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Synthesis of Hydrolitic Enzymes by *Xylotrophic macromycetes*

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Abstract

As a result of various strains of basidiomycetes screenings was revealed that fungi *Bjerkandera adusta* P-15, *Pleurotus ostreatus* F-18 and *Schizophyllium commune* N-17 have strong hydrolitic enzyme system. It was shaved that optimal conditions for these cultures which have been chosen as active producers are following: carbon source – used tea; nitrogen source – NH_4NO_3 (0,036-0,040% by nitrogen); temperature – 28-30^{0}C, pH – 5.2-5.7. In such conditions maximal synthesis time of hydrolitic enzymes for these fungi are 80-144 hours.

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Xylotrophic macromycetes, Hydrolitic enzyme system, Synthes, Carbon and nitrogen source.

Introduction

Biological approach to utilization of various plant wastes including 2 their method (microbiologic and enzymatic conversion) as source of main environmental pollution are supposed to be the problems of significant practical importance(5, 14). The number of investigations on this side is very much, but necessary results for high efficiency of processes especially enzymatic conversion are not enough because necessary quality enzyme preparations are absence. Powerful enzyme system and possibility to split the different polymers of complex composition by these enzymes (15) shows the actuality of basidiomycetes studies especially their wood-attacking representatives.

Therefore the main aim of present work was hydrolytic enzyme synthesis study of fungus belonging to various basidiomycetes and study of some peculiarities of synthesis process.

Materials and Methods

The cultures of fungi used during investigations were isolated from various regions of Azerbaijan (Lenkoran, Agdjabedi, Absheron, Terter, Ismailli, Guba). Isolation, storage and cultivation of fungi cultures were carried out by standard methods (8).

Hydrolytıc enzymes (amılase, cellulase, xylanase, protease, pectinase and lipase) activity was determined by known methods (2-3, 9, 11, 13) both in culture broth and in biomass (6). The nutrient medium used under determination of culture enzyme activity contains source of carbon - 10 g/l; peptone – 3 g/l; NH_4NO_3 – 1,5 g/l; $MgSO_4$ – 0,5 g/l; NaCl – 0,5 g/l and KH_2PO_4– 0,4 g/l.

Determination of protein quantity was made by known spectrophotometric method (7).

All the experiments were performed at least 4-6 times, the results were statistically analyzed and used only honest data (10).

Results and Discussions

The results on first phase analysis of active producers of hydrolase (Table 1) showed that all studied fungi possess enzymes activity of hydrolytic type and differ from each other in activity levels. For example, amylolytic activity of *T. versicolor* R-24 fungus is 1,2-3,0 times higher in comparison with other strains, but xylanolytic activity of ones is 1,2 time lower, than *P. ostreatus* F-118 strain. Such differences also observed during comparison of other strains, and therefore can be described as strain differences. Despite noted strain differences *P. ostreatus* F-118, *B. adusta* P-15 and *Sch. commune* M-17 have by comparison well-balanced hydrolytic enzyme system. It may be said that all enzymes catalyzed hydrolyze of main components (cellulose, hemycellulose, starch, pectine *etc.*) characterizes high activity (Table 1).

These components enter into composition of plant wastes. Therefore these strains were chosen for active producers of following investigations.

Investigations on influence of carbon source upon hydrolase synthesis of *P. ostreatus* F-118 fungus has showed, that all used sources impact on enzyme activity in some way or other (Table 2). Monosaccharides (glucose, xylose) reduce extracellular and intracellular activity of cellulase, xylanase and amylase to 1,25-1,67 times in other words they act as catabolic repressor. But monosaccharides are not able to act on pectinase, protease and lipase in such way. It may be said, that under addition of disaccharides as carbon sources to medium was observed uptrend of enzyme activity (Table 2).

It should be noted that a similar situation was observed with fungi *B. adusta* P-15 and *Sch. commune* M-17, although there were minor differences in the quantitative nature.

The uptrend of enzyme activity, especially cellulase, xylanase and amylase manifested itself clearly carbon source complicated. In some cases activity increases in 22,7 times (Table 2), that is typically for inductive enzymes (14). In general, one of the established facts is increase of concrete enzyme activity mainly during use of enzyme substratum as carbon source that is enzymes demonstrate peculiarities of substratum specificity. But it is not so much characteristically for amylase. Thus, the effect is high by addition of tea and filter paper as carbon sources to medium, whereas starch is substratum for catalyze of the amylase hydrolysis.

Table.1 Hydrolytic activity of basidiomycetes

N	Fungi (the number of strains)	Cellulasa	Xylanasa	Pektinasa	Proteasa	Amylasa
1	*Bjerkandera adusta* (3)	0,54-0,92	80-109	1,9-3,5	2,3-5,6	2,3-4,7
2	*Bjerkandera fumosa* (2)	0,21-0,31	84-93	1,0-1,7	4,9-5,9	1,7-3,5
3	*Cerrena unicolor* (3)	0,50-0,85	73-102	2,1-4,3	3,1-5,4	2,2-3,8
4	*Fomes fomentarius* (3)	0,22-0,37	60-69	1,2-2,1	5,1-6,2	1,7-2,7
5	*Fomitopsis pinicola* (3)	0,40-0,52	58-65	0,7-1,6	4,5-5,3	1,7-2,8
6	*Ganoderma aplanatum* (5)	0,25-0,34	67-78	1,7-2,2	4,7-5,6	1,9-3,2
7	*Ganoderma lucidum* (3)	0,36-0,64	65-87	2,3-3,9	2,4-5,0	1,6-3,5
8	*Hircshioporus pergamenus* (3)	0,30-0,58	74-90	2,1-4,1	1,2-5,1	2,4-3,2
9	*Phellinus igniarus* (3)	0,30-0,43	56-72	1,9-3,5	1,4-3,2	1,2-3,1
10	*Pleurotus ostreatus* (3)	0,55-0,97	70-120	5,7-8,5	3,7-6,6	2,3-4,1
11	*Schizophyllium commune* (3)	0,52-0,99	76-124	7,0-8,2	4,0-6,8	2,6-4,5
12	*Trametes hirsute* (3)	0,52-0,72	67-89	5,2-7,1	3,6-4,5	2,6-4,0
13	*T.versicolor* (3)	0,50-07,6	65-83	5,4-7,4	3,2-4,7	3,2-5,1

Table.2 Effect of carbon sources on hydrolase activity of *P. ostreatus* F-18 fungus

Carbon sources	Cellulasa		Xylanasa		Amylasa		Pektinasa		Proteasa	
	A*	B**	A	B	A	B	A	B	A	B
Glucose	0,5	0,3	58	43	1,0	0,7	8,6	6,0	9,8	5,7
Xylose	0,4	0,2	60	40	1,1	0,6	8,2	5,9	9,9	5,4
Sellobiose	1,4	0,6	70	58	2,6	1,4	9,2	6,3	9,5	6,0
Sacharosa	0,9	0,7	79	62	1,7	0,7	8,5	6,0	10,0	6,6
Maltosa	0,9	0,5	80	60	1,2	0,5	10,2	6,4	11,0	6,7
Laktosa	0,8	0	78	54	1,3	0,4	7,6	5,2	11,0	7,0
Na-CMC	10,6	6,8	160	106	7,7	5,0	10,0	7,0	12,0	6,2
Filter peper	4,0	2,9	144	113	12,6	8,9	8,2	6,3	6,0	6,0
MCC	3,0	2,4	129	101	10,7	6,0	7,0	5,4	9,9	6,4
Starch	2,6	1,7	107	78	10,1	6,5	10,1	6,8	8,7	7,4
Tea	8,7	5,0	180	128	13,0	10,2	16,2	10,4	14,6	9,5
Pektin	1,0	0,7	87	62	2,9	1,5	14,8	9,9	10,2	6,8
Kazein	0,8	0,6	90	60	2,0	1,4	7,7	6,0	16,0	10,4
Control	0,7	0,5	76	54	1,5	1,1	8,7	6,1	8,8	5,8

* - intracellular activity; ** - extracellular activity.

Table.3 Effect of azote sources on hydrolase activity (per cent by control) of *P. ostreatus* F-18 fungus

Nitrogen Sources	Cellulasa		Xylanasa		Amylasa		Pektinsa		Proteaza	
	A^1	B^2	A	B	A	B	A	B	A	B
$NaNO_3$	97	98	100	101	105	100	90	86	88	93
NH_4NO_3	117	125	116	117	118	113	118	112	115	112
KNO_3	92	93	100	99	100	94	95	100	80	84
$(NH_4)_2NO_3$	110	107	109	112	110	108	112	106	105	108
Peptone	113	102	107	110	106	107	110	106	109	105
Qlutamin	107	107	100	103	106	105	100	97	98	89
Asparagine	102	100	97	94	90	88	86	82	79	83
Urea	100	99	109	112	94	86	81	80	87	92
Control	100	100	100	100	100	100	100	100	100	100

1 - intracellular activity; 2 - extracellular activity.

It will be observed that influence of carbon source on extracellular secretion process of synthesized enzymes changes depending on the enzymes and kind of used carbon sources (Table 2). For example, using glucose as carbon source 37,5% cellulose, 41% pectinase and 27% lipase were secreted out of cells, whereas these index amounts to 42%, 43,4%, and 33% using filter paper as carbon source.

The results on influence of carbon sources to enzyme synthesis of *B. adusta* P-15 fungus showed, that they differ from data concerning *P. ostreatus* F-118. In that case only difference is quantification of some index or other. For instance, cellulose activity of *P. ostreatus* F-118 using CMC as sources increases in 12,5 times and xylanase activity of ones in 8,3 times. These index amount to 13,6 and 7,2 for *B. adusta* P-15.

In spite of noted distinction we have reasonable grounds to approve using tea as optimal carbon source for synthesis of balanced enzyme system for both cultures.

In general source of mineral form of nitrogen lowers activity in both cultures (Table 3). In some cases this

coefficient reaches to 20%. But ammonium form of nitrogen in all cases increases activity. Thus effect of mineral carbon sources on activity is selectively.

In distinction from mineral forms of nitrogen effect of organic nitrogen on enzyme activity changes depending on both the kind of nitrogen source, and enzyme. For example, carbamide increases amylase activity 1,09-1,12 times, whereas ones pectinase activity lowers 1,22-1,25 times.

Impact of organic sources of nitrogen as carbon source also can be estimated as one of the reasons of such comparatively different results.

It is appropriate mention here that impacts of both form of nitrogen sources on enzyme secretion, synthesized by studied fungi is connatural and quantity of secreted part of synthesized enzymes is not liable to variation.

As a results of investigations on carbon source selection was revealed that NH_4NO_3 is optimal source of nitrogen and one reaches to best index numbers at 0,04% density (by nitrogen) in the medium.

It was also established that pH for optimal enzyme synthesis of hydrolytic type must be equal of 5,2-5,7, but cultivation temperature – 28-30^{0}C. These index are optimal for growth and enzyme activity the majority of macromycetes and micromycetes (1, 4, 12).

During study of dynamics of enzyme activity by above said parameters (sources of carbon and nitrogen, pH and cultivation temperature) in suboptimized medium was established that beginning of all hydrolase synthesis except lipase is after 4-6 hours of cultivation. Lipase was registered in the medium later (after 8-10 h.). They are characterized by times of maximum activity and different index numbers. Thus, cellulase, xylanase and amylase reach to maximum activity (extra cellular) after 80 hours of incubation, pectinase and protease – after 96 hours, lipase – after 144 hours.

Thus, as a result of investigation on screening of various basidiomycetes strains showed, that *P. ostreatus* F-18, *B. adusta* P-15 and *Sch. commune* M-17 are active producers with powerful enzyme system. This system catalyses the hydrolysis of main components from composition of plant substratum. Isolated producers reach to maximum activity in the medium using tea as carbon source and NH_4NO_3 as nitrogen source, under cultivation for 80-144 hours, at temperature 28-30^{0}C and pH 5,2-5,7.

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