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PROPERTIES OF FRACTIONS CONTAINING NITROGENOUS SUBSTANCES FROM *Saccharomyces cerevisiae* AND *Trichoderma harzianum*

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Nitrogenous substances from Saccharomyces cerevisiae *and* Trichoderma harzianum *were found in the greatest amount in the biomass protein of the water-soluble fraction from the maximum stationary growth phase during a comparative study of the fraction compositions. The total amino acids in biomass protein of* S. cerevisiae *was 28.3%, of which 12.6% were essential.*

Key words: protein, nitrogen, amino acids.

Microbial conversion of cellulose is a very important research area in biotechnology. The selection of strains of microorganisms, among which protein producers attract special interest, is a critical problem.

Microorganisms added during fermentation of silage from agricultural wastes typically have high biomass yields, reaching 125-150 g/L [1-4].

The protoplasmic proteins albumin and globulin are considered the most balanced and readily assimilated. The reserve proteins prolamin and glutelin are less balanced [5]. We investigated the fractional composition of nitrogenous substances from biomass protein of the yeast *Saccharomyces cerevisiae* and the microscopic fungus *Trichoderma harzianum* and the amino-acid composition of the water-soluble biomass protein fraction of *S. cerevisiae*.

Table 1 compares the fractional composition of nitrogenous substances from *S. cerevisiae* and *T. harzianum*. The latter contains more nitrogenous substances (66.38%) than *S. cerevisiae* in the water-soluble fraction. Thus, the water-soluble fraction of *T. harzianum* contains 29.22% non-proteinaceous N; *S. cerevisiae*, 25.40%. The lowest content in the studied cultures was found in the alcohol-soluble fraction, 4.35% for *T. harzianum* and 3.44% for *S. cerevisiae*.

Table 2 shows the distribution by fractions of non-proteinaceous N in *S. cerevisiae* biomass protein. The amount in the culture fractions depends on the growth phase. The least amount of non-proteinaceous N in all studied fractions of *S. cerevisiae* and *T. harzianum* was found in the exponential growth phase; the greatest, in the maximum stationary phase. Thus, the distribution by fractions of non-proteinaceous N in *S. cerevisiae* was: 38.40% in the water-soluble; 6.20%, salt-soluble; 4.30%, alcohol-soluble; 15.40%, base-soluble.

TABLE 1. Fractional Composition of Nitrogenous Substances from *S. cerevisiae* and *T. harzianum*, % of Total Nitrogenous Substances

Fractions	*S. cerevisiae*		*T. harzianum*	
	Proteinaceous N	Non-proteinaceous N	Proteinaceous N	Non-proteinaceous N
Water-soluble	3.02	25.40	5.42	29.22
Salt-soluble	2.16	3.70	3.67	10.32
Alcohol-soluble	0.25	3.44	0.38	4.35
Base-soluble	5.20	3.55	10.52	5.45
Total	11.63	36.09	16.93	49.36

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TABLE 2. Distribution of Non-proteinaceous (% of Total Non-proteinaceous N) and Ammonium N (% of Non-proteinaceous N-fraction) Content in *S. cerevisiae* and *T. harzianum* Biomass by Fractions

Fraction	*S. cerevisiae*			*T. harzianum*		
	Growth phase					
	exponential	slow growth	maximum stationary	exponential	slow growth	maximum stationary
	Distribution of non-proteinaceous N					
Water-soluble	31.20	35.40	38.40	36.2	41.40	44.40
Salt-soluble	4.02	5.40	6.20	4.32	5.40	8.70
Alcohol-soluble	2.11	3.12	4.30	3.54	4.50	6.80
Base-soluble	11.20	12.40	15.40	5.20	7.20	11.40
	Ammonium N content					
Water-soluble	42.0	55.0	62.0	38.0	41.0	48.0
Salt-soluble	38.0	51.0	54.0	27.0	45.0	39.0
Alcohol-soluble	0.40	1.70	2.10	0.60	1.70	1.80
Base-soluble	29.0	44.0	48.0	24.0	34.0	38.0

TABLE 3. Amino-acid Composition (%) of Protein Fraction of *S. cerevisiae* Mycelium

Amino acid	Protein fractions			
	albumin	globulin	prolamin	glutelin
Lys	2.32	1.68	1.24	0.16
His	1.94	1.38	1.16	1.36
Arg	1.76	1.42	1.18	1.32
Asp	2.74	1.94	0.32	0.42
Thr	1.68	1.48	0.94	0.48
Ser	0.52	0.44	0.36	0.96
Glu	4.74	3.14	2.08	2.21
Pro	0.34	0.18	0.22	0.68
Gly	1.28	0.98	0.48	1.08
Ala	2.34	1.84	1.22	1.33
Val	0.98	0.62	0.56	0.94
Cys+Met	1.78	1.32	1.12	1.68
Leu	1.86	0.56	0.54	0.42
Ile	1.04	0.64	0.14	0.44
Tyr+Phe	2.98	1.78	0.52	0.54
Σamino acid	28.30	18.40	13.08	14.02
Of these:				
essential	12.64	8.08	5.06	4.66
nonessential	15.66	10.32	8.02	9.36

The amount of non-proteinaceous N in the maximum stationary growth phase of *T. harzianum* was 44.40% in the water-soluble fraction; 6.80%, salt-soluble; 11.40%, base-soluble.

Table 2 also gives the content of ammonium N in biomass protein of *S. cerevisiae* and *T. harzianum.* It was found that the cultures differ substantially in content of ammonium N. Thus, it is greater in all studied fractions of *S. cerevisiae* than in the corresponding fractions of *T. harzianum.* The greatest amount of ammonium N in the studied cultures was found in the

water-soluble fraction of the maximum stationary phase of *S. cerevisiae* (62.0%) and *T. harzianum* (48.0%). It was slightly less in the salt-soluble fraction of both cultures. A low content of ammonium N was found in the alcohol-soluble fraction of the maximum stationary growth phase of *S. cerevisiae* (2.10%) and *T. harzianum* (1.80%).

A study of the amino-acid composition of the water-soluble fraction of *S. cerevisiae* biomass protein showed that the total amino-acids was 28.30%, of which 12.64% were essential (Table 3).

The predominant essential amino acids were Lys, Thr, Cys+Met, Leu, Ile, and Tyr+Phe.

The amount of nonessential amino acids was 15.66%. Of these, Asp, Glu, Ala, and His dominated.

Thus, the study of the composition of nitrogenous substances of *S. cerevisiae* and *T. harzianum* biomass protein found that the cultures have a high content of nonproteinaceous and ammonium N, the amounts of which depend on the growth phase.

The greatest amount of these nitrogenous substances are found in the biomass protein in the water-soluble fraction of the maximum stationary growth phase.

The total amino acids in the water-soluble fraction of *S. cerevisiae* biomass protein is 28.3%, of which 12.6% are essential.

EXPERIMENTAL

The yeast *S. cerevisiae* and microscopic fungus *T. harzianum* were isolated from fermented agricultural silage wastes. Cultures were grown by an immersion method in Czapek medium in which the carbon source was filter paper in order to produce biomass.

Nitrogenous substances from biomass of the studied microorganisms were fractionated successively at room temperature by water (water-soluble), NaCl solution (7%, salt-soluble), ethanol solution (70%, alcohol-soluble), and aqueous NaOH (0.2%, base-soluble) [6].

Protein from the extract was precipitated by TCA solution (2%). Nitrogen in the precipitate was determined by the Kjeldahl method [6].

The content of non-proteinaceous protein was determined after precipitation of the protein in the filtrate [7].

The amino-acid content in the water-soluble fraction of biomass protein was determined as usual [8]; the amount of amino acids, on an AAA-881 automated amino-acid analyzer.

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