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KINETICS OF THE QUALITATIVE AND QUANTITATIVE CHANGES OF VARIOUS PROTEIN FRACTIONS IN THE ONTOGENESIS OF PROTOPLAST AND HYBRID CULTURES OF *Trichoderma harzianum*

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The protein composition and the kinetics of the quantitative changes in the various protein fractions during the ontogenesis of protoplast and hybrid cultures of Trichoderma harzianum *have been studied. In the exponential growth phases of both cultures the highest amount of protein was contained in the water-soluble (33.0-34.2%) and salt-soluble (31.0-32.0%) fractions. The protein contents of the water-soluble fractions of the cultures studied were similar to those in meat and clover and were 3.5 times greater than in yeast.*

The fractional composition of proteins serves as an index of their quality, and its study is of theoretical and practical importance.

Our task was to study the protein composition of protoplast and hybrid cultures of *Trichoderma harzianum* and a comparative evaluation of the fractional compositions of the proteins of these cultures with protein fractions from fungi, yeasts, meat, and clover, and also a study of the kinetics of the quantitative changes in various protein fractions in the ontogenesis of the cultures studied [1-3].

In the investigation of the quantitative and qualitative changes of various protein fractions of protoplast and hybrid cultures of *T. harzianum*, we fractionated the protein from cells of 2-, 3-, and 5-day mycelia (Table 1).

In the exponential growth phases of the protoplast and hybrid cultures of *T. harzianum* the highest protein content was found in the water-soluble and salt-soluble fractions (I) and (II). The alcohol-soluble fraction contained 2.2-11.4% [sic] less protein than the water-soluble fraction. In the early stages of the development of the cultures there was a predominance of fractions I and II, belonging to the readily enzyme-hydrolyzable proteins and together making up about 70% of the total amount of proteins. In the later stages of growth, the proteins of the alcohol-soluble (III) and alkali-soluble (IV) fractions, belonging to the difficultly hydrolyzable proteins, began to predominate.

The largest amounts of protein in fractions I and II were found in the exponential growth phase and made up 34.2 and 32.0% of the total amount of protein, respectively. Fractions III and IV amounted to 22.8 and 11.8%, respectively.

It was noted that in the growth retardation phase of the protoplast culture of *T. harzianum* the protein fraction I diminished appreciably and fraction IV increased. For the hybrid culture of *T. harzianum*, in the exponential growth phase the amount of proteins in fraction I was similar to their amount in meat and clover and was 3.5 times greater than in yeast (Table 2).

The amount of proteins in fraction I of the protoplast culture was 3.5 times greater than in the yeast and was similar to its amount in clover. The investigations showed that in the exponential growth phase of the *T. harzianum* cultures that were investigated the amount of proteins in fraction IV was 4 times less than in clover and 2.5 times less than in yeast.

Thus, as cultures age, the intensity of their metabolism falls, the amount of water-soluble fractions increases and that of alkali-soluble fractions increases. The results obtained show that the fractional composition of the proteins of protoplast and hybrid cultures of *T. harzianum* depends on individual features of the producing agents.

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TABLE 1. Fractional Compositions of the Proteins of Micromycetes, % of the Total Amount of Protein

Protein fraction	Phase of development of the micromycetes								
	Exponential			Retardation of growth			Maximum stationary		
	1	2	3	1	2	3	1	2	3
I. Water-soluble	30.4	33.0	34.2	29.4	28.8	32.6	22.2	23.4	24.0
II. Salt-soluble	31.0	31.0	32.0	27.0	29.4	31.2	24.0	24.0	25.4
III. Alcohol-soluble	26.2	22.4	22.8	24.5	23.4	24.0	27.6	27.6	28.0
IV. Alkali-soluble	12.3	13.3	11.8	19.1	18.4	12.2	25.6	25.3	22.6

*1) *Trichoderma harzianum*; 2) protoplast culture of *T. harzianum*; 3) hybrid culture of *T. harzianum*.

TABLE 2. Fractional Compositions of Proteins with Different Origins

Object of investigation	Total protein content, % on the total weight of the preparations	Amounts of protein in the fractions, % of the total amount of proteins			
		I	II	III	IV
Clover	9.0	35.0	13.0	12.0	40.0
Muscle tissue (meat)	73.0	27.0	62.0	8.0	3.0
The yeast *C. tropicalis*	42.00	9.0	27.0	35.0	28.0
Hybrid culture of *T. harzianum*	38.0	34.2	32.0	22.0	11.8
Protoplast culture of *T. harzianum*	37.6	33.0	31.0	28.0	13.3

EXPERIMENTAL

The biomasses of hybrid and protoplast cultures of *Trichoderma harzianum* were used. The micromycetes were grown under deep cultivation conditions at 28°C for 96 h. The sole source of carbon used was 2% wheat straw with a particle size of about 0.2 mm [4, 5]. The micromycete biomasses were washed with water, and the preparations were dried at 50-60°C.

Fractionation of the Proteins. Dried biomass (10 g) was disrupted with liquid nitrogen, and the cells remaining undisrupted were treated by freezing–thawing. The protein fractions were extracted successively from the disrupted cells [6]. Fraction I was extracted with water. The disrupted cells were covered with water (300 ml in each case), and the mixture was shaken for 1 h and left in a refrigerator at 0-4°C for 18 h, and then centrifuged. The protein solution was decanted into a collecting flask and the deposit was again treated with water. Such extraction followed by centrifugation was repeated 5-6 times, until the reaction with the Folin reagent [6] had disappeared. The supernatants were collected in one flask.

After complete extraction of the water-soluble proteins, the biomass was transferred to a flask and covered with 300 ml of 1 N KCl to extract the proteins of fraction II, and the mixture was placed in a refrigerator for 15 h, after which it was shaken for 1 h and centrifuged. The supernatant was decanted into a collecting flask, and the deposit of biomass was treated again with KCl solution until the reaction for protein had disappeared. Fraction III was extracted with alcohol. The homogenate was treated five times (250 ml each time) with 80% ethyl alcohol. After each addition of alcohol the contents of the flask were shaken for 1 h and placed in the refrigerator for 18 h and were then centrifuged, and the solution of proteins was decanted into a collecting flask.

To isolate fraction IV, the biomass was treated with a 1 N solution of NaOH [7, 8]. Extraction with this solution was carried out 5 times in the same way as for the isolation of the other protein fractions. The preparations were dried in a vacuum

desiccator and a drying chest at 50-60°C, and were weighed. The percentage of each fraction was calculated by taking the combined weight of all the fractions as 100%.

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