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FRACTIONAL AND AMINO ACID COMPOSITIONS OF MICROMYCETE PROTEINS

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A comparative study has been made of the fractional and amino acid compositions of the proteins of the biomasses of Trichoderma harzianum, Fusarium moniliforme, *and the hybrid culture FT-2. It has been established that the micromycete strains investigated are high-protein cultures containing from 36.0 to 49.7% of protein. A study of the amino acid compositions of the micromycetes showed that the hybrid culture FT-2 possessed the largest amount of amino acids among the water-soluble proteins of the biomass (61.3 g/100 g of biomass protein).*

Micromycetes belonging to the genera *Trichoderma* and *Fusarium* may be a potential source of new types of protein preparations for fodder purposes. According to the literature on synthetic media these fungi form 10-20 g/liter of biomass containing from 30 to 50% of protein [1].

We have studied the fractional and amino acid compositions of the proteins formed by the cellulose-destroying micromycetes *Trichoderma harzianum* and *Fusarium moniliforme* and by the hybrid culture FT-2.

On growth under deep fermentation conditions, the micromycetes taken for the investigations formed considerable amounts of proteins: *T. harzianum* — 36%; *F. moniliforme* — 40.8%; the hybrid culture FT-2 — 49.7% of the biomass (Table 1). On determining the total proteins in the freeze-dried biomass it was established that the yield of protein depended to a considerable degree on the composition of the solvent (Table 2). The largest amount of protein was present in the water-soluble fraction. The salt- and alcohol-soluble fractions of protein from the freeze-dried micromycete biomass amounted to

TABLE 1. Formation of Biomass by Micromycetes and Its Protein Content

Culture	Amount of biomass, g/liter	Protein content, %
Trichoderma harzianum	30.4	36.0
Fusarium moniliforme	32.2	40.8
Hybrid FT-2	38.4	49.7

TABLE 2. Fractional Composition of the Proteins of the Micromycete Biomass, % of the Absolutely Dry Weight

Protein fraction	Sum of the soluble fractions, %		
	T.harzianum	F.moniliforme	Hybrid FT-2
Water-soluble	21.2	23.8	26.4
Salt-soluble	2.0	2.4	2.8
Alcohol-soluble	3.0	3.4	3.6
Alkali-soluble	7.2	7.7	8.5
Sum of the soluble substances	33.4	37.3	41.3

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TABLE 3. Amino Acid Compositions of the Water-Soluble Micromycete Proteins

Amino acid	FAO standard, g/100 g of protein	Hybrid FT-2			T. harzianum			F. moniliforme		
		Amino acid content, g/100 g of total biomass (a), of the protein of the total biomass (b), and of the total essential amino acids (c)								
		a	b	c	a	b	c	a	b	c
Lysine	5.5	3.2	7.0	8.3	3.0	9.5	6.2	2.6	7.3	5.8
Histidine		2.6	1.62		1.3	2.0		1.1	1.34	
Arginine		0.9	3.5		0.7	6.2		0.6	3.10	
Aspartic acid		3.2	4.56		2.9	4.6		2.8	9.2	
Threonine	4.0	4.8	5.10	4.8	4.5	4.8	4.6	4.2	4.9	3.6
Serine		2.3	2.39		1.9	5.2		2.2	2.1	
Glutamic acid		13.4	19.08		11.5	21.2		13.4	19.4	
Proline			3.8		0.5	3.12			2.12	
Glycine		0.3	6.3		3.8	5.42		0.4	2.8	
Alanine			10.25			9.67		3.2	9.26	
Valine	5.0	4.2	6.03	6.8	3.2	7.09	6.1	4.0	5.1	4.7
Cystine + methionine	3.5	4.6	4.53	4.42	4.3	3.04	4.0	3.4	4.8	4.2
Isoleucine	4.0	3.5	4.83	4.52	3.5	3.72	3.7	7.8	6.04	4.3
Leucine	7.0	8.8	9.02	8.4	9.8	7.64	6.4	8.7	9.14	6.6
Tyrosine + phenylalanine	6.0	9.5	8.5	9.04	4.4	6.8	6.9	4.8	9.8	7.4
Sum of essential amino acids				46.28			37.9			36.6
Total sum of biomass amino acids		61.3			55.3			59.2		
Total sum of biomass protein			100.0			100.0			100.0	
FAO standard	35.0									

TABLE 4. Biological Value of the Water-soluble Proteins Formed by Micromycetes

Essential amino acid	Amino acid score, % of total		
	T.harzianum	F.moniliforme	hybrid FT-2
Lysine	105.4	116.3	150.9
Threonine	90.0	110.0	120.0
Valine	90.4	122.0	136.0
Cystine + threonine	120.0	114.0	120.5
Isoleucine	105.0	90.2	113.0
Leucine	90.4	90.1	120.0
Tyrosine + phenylalanine	120.3	115.0	150.6

2-3.6% of the total water-soluble proteins. The fractional composition of the proteins from the cultures investigated depended on individual features of the producing agent. Thus, the biomass of the hybrid culture FT-2, as compared with *T. harzianum* and *F. moniliforme*, contained the largest amount of water-soluble protein (26.4%). A comparison of the sums of the water-soluble proteins with the total protein content of the biomass showed that the hybrid culture FT-2 contained the largest amount of water-soluble proteins.

The water-soluble fractions of the proteins were studied in more detail. As can be seen from Table 3, the water-soluble proteins of the micromycetes under investigation contained 17 amino acids, of which seven were essential. The highest total amounts of amino acids of the micromycete biomass and of the essential amino acids were found for the hybrid culture FT-2: 61.3 and 46.28 g/100 g of biomass protein, respectively.

Table 4 gives indices of the biological value of the water-soluble fractions of the freeze-dried proteins of the micromycetes under investigation according to the results of amino acid analysis, and also indices of the amino acid score for such amino acids as lysine, threonine, valine, cystine + methionine, isoleucine, leucine, and tyrosine + phenylalanine. As the standard we used the FAO ideal amino acid scale [2]. In the case of the hybrid culture, lysine, threonine, valine, cystine + methionine, isoleucine, and tyrosine + phenylalanine had amino acid scores exceeding 100%. In the case of *T. harzianum*, threonine, leucine, and valine had low amino acid scores.

It can also be seen from Table 4 that *T. harzianum, F. moniliforme*, and the hybrid culture FT-2 had good amino acid scores. With respect to the total essential amino acids they exceeded the FAO standard. Thus, for the levels of such essential amino acids as lysine, isoleucine, and tyrosine + phenylalanine, *T. harzianum* exceeded the FAO standard by 5.4, 5.0, and 20.3%, respectively. The hybrid culture FT-2, unlike *T. harzianum* and *F. moniliforme,* exceeded the FAO standard for the levels of lysine, valine, and tyrosine + phenylalanine by 50.9, 36.0, and 50.6%, respectively, and the sum of the essential amino acids in this culture amounted to 46.28 g/100 g of biomass protein at a FAO standard of 35 g/100 g of protein. Thus, the micromycete proteins investigated are promising for the preparation of new fodder products.

EXPERIMENTAL

We investigated the cellulose-destroying fungi *Trichoderma harzianum* and *Fusarium moniliforme* isolated from the soil of Tashkent oblast, and also the hybrid culture FT-2 obtained by the fusion of protoplasts of the above-mentioned cultures.

In order to obtain deep biomass [3], *T. harzianum, F. moniliforme*, and the hybrid culture FT-2 were grown under deep fermentation conditions at 28°C for seven days in Mandels nutrient medium (pH 5.5) [4] having the following composition (g/liter): $(NH_4)_2PO_4$, 2.3; KH_2PO_4, 1.0; $MgSO_4$, 0.5; $CaCl_2$, 0.3; acetic acid, 1 ml; comminuted wheat straw, 20; mains water, 1 liter.

The true protein contents of the freeze-dried biomasses of the cultures investigated were calculated by the method of Romanov et al. [5] on the basis of the formula that they have proposed in comparison with a standard preparation of microorganism biomass, the protein content of which we determined by Lowry's method [6].

To determine the quantitative content of amino acids in the fungal mycelium [7], the biomass was hydrolyzed in sealed glass tubes in 6 N HCl at 105-120°C for 24 h (2 ml of HCl per 100 ml of protein). After the end of hydrolysis the humins were filtered off, the hydrochloric acid was repeatedly evaporated under vacuum, and the hydrolysate was desalted. For this, it was evaporated on the water bath until a syrup had formed. This was dissolved in acidified 96% ethanol (0.5 ml of concentrated HCl to 100 ml of ethanol) and kept for 12 h for the extraction of the amino acids. The precipitate was separ-

ated off by centrifugation and the solution was again evaporated, and a solution of the residue in acidified alcohol was kept in a refrigerator for 16 h. This operation was repeated three times.

In order to eliminate lipids, the hydrolysate was shaken with ethyl ether in a separatory funnel several times. Then the hydrolysate was evaporated on the water bath, and the dry residue was dissolved in standard pH 2.2 buffer. The quantitative determination of the amino acids was carried out on an AAA-881 automatic amino acid analyzer.

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