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PRODUCTION OF EXTRACELLULAR ENZYMES BY LOW-PROTEASE MUTANTS OF *TRICHODERMA REESEI*

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Abstract. Cultivation has been performed of isolated earlier low-protease mutants and parental strain in the presence of novel inducers of cellulases production – lactulose and lactobionic acid mixed with lactose in the ratio 1:1. Nine among ten tested during batch cultivation low-protease mutants exhibited lower ability for the production of proteases than parental strain M-7. Higher enzymatic activities have been estimated (cellulolytic, xylanolytic and beta – galaktosidase) of culture filtrates during cultivation in the presence of mixtures of lactobionic acid and lactose in comparison to lactulose and lactose. Tested, during continuous cultivation, low-protease mutant *T. reesei* Mp5 exhibited high resistance for the temperature shifting. FPU activities of culture filtrates were stable in the high range of temperature of cultivation (26-34°C). In addition current knowledge about correlation between protease and other enzymes of culture fluids of selected filamentous fungi has been reviewed. The ways of preventing homologous and heterologous protein produced by fungi and bacteria from enzymatic cleavage by proteases have been also described. This work is an introduction for future studies on correlation between proteases and other enzymes in culture filtrates of *Trichoderma reesei*.

Key words: *Trichoderma reesei*, low-protease mutants, deficient-protease strain, cellulases, lactose, lactulose, lactobionic acid

INTRODUCTION

One of the favourite producers of enzyme hydrolysing ligninocellulose, which has found industrial application, is *Trichoderma reesei* QM 6a and its mutants. Apart cellulases and xylanases other enzymes, which occur in the culture filtrates (proteases, chitinases and beta 1,3-glucanases), are also involved in the hydrolysis of plant biomass and play other significant roles. In recent years attention is focused on proteases, which can cause postsecretional modification of cellulases and affect their activities and stability in culture filtrates during cultivation. Proteases may also be involved with release of intracellular enzymes, e.g., beta-glucosidase and may play a role in the production of multiple forms of cellulases components. Same authors have reported the isolation of truncated form of cellulases enzymes which can be caused by proteolytic modification by

proteases present in the culture fluids. These enzymes can be also involved in unwanted degradation of heterologous protein [Calmels et al. 1991]. Several studies failed to demonstrate proteolysis of cellulases during cultivation of *Trichoderma* reesei performed at steady pH of cultivation medium [Dunne 1992, Labudova and Farkas 1983, Kamel and Kubicek 1985] Biosynthesis of cellulases in most cellulolytic fungi is connected with the presence of inducer in cultivation medium. Cellulose has usually been considered to be the best inducer for producing a well-balanced cellulase system but this substrate is expensive and also causes operational and rheological problems during cultivation in bioreactor. Lower cost of production and greater control of fermentation allows the use of soluble carbon source such as lactose or cellobiose. New more effective inducers of cellulases production are still being searched. Bruchman et al. [1978] reported on stimulation of cellulases formation in cellulose degrading *Trichoderma reesei* by cellobionolactone a component found among cellulose degradation products in culture filtrates of *Trichoderma reesei*. Janas et al. [2002] described inductive effect of other aldonic acid - lactobionic acid and disaccharide lactulose on the production of cellulases by mutant-strain *Trichoderma reesei* M-7.

The aim of work was to examine the influence of lactobionic acid and lactulose on the production of extracellular enzymes by isolated earlier [Janas et. al. 2003] low protease mutants and parental strain M-7 of *Trichoderma reesei* during batch and continuous cultivation.

MATERIALS AND METHODS

Following mutant-strains are used in the studies:

1. Mutant-strain *Triichoderma reesei* M-7 (parental strain) from Collection of Microorganisms Department of Food Technology and Storage, Agricultural University in Lublin which exhibited high ability for the production of extracellular enzymes 9cellulases, xylanases and autolytic enzymes) during cultivation on liquid mineral medium. Strain has been isolated after mutagenisation by uv-radiation of *Trichoderma reesei* QM 9414.

2. Low-protease mutants of *Trichoderma reesei* M-7.

Induction of low -protease mutants has been performed by uv-irridiation of suspension of conidia of mutant M-7 and by 0.01% solution of NTG. Low-protease mutants have been isolated during cultivation on liquid and solid medium with 1% addition of gelatine. Among 960 obtained mutants 10 have been tested during batch and continuous cultivation in the presence of novel inducers of cellulase production – lactobionic acid and lactulose. Batch cultivation of mutants were performed in 500 cm^3 Erlenmeyer flasks containing 100 cm^3 of medium according to Mandels and Weber [1969] fortified with 1% mixtures of lactose with lactobionic acid and lactulose at the ratio: 1:1. This medium was sterilised by autoclaving at 0.05 MPa for 30 min, cooled and inoculated with 2% (v/v) of pre-grown cells. Cultivation was performed on a rotary shaker (220 rpm) at 26°C.

Fed-batch (pre-cultivation) and continuous cultivation was run in a 5 dm^3 capacity bio-reactor Bioflo III- New Brunswick (USA) on the mineral medium composition described by Mandels and Weber [1969], fortified 1% mixture of lactose with lactobionic acid and lactulose. This medium was sterilised (at 0.05 MPa for 30 min), cooled

and inoculated with previously prepared 200 cm^3 inoculum. Cultivation was performed at a constant pH value of 4 adjusted with 5% NH_4OH and 2.5% H_3PO_4 at temperature 26°C, 34°C and 38°C. Continuous cultivation was carried out with a dilution rate of 0.024 h^{-1} (D = 0.024 h^{-1}). After cultivation, cells were separated by centrifugation (6000 x g, 10 min) at 4°C and the obtained supernatant was used for enzyme analyses. The activity of cellulases (FPU) of culture filtrates was assayed according to the method described by Mandels et al. [1976] and expressed in International Unit (IU), using Whatman No. 1 filter paper. The activity of xylanases of the culture filtrates was assayed accordingly: 0.9 cm^3 of 5.5% birchwood xylan solution in 0.1 M sodium acetate buffer (pH = 4.8) was used and 0.1 cm^3 of an appropriate enzyme dilution of the culture filtrate was added. Incubation was performed for 30 min at a temperature of 50°C. The reducing sugars released were measured with DNS method [Miller 1959]. Activities were expressed in μmol/cm^3 x min. The activity of protease of culture filtrates was assayed according to the azocasein method described by Lovrien et al. [1985]. One unit of proteolytic activity was expressed as increased in absorbance of reaction mixture at 366 nm in minutes per 1 cm^3. Beta-galactosidase activity was assayed according to the method described by Colowick and Kaplan [1955], using 0,1 M sodium acetate buffer (pH = 4.8) instead of 0.2 M phosphate buffer (pH = 7.25). One unit of enzyme activity was defined as increase in absorbance of a reaction mixture per 1 minute in 1 cm^3 of the culture filtrate.

RESULTS AND DISCUSSION

Activities of enzymes estimated in culture filtrates of almost all low-protease mutants were lower than parental strain. Only two mutants among ten cultivated on mixture of lactose with lactulose exhibited 7 and 20% higher activities than strain M-7. Filtrates of the remaining 8 mutants had from 10 to 37% higher non-specific cellulolytic activities FPU (Table 1). FPU activity of culture fluids of mutant marked Mp5 obtained during cultivation in presence of mixture of lactose with lactobinic acid as a source of carbon was comparable to control strain M-7. Proteolytic activities were similar after cultivation on two combinations of medium with lactobionic acid and lactulose and were lower than parental strain (Table 2). An exemption was mutant Mp8, which exhibited over 60% higher activity of culture, filtrate then *T. reesei* M-7. Same of low-protease mutants produced proteases with even 30 – times lower activities of culture fluids then M-7. The medium composed of lactose and lactobionic acid had better unductive effect on the production of xylanases then that of lactose and lactulose (Table 3). Because of the assumable function in induction process of cellulases of *T. reesei* beta-galactosidase was the next studied enzyme. Activities of culture filtrates of this enzyme obtained after cultivation on mixture of lactose with lactulose were many times lower than in presence of lactose and lactobionic acid (Table 4). Low-protease mutant Mp5 which characterized by high activity of cellulases and xylanases of culture filtrates has been chosen for the next stage of investigations during batch and continuous fermentor cultivation. Cultivation have been performed on mixture of lactose and lactobionic acid at pH = 4 in temperatures 26.34 and 38°C. In the first part of fermentor cultivation – batch cultivation all estimated activities were lower than during performed in Erlenmeyer flasks. Maximal activities of cellulases, xylanases and beta-galactosidase have

been achieved at 18 days of continuous cultivation at 26°C with simultaneous protelytic activities 1,1 $U \cdot cm^{-3} \cdot 10^3$ (Table 5). The most interesting is, that tested during continuous cultivation low-protease mutant exhibited high resistance to the temperature shifting. Non-specific cellulolytic activities were stable in high range of temperature of cultivation (26-34°C) whereas proteolytic activities of culture filtrates increased to 2.5-2.7 $U \cdot cm^{-3} \cdot 10^3$. Decreased cellulytic activities has been observed of culture fluids and simultaneous increase proteolytic activities in the highest temperature of continuous cultivation (38ºC).

Akiba et al. [1995] have purified endo beta-1,4-glucanase from a culture filtrate of *Aspergillus niger* IFO031125. This enzyme was very stable thermally and no loss activity was observed after incubation at 60°C for 2 hours. Endo-glucanase had also very strong protease resistance and exhibited stable activity during incubation with protease such as savinase at 40ºC even for two weeks. The results of the earlier studies, as it was mentioned in the introduction to this work, failed to demonstrate the effect of proteases on cellulases in culture fluids during cultivation of *Trichoderma reesei* performed at steady pH. It is probably connected with properties of proteases of *Trichoderma reesei* which resemble pepstatin-insensitive, N-chlorosuccinimide aspartate proteases exhibits the highest activities in acidic pH [Haab et al. 1990]. Difficulties in performing studies on correlation between these two groups of enzymes are connected with low concentration of protein of proteases in culture fluids and strong bounding to cellulases and other enzymes. Various workers observed multiple forms of cellulases in culture filtrates of *T. ressei*, but their origin was not satisfactory explained [Kamel and Kubicek 1985]. By using monoclonal, domain specific antibodies against cellobiohydrolase I and II group of Kubicek [Hagspiel et al. 1989] demonstrated presence of proteolytic degradation product of CBH I and CBH II. CBH I was modifying from carboxy-terminal AB region whereas CBH II was attacked from amino – ABB and carboxy – terminal.

Proteases of *Trichoderma reesei* and other filamentous fungi can also play positive role in secretion of extracellular proteins. It has been stated that cell extracts of this fungus exhibited dibasic endopeptidase activity similar to yeast Kex 2 protease. Activity of this enzyme was strongly inhibited by pAPMSF so this substrate was used to study the role of dibasic endopeptidase on the secretion of protein by *T. reesei*. Secretion of xylanase II and I was strongly inhibited by pAPMSF. Secretion of cellobiohydrolases II was slightly inhibited by pAPMSF but in contrast CBHI was stimulated by pAPMSF and simultaneous decrease in the concentration of intracellular enzyme was detected [Goller et al. 1998].

Studies on correlation between proteases and other enzymes in culture fluids have been performed also in other microorganisms (filamentous fungi and bacteria). Fiedurek et al. 1985 after mutagenisatian by uv radiation and NTG of parental strain of *Asprgillus niger* C obtained 6 mutants, 5 of which were characterized by lower or lack proteolytic activity. Glucoamylase activity and protein synthesis in the mutants examined were proportional to their proteolytic activity. Electrophoretic analysis showed that protease reduced mutants had only one or two glucoamylase fraction of four occurring in the parent strain. It is suggested that proteases can be responsible for generating isoenzymatic forms of glucoamylase. To explain the role of vacular pepsastin-sensitive protease in *Neurospora crassa*, the protease-deficient mutant-strains were generated by the gene Riping procedure [Vasquez-Laslop et al. 1996]. Obtained mutants had elevated levels of vacuolar proteinase B and carboxypeptidase activities.

The expression and action of proteases in high levels often limit production of homologous and heterologous protein production by filamentous fungi and bacteria. Different strategies can be developed to solve these problems. Papagianni and Moo-Young [2002] investigated the effect of fungal morphology on protease secretion by wild-type *Aspergillus niger* strain producing homologous glucoamylase. Changes of level and quality of inoculum manipulated morphology of the fungus. Growth of mycelium in the large pellets was connected with lower protease activities and increase glucoamylase activities. Reduction of protease secretion has been also observed with immobilized cultures of the *Aspergillus niger* as reported in another work [Liu et al. 1998]. Lehmbeck [1997] has inactivated an alkaline protease genes by a deletion, insertion or substitution or by antisense expression in a new fungus host e.g. *Aspergillus oryzae, Aspergillus niger, Aspergillus awamori, Aspergillus phoenicis, Aspergillus nidulans, Trichoderma reesei, Trichoderma harzianum, Humicola insolens, Candida sp, Fusasarium solani* and another. The host may be used to secrete many recombinant proteins for example: alpha and beta amylases, cellulase, endo-1,3-beta-D-glucanase, endo-1,4-beta-D-xylanase, alpha-galactosidase, cellobiohydrolase, polygalacturonase. Bacterium *Escherichia coli* is also a good host for the expression of recombinant proteins. A number of proteases is present in different cellular fraction of *E. coli*. (cytoplasm, periplasm and inner and outer membrane). Endoproteases can play very important roles. For example are responsible for degradation of misfolded or abnormal proteins in periplasmatic space of bacteria. These enzymes are also involved in the degradation of many recombinant proteins synthesized in *E. coli* (Protease OmT). Jiang et al. [2002] replaced OmT gene with chloramphenicol resistance gene what was confirmed by Southern blot analysis. Recombinants obtained in this way exhibited an increased stability of in vitro biosynthesis of protein of phospholipase D.

CONCLUSIONS

1. Nine among ten tested during batch cultivation low-protease mutants exhibited lower ability for the production of proteases than parental strain M-7 (Table 2).
2. Enzymatic activities has been estimated higher (cellulolytic and xylanolytic) of culture filtrates during cultivation in the presence of 0.5% lactobionic acid and 0.5% lactose in comparison to 0.5% lactulose and 0.5% lactose as a source of carbon (Table 1, 3).
3. Tested during continuous cultivation low-protease mutant Trichoderma reesei 18/Mp5 exhibited high resistance to the temperature shifting. Non-specific cellulolytic activities of culture filtrates were stable in high range of temperature of cultivation (Table 5).
4. This work is an introduction to future studies on correlation between proteases and other enzymes in culture filtrates of *Trichoderma reesei*.

Table 1. Cellulolytic activities of culture filtrates obtained during batch cultivation of low-protease mutant and parental strain M-7
Tabela 1. Aktywność celulolityczna filtratów pohodowlanych otrzymanych podczas hodowli okresowych niskoproteazowych mutantów i szczepu rodzicielskiego M-7

Source of carbon in cultivation medium Źródło węgla w podłożu hodowlanym	Time of cultivation days Czas hodowli dni	Cellulolytic activities FPU, $\mu M \cdot cm^{-3} \cdot min^{-1}$ Aktywność celulolityczna, $\mu M \cdot cm^{-3} \cdot min^{-1}$										
		18/Mp1	18/Mp2	18/Mp3	18/Mp4	18/Mp5	18/Mp6	18/Mp7	18/Mp8	18/Mp9	18/Mp10	M-7
0.5% lactose + 0.5% lactulose 0,5% laktoza + 0,5% laktuloza	2	–	–	–	–	–	–	–	–	–	–	0.231
	4	0.254	0.205	0.222	0.201	0.162	0.213	0.208	0.336	0.247	0.259	0.238
	6	0.284	0.211	0.236	0.236	0.208	0.259	0.247	0.379	0.277	0.284	0.303
	8	0.358	0.250	0.275	0.259	0.286	0.273	0.259	0.340	0.307	0.307	0.335
	10	0.307	0.183	0.264	0.264	0.312	0.240	0.286	0.347	0.317	0.384	0.373
	12	0.425	0.250	0.303	0.273	0.323	0.256	0.287	0.388	0.326	0.481	0.402
0.5% lactose + 0.5% lactobionic acid 0,5% laktoza + 0,5 kwas laktobiono-wy	2	0.245	0.181	0.234	0.267	0.345	–	0.278	–		0.192	0.347
	4	0.416	0.243	0.401	0.312	0.370	0.300	0.326	0.314	0.397	0.382	0.392
	6	0.485	0.328	0.428	0.347	0.482	0.547	0.409	0.397	0.416	0.458	0.514
	8	0.508	0.358	0.463	0.416	0.555	0.601	0.405	0.367	0.474	0.578	0.593
	10	0.555	0.443	0.555	0.382	0.539	0.597	0.416	0.393	0.493	0.634	0.630
	12	0.463	0.416	0.520	0.347	0.682	0.589	0.450	0.393	0.589	0.508	0.670

Table 2. Proteolytic activities of culture filtrates obtained during batch cultivation of low-protease mutant and parental strain M-7
Tabela 2. Aktywność proteolityczna filtratów pohodowlanych otrzymanych podczas hodowli okresowych niskoproteazowych mutantów i szczepu rodzicielskiego M-7

Source of carbon in cultivation medium Źródło węgla w podłożu hodowlanym	Time of cultivation days Czas hodowli dni	Proteolytic activities, $U \cdot cm^{-3} \cdot 10^3$ Aktywności proteolityczne, $U \cdot cm^{-3} \cdot 10^3$										
		18/Mp1	18/Mp2	18/Mp3	18/Mp4	18/Mp5	18/Mp6	18/Mp7	18/Mp8	18/Mp9	18/Mp10	M-7
0.5% lactose + 0.5% lactulose 0,5% laktoza + 0,5% laktuloza	2	1.5	1.5	1.4	2.3	0.3	0.8	2.1	0.3	0.9	2.2	2.8
	4	2.4	1.7	2.3	4.2	0.9	1.2	3.6	0.9	1.7	3.7	5.9
	6	3.6	2.8	3.2	2.9	1.3	3.0	3.9	1.7	2.2	1.1	6.7
	8	6.0	3.4	3.0	6.2	1.9	3.4	4.3	1.4	2.9	1.6	7.9
	10	6.3	3.7	3.4	6.1	3.2	3.2	4.4	5.1	3.3	2.1	8.2
	12	5.8	6.5	4.0	5.8	2.7	3.4	5.4	2.4	3.8	2.9	6.1
0.5% lactose + 0.5% lactobionic acid 0,5% laktoza + 0,5 kwas laktobiono-wy	2	2.1	1.7	1.8	3.9	0.9	4.7	2.1	3.7	2.4	1.2	3.4
	4	2.7	2.3	2.3	4.5	1.4	5.4	2.0	4.3	3.6	1.8	5.7
	6	3.3	3.0	4.8	4.4	2.0	5.4	4.6	6.7	4.8	2.5	8.1
	8	5.9	3.6	5.3	5.5	3.9	6.6	4.8	13.4	5.3	3.9	9.5
	10	5.7	4.7	6.0	4.5	3.4	4.7	5.0	13.0	4.9	5.3	4.9
	12	5.5	3.4	5.4	5.0	3.7	5.5	4.3	16.0	5.1	6.5	7.2

Table 3. Xylanolytic activities of culture filtrates obtained during batch cultivation of low-protease mutant and parental strain M-7
Tabela 3. Aktywność ksylanolityczna filtratów pohodowlanych otrzymanych podczas hodowli okresowych niskoproteazowych mutantów i szczepu rodzicielskiego M-7

Source of carbon in cultivation medium Źródło węgla w podłożu hodowlanym	Time of cultivation days Czas hodowli dni	Xylanolytic activities, $\mu M \cdot cm^{-3} \cdot min^{-1}$ Aktywności ksylanolityczne, $\mu M \cdot cm^{-3} \cdot min^{-1}$										
		18/Mp1	18/Mp2	18/Mp3	18/Mp4	18/Mp5	18/Mp6	18/Mp7	18/Mp8	18/Mp9	18/Mp10	M-7
0.5% lactose + 0.5% lactulose 0,5% laktoza + 0,5% laktuloza	2	–	–	0.67	2.34	2.67	–	–	–		–	11.96
	4	1.56	2.74	4.37	7.52	4.43	1.58	1.11	0.55	1.68	0.40	13.92
	6	1.62	2.40	5.94	10.01	5.34	2.37	1.98	1.68	2.13	1.82	13.21
	8	2.04	2.92	2.34	7.94	7.53	5.53	7.84	2.98	3.76	4.62	18.12
	10	1.50	3.13	3.76	5.39	6.24	4.31	6.76	9.50	2.50	4.68	18.41
	12	1.05	3.66	4.35	3.60	3.80	4.58	4.91	2.93	2.33	4.74	5.02
0.5% lactose + 0.5% lactobionic acid 0,5% laktoza + 0,5 kwas laktobiono-wy	2	–	–	0.79	0.89	4.57	3.23	2.34	3.97	0.58	1.78	12.29
	4	2.37	2.20	3.84	4.20	7.40	6.47	3.74	6.12	2.19	2.47	13.66
	6	1.96	3.80	3.82	7.53	8.04	10.17	5.74	4.43	2.74	2.08	19.65
	8	2.08	1.24	9.13	13.88	10.25	14.68	9.53	10.25	7.84	6.19	20.15
	10	1.60	1.06	10.05	14.76	9.62	9.56	7.64	4.27	3.58	9.10	20.20
	12	1.05	0.45	5.51	16.05	7.02	5.94	7.02	3.43	1.96	10.96	17.48

Table 4. Beta-galactosidase activities of culture filtrates obtained during batch cultivation of low-protease mutant and parental strain M-7
Tabela 4. Aktywność beta-galaktozydazy filtratów pohodowlanych otrzymanych podczas hodowli okresowych niskoproteazowych mutantów i szczepu rodzicielskiego M-7

Source of carbon in cultivation medium Źródło węgla w podłożu hodowlanym	Time of cultivation days Czas hodowli dni	Beta-galactosidase activities, $U \cdot cm^{-3} \cdot 10^3$ Aktywności beta-galaktozydazy, $U \cdot cm^{-3} \cdot 10^3$										
		18/Mp1	18/Mp2	18/Mp3	18/Mp4	18/Mp5	18/Mp6	18/Mp7	18/Mp8	18/Mp9	18/Mp10	M-7
0.5% lactose + 0.5% lactulose 0,5% laktoza + 0,5% laktuloza	2	0	0	0	0	0	0	0	0	0	0	0.30
	4	0	0	0	0	0	0	0	0	0	0	0.70
	6	0.40	0.60	0.70	0.44	0	0	0	0.40	0	0	0.91
	8	0.28	1.20	1.13	1.06	0.93	0.86	0.40	041	0.20	0.13	1.45
	10	0.80	0.44	0.53	0.96	0.66	1.06	0.33	0.13	0.22	0.30	2.80
	12	0.53	0	0	0	0.60	0	0.46	0	0	0.40	1.40
0.5% lactose + 0.5% lactobionic acid 0,5% laktoza + 0,5 kwas laktobiono-wy	2	0	0	0	0	0	0	0	0	0	0	0.90
	4	0	0	2.20	0.33	0.80	3.60	0.73	0	2.23	0.73	10.90
	6	2.20	2.10	24.60	2.60	3.40	8.44	53.60	4.40	35.80	7.10	16.20
	8	23.8	12.40	26.00	8.60	32.80	48.40	41.40	13.80	41.70	51.20	26.90
	10	10.8	26.60	43.20	13.50	33.70	46.20	45.70	6.60	39.70	27.00	59.90
	12	45.0	27.20	16.00	16.00	65.90	47.90	56.80	23.20	55.90	1.60	58.50

Table 5. Characteristic of culture filtrates obtained during batch and continuous-bioreactor cultivation of low-protease mutant *Trichoderma reesei* 18/Mp5 in different temperatures
Tabela 5. Charakterystyka filtratów pohodowlanych otrzymanych podczas okresowych i ciągłych hodowli bioreaktorowych przez niskoproteazowego mutanta *Trichoderma reesei* 18/Mp5 w różnych temperaturach

Kind of cultivation Rodzaj hodowli	Temperature Temperatura °C	Time of cultivation days Czas hodowli dni	Enzymatic activities of culture filtrates Aktywność enzymatyczna filtratw pohodowlanych			
			cellulase FPU celuloza $\mu M \cdot cm^{-3} \cdot min^{-1}$	xylanase ksyloza $\mu M \cdot cm^{-3} \cdot min^{-1}$	protease proteoza $U \cdot cm^{-3} \cdot 10^3$	beta--galactosidase beta--galaktozydoza $U \cdot cm^{-3} \cdot 10^3$
Batch Okresowa	26	2	0.12	2.78	0.82	0.6
		4	0.31	7.00	1.21	3.3
		6	0.34	8.70	0.79	3.1
		7	0.41	9.64	0.75	3.4
		8	0.37	11.76	0.88	7.0
Continuos Ciągła	26	9	0.355	4.96	0.61	12.0
		10	0.36	4.17	0.25	14.1
		11	0.375	5.17	0.43	19.6
		12	0.34	3.94	0.16	22.8
		13	0.38	12.50	0.24	26.4
		14	0.33	11.50	0.45	26.5
		15	0.325	13.35	0.68	29.1
		16	0.37	18.65	1.05	30.8
		17	0.42	16.43	1.18	33.8
		18	0.43	19.01	1.1	34.4
		19	0.39	15.68	0.65	28.4
		20	0.41	13.09	0.69	23.4
	34	21	0.42	10.58	2.04	23.1
		22	0.425	8.60	2.52	23.0
		23	0.445	7.90	2.53	22.6
		24	0.44	4.06	2.50	21.0
		25	0.41	5.62	2.54	20.0
	38	26	0.40	7.70	2.72	17.7
		27	0.34	8.09	2.76	14.0
		28	0.28	11.62	3.31	13.5
		29	0.25	9.62	2.85	12.0
		30	0.235	7.31	2.69	10.2
		31	0.21	8.27	3.50	10.0
		32	0.205	6.94	2.31	9.0

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PRODUKCJA ENZYMÓW ZEWNĄTRZKOMÓRKOWYCH PRZEZ NISKOPROTEAZOWE MUTANTY *TRICHODERMA REESEI*

Streszczenie. Prowadzono hodowle wyizolowanych wcześniej niskoproteazowych mutantów i szczepu wyjściowego w obecności nowych induktorów produkcji celulaz – laktu-

lozy i kwasu laktobionowego zmieszanych z laktozą w stosunku 1:1. Dziewięć spośród dziesięciu testowanych podczas hodowli okresowych niskoproteazowych mutantów wykazywało niższe uzdolnienia do produkcjii proteaz niż szczep rodzicielski M-7. Wartości aktywności enzymatycznej (celulolityczne, ksylanolityczne i beta-galaktozydazy) filtratów pohodowlanych oznaczane podczas hodowli w obecności mieszanin kwasu laktobionowego i laktozy były wyższe w porównaniu z laktulozą i laktozą. Testowany podczas hodowli ciągłej niskoproteazowy mutant Mp5 charakteryzował się dużą odpornością na zmiany temperatury. Aktywności FPU filtratów pohodowlanych były stabilne w szerokim zakresie temperatury hodowli (26-34°C). Dodatkowo w pracy przedstawiono aktualny stan wiedzy na temat zależności między proteazami i innymi enzymami cieczy pohodowlanych u wybranych grzybów strzępkowych. Opisano również sposoby zabezpieczenia białek heterologicznych i homologicznych, produkowanych przez grzyby i bakterie przed enzymatycznym rozkładem przez proteazy.

Słowa kluczowe: *Trichoderma reesei,* niskoproteazowe mutanty, celulazy, laktoza, laktuloza, kwas laktobionowy

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