a source of microbiological protein. *Appl. Microbiol.*, **6** : 5-8.

Vogel, A. I. (961). *Determination of Ammonia*. In : "*A Textbook of Quantitative Inorganic Analysis*". The English Language Book Society and Longman, pp. 783-784.

Wang, Lin and Li, Yuyne (1985). Amino-acid analysis of 20 kinds of edible mushrooms. *Shipin Kexue*, **61** : 10-12.

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