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PROTEIN BIOMASS OF THE THERMOTOLERANT CELLULOLYTIC FUNGUS *Penicillium atrovenetum* MIXED WITH AN INSOLUBLE SUBSTRATE CONTAINING CELLULOSE

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The biosynthesis of protein biomass of the fungus Penicillium atrovenetum *on substrates containing cellulose was studied. It was found that protein formation depends on the substrate and its content in the culture medium. The largest amount of protein biomass (16.4%) as a carbon source was produced by cultivating fungus on corn stalks ground to a particle size of 90 μm. Investigation of the fractional composition of* P. atrovenetum *protein biomass showed that 36.2% of it is formed in the water-soluble fraction during the exponential growth phase. The total amino-acid content was greatest in the albumin fraction (26.40%).*

Keywords: thermotolerant fungus, protein, amino acids, fractions.

The utilization of a substrate containing cellulose depends on its physicochemical properties [1-3].

We studied the dependence of protein biomass accumulation of the thermotolerant fungus *Penicillium atrovenetum* on the particle size of a substrate containing cellulose in the nutrient medium during growth on a medium with fractions of ground wheat straw and corn stalks of particle sizes 90, 200, 300, and 400 μm.

It was found that the nature of the substrate has a significant effect on the formation of biomass and the protein content during growth of *P. atrovenetum* on these substrates. Thus, the greatest *P. atrovenetum* biomass and protein was observed during growth on medium with corn stalks as the natural carbon source (Table 1) because they contain more sugars and starches necessary for fungal growth than wheat straw.

The particle size of the studied substrates containing cellulose was just as important for biomass and protein formation in *P. atrovenetum* biomass.

As the size of the wheat-straw and corn-stalk fractions decreased from 400 to 90 μm, the percent utilization of substrate by the fungus increased and a larger amount of biomass with a higher protein content was formed.

A comparison of the fractional composition of *P. atrovenetum* protein biomass during its cultivation on the studied substrates showed that its content is higher for growth on ground corn stalks than in all fractions from nutrient medium with wheat straw. The protein content in biomass of the water-soluble fraction varied after cultivation for 120 h of *P. atrovenetum* on medium with ground corn stalks of particle size 90 μm (Table 2).

The protein content in the biomass during the maximal stationary growth phase was greatest in the base-soluble fraction, 24.1% of its total mass.

The results indicate that the water-soluble fraction of biomass protein decreases gradually as *P. atrovenetum* ages whereas the alcohol-soluble and base-soluble fractions increase. It was noted that the formation of protein biomass during the exponential growth phase is most vigorous in the water-soluble fraction.

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TABLE 1. Biochemical Characteristics of *P. atrovenetum* Cultivated on Medium Containing Cellulose

Characteristic	Particle-size of fractions, μm			
	90	200	300	400
	Wheat straw			
Protein content in mixture, %	18.7	14.3	12.1	10.6
Biomass, mg/g	234	202	182	148
Straw utilization, %	38.4	26.6	22.6	20.2
	Corn stalks			
Protein content in mixture, %	16.4	14.8	13.6	12.5
Biomass, mg/g	262	224	196.2	174.2
Corn-stalk utilization, %	39.4	29.4	26.6	23.2

TABLE 2. Fractional Composition of *P. atrovenetum* Protein Biomass Cultivated on Substrates Containing Cellulose

Fraction	Exponential phase			Slow-growth phase			Maximal stationary phase		
	Cultivation time, h								
	64	98	120	64	98	120	64	98	120
	Wheat straw								
Water-soluble	22.2	27.8	28.4	16.4	24.2	26.4	12.4	14.2	20.8
Salt-soluble	14.8	16.2	20.6	18.2	22.4	24.0	14.2	16.2	18.6
Alcohol-soluble	13.2	18.4	19.4	6.4	8.4	16.8	14.2	17.2	26.2
Base-soluble	10.2	10.4	13.6	6.2	7.4	14.6	13.0	15.2	21.4
	Corn stalks								
Water-soluble	20.4	25.5	36.2	14.7	20.4	22.3	9.7	12.2	19.4
Salt-soluble	12.2	14.4	19.7	16.8	23.4	28.4	10.2	11.4	14.2
Alcohol-soluble	11.7	14.0	15.4	7.5	7.2	18.5	13.8	14.6	21.4
Base-soluble	9.7	10.4	11.5	5.8	6.2	15.2	11.0	13.6	24.1

A study of the amino-acid content in *P. atrovenetum* protein biomass grown on medium with corn stalks (particle size 90 μm) showed (Table 3) that the amount of amino acids in the albumin fraction is 26.40% of the total content; in the globulin fraction, 21.00; in prolamine, 22.64; and in glutelin, 22.93. The essential amino acids in the albumin fraction were 11.33% of the total protein biomass; in globulin, 9.62; in prolamine, 9.08; in glutelin, 9.79. The amounts of nonessential amino acids in these fractions were 15.07, 11.38, 13.56, and 13.14% of the total protein biomass. The predominant essential amino acids were Thr (2.52%), Leu (1.34%), Tyr+Phe (1.76%), and Lys (1.32%); nonessential, Glu (3.72%), Asp (2.74%), Ala (2.64% of the total protein biomass).

Thus, the results showed that growing *P. atrovenetum* on medium with corn stalks (particle size 90 μm) can increase the nutritive value of the substrate containing cellulose and that the fungus *P. atrovenetum* is a promising culture for producing protein biomass from agricultural wastes.

TABLE 3. Amino-acid Composition (%) of *P. atrovenetum* Protein Biomass Fractions

Amino acid	Protein fractions			
	albumin	globulin	prolamine	gluteolin
Lys	1.07	0.75	1.44	1.65
His	0.54	1.75	1.17	1.42
Arg	0.97	1.37	1.38	1.45
Asp	2.74	1.55	1.72	2.24
Thr	2.52	1.74	1.46	1.53
Ser	1.78	0.44	1.68	1.64
Glu	3.72	3.02	3.92	30.4
Pro	0.54	0.25	0.15	0.26
Gly	2.14	1.26	1.54	1.74
Ala	2.64	1.74	2.00	1.35
Val	1.44	1.01	1.02	1.58
Cys+Met	1.64	1.36	1.17	1.38
Leu	1.34	0.78	0.44	1.26
Ile	1.55	1.96	1.21	0.74
Tyr+Phe	1.76	2.02	2.34	1.65
Σamino acids	26.40	21.00	22.64	22.93
Of these:				
essential	11.33	9.62	9.03	9.79
nonessential	15.07	11.38	13.56	13.14

EXPERIMENTAL

We used four fractions of wheat straw and corn stalks that were disintegrated and passed through sieves of pore diameters 90, 200, 300, and 400 μm. Substrate (1 g) of each fraction was sterilized for 1 h at 1 atm in rocker flasks in tapwater acidified to pH 5.0. The volume of medium was set so that it contained 2% substrate.

An equal volume of Czapek medium of doubled concentration was added after sterilization in the flask. Then the flasks with substrate were shaken for 1 d on a rocker at 40°C, i.e., under the same conditions at which the fungus was grown. Next the substrate was absorbed onto a paper filter and dried to constant mass. The solid on the filter was washed with an equal volume of medium that was used to grow the thermotolerant fungus *P. atrovenetum.*

Biomass of *P. atrovenetum* was produced by cultivation for 120 h at 40°C in rocker flasks filled with liquid nutritive medium (100 mL) for fungus cultivation containing glucose (0.5%) and potassium phosphate buffer (pH 6.5, 0.1 M). The fungus was grown on rockers turning at 220 rpm. The resulting biomass was collected by filtration, washed, and dried. Protein was determined by the Romanov method [4]; protein fractionation, by the literature method [5]; amino-acid composition of protein fractions, by the usual methods [6, 7].

The amount of amino acids was calculated on an AAA-881 automated amino-acid analyzer.

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