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Protein value of mycelial biomass of some edible mushrooms

S. RAY * AND N. SAMAJPATI
Mycology Laboratory, Department of Botany, Calcutta University.
Calcutta-700 019

Proetin production in optimal liquid synthetic medium by the four test fungi revealed that yields were 33.62, 28.62, 25.62 and 42.16 percent for *L. squarrosuluss, L. polychrous, A. broadwayi,* and *A. hygrometricus* respectively. Amino acid composition of mycelial protein of four test fungi also revealed that all these fungi were good sources of microbial protein of these, *A. hygrometricus* was a promising one for its high content of sulpher amino acid.

Key words : Edible fungi, protein content of the mycelia, amino acid composition of mycelial protein

INTRODUCTION

Astreaeus hygrometricus is highly prized edible ,ushrooms in the many localities of southern Bengal. It grows wild and is collected and eaten by the common people of the aforesaid areas. Though mushrooms are eaten primarily as condiments especially in western countries. *A hygrometricus* as well as its mycelia and the mycelia of other three test fungi viz. *Lentinus squarrosulus, L. polychrous* and *Agrocybe broadwayi* have sufficient nutritive value to serve as a supplementary source of protein.

MATERIALS AND METHODS

Mycelial cultures of the test species were prepared from the fresh basidiocarps collected from the different sal forest of southern Bengal. Mycelia of four test fungi, were grown in four separate optimal liquid synthetic medium i.e. Starch - L-glutamic acid medium for *Lentinus squarrosulus* (starch–20 g., L-glutamic acid-3.5 g., ammonium phosphate - 1.0 g., magnesium sulphate - 0.1 g., thiamine hydrochloride - 100 mg, - manganese sulphate - 0.01 mg., zinc sulphate - 0.01 mg., distilled water - 1000 ml., *p*H -6.5); "Maltose - L - Lysine "medium for *L. polychrous*,, (Maltose - 35 g., L-Lysine - 1.8 gm., Ammonium phosphate - 1.0 g., magnesium sulphate - 0.1 g., manganese sulphate - 0.01 mg., zinc sulphate - 0.01 mg., copper sulphate - 0.01 mg., thiamine hydrochloride - 100 mg., distilled water - 100 ml., *p*H-6.5); "Glucose–L–Asparagine" medium for *Agrocybe broadwayi* (Glucose-40 g., - ammonium phosphate - 1.0 g., L- asparagine - 1.4 g., - 0.1

g., thiamine hydrochloride - 100 mg., manganese sulphate - 0.01 mg., Distilled water - 1000 ml., *p*H - 7.5) and "Sucrose. L - Asparagine" medium for *Astraeus hygrometricus* (Sucrose 35 g., L - Asparagine - 1 g., maguesium sulphate - 0.5 G., - 0.5 g., 10 drops of a 1% solution of ferric chloride, - 0.01 mg., zinc solphali - 0.01 mg., thiamine hydrochloride - 100 mg., Pyridoxine - 100 mg., Distilled water - 1000 ml., *p*H - 6.0).

50 ml of the medium were dispensed in 250 ml. Erlenmeyer flasks, autoclaved, inoculated and incubated in a shaking incubator (120 - r.p.m.) for 7 days at optimum temperature (30°C) in complete darkness. *Agrocybe broadwayii* and *Astraeus hygrometricus*, however, were harvested after 8 and 10 days respectively.

After the incubation period the medium and mycelium were separated by filtration through a tarred funnel. The filtered mycelium was washed repeatdely with distilled water to mak them free from adherent medium and dried to constant weight at 60°C. for 72 hours. The dry weight of the mycelium thus obtained was taken as an index of growth.

The protein content of the dried mycelium was measured by determining the total nitrogen by colorimetric method (Follin and Wu, 1919 and Vogel, 1962). Colorometric readings were taken using blue filter (420 mm).

Amino acid composition

Both qualitative and quantitative composition of mycelial protein of four test fungi in submerged culture was estimated. The dry mycelial mats were powdered in a wiley mill and passed through 60 screen mesh. Then the powdered mycelia were used for isolation and purification of protein.

Isolation and purification of mycelial protein

Crude protein was extracted with 0.4 (N) NaOH in cold from the dried powder of *Lentinus squarrosulus, L. polychrous, Agrocybe broadwayi, Astraeus hygrometricus*. The insoluble materials were discarded by centrifugation. The protein in clear supernatent solution was precipitated out by adjusting its *p*H to 4.5 with 6% trichloro–acetic acid (TCA). The precipitate was collected by centrifugation and washed once with 6% TCA. Nucleic acid contaminants were removed by heating the suspension of the precipitate in the same acid. The residue was washed successively with 6% TCA at room temperature (25° - 26°). cold ethanol, warm (45°–60°C) ehtanol : diethyl ether mixture (1:1) by centrifugation. The protein thus recovered in final sediment was dried under air current.

Hydrolysis of protein

A measured amount of dried protein was hydrolyzed with 6(N) HCl at 110°C in sealed evacuated tubes for 24-36 h (Burner *et al.*, 1969). After the hydrolysis was complete acid was removed by putting in an vaccum dessicator over NaOH at 25°C and the final residue was dissolved in 1 ml of 10% aq. isopropanol solution for chromatographic fractionation.

For alkaline hydrolysis a measured amount of protein was digested with a suspension of hydrated $Ba(OH)_2$ solution in water at 125°C for 4 h (Burner *et al.*, 1969). The *p*H of the digest was adjusted to 6.0 with 2 N H_2SO_4 and Ba^{++} was precipitated as $BaSO_4$. The resultant suspension was boiled and centrifuged. The sediment was washed with distilled water and the combined supernatant and washing were evaporated to dryness under

vacuum. The residue was dissolved in 1 ml. of distilled water for further fractionation.

Chromatographic separation of amino acid mixture

Amino acid mixture from cell pool was subjected to two dimensional thin layer chromatography on activated silica gel G (E. Merk) using chloroform : methanol : 17% NH_4OH (2:2:1) and phenol saturated with water as solvent system (Fahny *et al.*, 1961). After chromatography solvents were removed by evaporation and the chromatogram was developed by spraying 0.5 percent ninhydrin solution in 95 percent acetone followed by acetone followed by heating at 80°C for 5 minutes. The developed colour spots were identified comparing the mobilities of standard amino acid under identical condition.

Quantitative estimation of amino acid

The coloured spots were separated off from the chromatogram, mixed thoroughly with definite volume (1.5-2.5 ml.) of 0.005 percent $CuSO_4$ solution in 50 percent aqueous ethanol and centrifuged (Giri *et al.*, 1952).

The clear supernatant obtained after centrifugation at 2000 r.p.m. for 10 minutes was assayed colorimetrically in Beckman Du spectrophotometer at 540 mm. The colorimetric blank was prepared by treating the scrapings taken from unstained area of TLC plate equal in size as that of amino acid spots similarly. The amount of different amino acid was calculated by using individual amino acids as 'standards'.

RESULTS AND DISCUSSION

The protein content of the mycelium in optimal liquid synthetic medium reached its maximum on 7th day which is as high as 33.62 perent in *Lentinus squarrosulus* and 28.62 percent in L. *polychrous*. Protein production in optimal liquid synthetic medium also reached its maximum i.e. 25.62 percent in *Agrocybe broadwayi* after 8 days and 42.16 percent in *Astraeus hygrometricus* after 10 days.

From the data on the protein production in submerged culture it is evident that *A. hygrometricus* proved to best among the four. The growth of three test fungi though comparatively faster than the *A. hygrometricus* in optimal liquid synthetic medium yet the yield of protein by this fungus was much more in comparasion to the other three.

The data in Table–2 indicate that the amino acid composition of the mycelia protein of all the four test fungi are quite good. In case of *Lentinus squarrosulus*, among the essential amino acids, it was a good source of lysine, leucine, arginine, valine, phenylalanine, tryptophan, histidine and alanine while threonine and isoleucine are present in appreciable amounts. Though cystine content was quite low yet methionine is present as 2.5g/100g of protein which is quite good so far the micorbiol protein is concerned. Among the other amino acid were present in appreciable amount but glycine and serine contents are quite low. Among the rest three test fungi similar pattern of distributions were noticed. But in case *A. hygrometricus* the cystine content (0.66g/100g) and methionine content (2.80g/100g) appeared to be quite promising as normally microbiol protein are deficient in sulpher amino acid.

From the data amino acid composition (table-2) of mycelial protein of the four test fungi it was evident that all the four test fungi are good sources of protein. It is well known that microbiol protein is deficient in sulpher amino acid in general but in Astraeus

hygrometricus it is quite promising.

Considering sulpher amino acid as limiting one, viz. methionine and cystine, the chemical assessment of proteins are 58.86, 56.41, 50.56 and 65.28 for Lentinus squarrosulus, L. polychrous, Agrocybe broadwayi and Astraeus hygrometricus respectively (Table-3).

The results obtained during the experimental period are given in Tables 1, 2 and 3.

Table 1. Data (mean)* showing the growth of mycelia (dry weight basis) and protein content of *Lentinus squarrosulus* in "Starch-L-Glutamic acid" medium, *L. polychrous* in "Maltose-L-Lysine" medium and *Astraeus hygrometricus* in "Sucrose-L-Asparagine" medium under submerged condition

Test fungi	Incubation period (days)	Dry Wt. of mycelium	Protein content of mycelium (g/l)	Protein mycelium mycelium (%)	Final *p*H medium (%)
Lentinus squarrosulus	7	10.36 ± 0.46	3.48 ± 0.44	33.62	6.5
L. polychrous	7	10.46 ± 0.46	2.99 ± 0.36	28.62	6.6
Agrocybe broadwayi	8	11.36 ± 0.42	2.91 ± 0.26	25.62	7.7
Astraeus hygrometricus	10	11.26 ± 0.24	4.74 ± 0.38	42.16	6.5

* Data are expressed as average of five replicates

Table 2. Data (mean)* showing the amino acid composition of mycelial protein of the four test fungi grown in optimum media under submerged condition

Amino acid	Amount g/100g of protein			
	Lentinus squarrosulus	*L. polychrous*	*Agrocybe broadwayi*	*Astraeus hygrometricus*
Alanine.	3.20	3.46	3.16	3.20
Aspertic acid.	4.81	4.62	4.86	4.71
Glutamic acid.	5.62	5.86	6.36	6.16
Glycine.	1.64	1.52	2.16	2.30
Histidine.	3.62	3.81	4.16	4.00
Methionine.	2.50	2.40	2.00	2.80
Cystine.	0.62	0.59	0.68	0.66
Isoleucine.	2.46	2.71	2.64	2.78
Leucine.	6.52	6.62	6.96	7.10
Arginine	4.62	4.42	4.10	4.68
Tyrosine.	2.64	2.71	2.51	3.16
Phenylalanine	4.26	4.36	4.16	4.60
Serine.	1.56	1.48	1.58	1.92
Lysine.	4.62	4.81	5.26	6.16
Threonine.	1.62	1.60	1.71	1.80
Tryptophan.	3.64	3.40	3.20	3.46
Valine.	4.26	4.00	4.31	4.20
Proline.	6.62	6.91	6.76	5.16

* The data are average of five replicates.

Table 3: Data showing the nutritive value of mycelial protein of *Lentinus squarrosulus, L. polychrous, Agrocybe broadwayi* and *Astraeus hygrometricus* on the basis of essential amino acid composition in comparison to these of whole egg

Amino Acids	Whole egg protein. (Sheffner 1967) protein	*Lentinus squarrosulus* protein (g/100g. protein)**	Egg ratio (%)*	*L. polychrous* protein (g/100g) protein)**	Egg. ratio (%) *	*Agrocybe broadwayi* (g/100g) protein)**	Egg ratio (%)*	*Astreaus hygrometricus* (g/100g)	Egg ratio (%) *
Histidine	2.6	3.62	100.00	3.81	100.00	4.16	100.00	4.00	100.00
Lysine	7.8	4.62	59.23	4.81	61.66	5.26	67.43	6.16	78.97
Methionine-cystine	5.3	3.12	58.86	2.99	56.41	2.68	50.56	3.46	65.28
Phenlalanine-tyrosine	9.3	6.90	74.19	7.07	76.02	6.67	71.72	7.76	80.21
Leucine-Isoleucine	14.7	8.98	60.68	9.33	63.46	9.60	65.30	9.88	67.21
Valine	7.1	4.26	60.00	4.00	56.25	4.31	60.70	4.20	59.15
Threonine	4.9	1.62	33.06	1.60	32.65	1.71	34.89	1.80	36.73
Tryptophan	1.4	3.64	100.00	3.40	100.00	3.20	100.00	3.46	100.00
*Total	53.1	36.76		37.01		37.59		40.72	

*The ratio of the quantity of each essential amino acid of the test fungi protein to the quantity of the same amino acid in the same amount of egg. protein..

**Chemical score of the test fungi mycelial protein is 58.86, 56.41, 50.56 and 65.28 of *Lentinus squarrosulus, L. polychrous, Agrocybe broadwayi* and *Astraeus hygrometricus* respectively.

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