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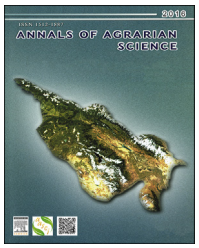

Induction and catabolite repression of cellulase and xylanase synthesis in the selected white-rot basidiomycetes

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ABSTRACT

This paper reports regulation of endoglucanase (EC 3.2.1.4) and xylanase (EC 3.2.1.8) production in submerged cultivation of four white-rot basidiomycetes. Among carbon sources tested, the Avicel-based medium provided the highest levels of both hydrolases activities in all fungal cultures. However, the maximum endoglucanase and xylanase activities of the tested basidiomycetes varied from 3.9 U/ml and 7.4 U/ml in *Fomes fomentarius* to 34.2 U/ml and 29.5 U/ml in *Pseudotrametes gibbosa*, respectively (*P. gibbosa* specific cellulase and xylanase activities achieved 8.55 and 7.38 U/mg, respectively). Replacement of Avicel in the medium with carboxymethyl cellulose or xylan significantly lowered the enzyme yield of the tested fungi. Moreover, xylan did not ensure high xylanase activity of these fungi. Lignocellulosic substrates used as a carbon source provided poorer productivity (the specific CMCase activity was 1.12–3.62 U/mg and the specific xylanase activity was 1.95–3.32 U/mg). Expression of endoglucanase and xylanase synthesis in *Panus lecometei* and *P. gibbosa* was inducible; supplementation of the glycerol-containing medium with Avicel accompanied with a sharp increase of the fungal specific CMCase and xylanase activities from 0.02–0.04 U/mg to 1.30–8.55 U/mg. Supplementation of the Avicel-induced cultures with glucose or glycerol caused a catabolite repression of the cellulase and xylanase formation by *P. gibbosa* and *P. lecometei*. The enzyme synthesis resumed only after depletion of easily metabolizable carbon source, glucose or glycerol, from the medium. The data received suggest that in the tested fungi endoglucanase and xylanase synthesis is under control by a common regulatory mechanism.

Introduction

White-rot basidiomycetes (WRB) are one of the most efficient decomposers of lignocellulosic biomass due to their capability to synthesize all relevant hydrolytic and oxidative extracellular enzymes required to degrade the major polymers of plant raw materials [1,2]. These fungi synthesize a variety of cellulases and hemicellulases that catalyze the hydrolysis of the plant polysaccharides to sugars in order to ensure

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microorganisms with carbon and energy sources for sustainable growth. These hydrolytic enzymes are of fundamental importance for the efficient bioconversion of plant raw materials and for various biotechnological applications [3]. Therefore, search for the exceptionally potent enzyme producers and a detailed understanding of mechanisms regulating the hydrolases synthesis are mandatory.

WRB have evolved and specialized to metabolize their woody substrates; therefore, it is expected that the rational prospecting of these organisms will result in potentially useful microorganisms and enzymes with properties relevant for their industrial application. Recently, several species of WRB have been studied in submerged and solid-state fermentation of lignocellulose, of which some have shown high potential for the production of individual groups of hydrolytic enzymes at the appropriate cultivation conditions [4–7]. Thus, *Armillaria gemina* secreted 146 U endoglucanase/ml, 15 U β-glucosidase/ml, and 1.72 U FPA(filter paper activity)/ml when the fungus was grown in medium containing rice straw as growth substrate and yeast extract as the best nitrogen source [7]. Moreover, reactions with Celluclast 1.5 L and Novozyme 188 supplemented with *A. gemina* endoglucanase were 20% more efficient in the production of reducing sugars than the Celluclast 1.5 L and Novozyme 188 alone. *Coprinellus disseminatus* produced 469 U/ml of alkali-thermo-tolerant xylanase along with negligible cellulase activity [5]. Furthermore, the literature data also prove that WRB secrete a full range of hydrolytic enzymes required for the biomass polysaccharides deconstruction [2,8].

Previously, we have found that in submerged fermentation of lignocellulosic materials *Panus lecometei*, *Fomes fomentarius*, *Pseudotrametes gibbosa*, and *Trametes versicolor* produce significant amounts of cellulases and xylanases which are part of the lignocellulose-degrading enzyme system of these fungi [4,9]. The capacity of WRB to produce high levels of hydrolytic enzymes along with ligninolytic enzymes is important to steadily supply the growing cultures with carbon and energy and indicates that they may be promising for the plant raw materals saccharification. However, unlike lignin-modifying enzymes of WRB, surprisingly little is known about mechanisms regulating their hydrolases synthesis. The present study' goal was to give an insight into the regulation of endoglucanase and xylanase production by the selected fungi, which has not yet been reported.

Materials and methods

Organisms and inoculum preparation

The following WRB isolated from the forests of Georgia have been used in this study: *F. fomentarius* BCC 38, *P. lecometei* (former *Pleurotus dryinus*) BCC 903, *P. gibbosa* BCC 17, and *T. versicolor* BCC 13. These fungi are typical WRB belonging to different families of order *Polyporales* Gäum. For preparation of inoculum, the macromycetes were grown on a rotary shaker at 150 rpm and 27 °C in 250 ml flasks containing 100 ml of basal medium of following composition (g/l): glucose 10; ammonium tartrate 2; KH_2PO_4 1; $MgSO_4 \cdot 7H_2O$ 0.5; yeast extract 2.0, pH 6. After 7 days of cultivation mycelial pellets were harvested and homogenized with a Waring laboratory blender.

Cultivation conditions

Submerged cultivation of fungi was carried out on a rotary shaker at 150 rpm and 27 °C in 250 ml flasks containing 50 ml of the above-mentioned standard medium. The effect of carbon sources on fungi growth and enzyme production was studied using a basal medium supplemented with crystalline cellulose (Avicel), soluble carboxymethyl cellulose (CMC, low viscosity), xylan from beech wood, glucose, cellobiose, lactose or glycerol at a concentration of 10 g/l. Lignocellulosic growth substrate, ethanol production residue (EPR), providing efficient secretion of hydrolases by majority of recently studied fungi, was used for comparison. Control without carbon source was run in parallel. The initial pH of the media was adjusted to 6.0 prior to sterilization by adding 2 M NaOH.

In the experiments designed to study the induction of cellulase and xylanase synthesis, the fungi were grown in media containing 0.3% or 0.6% glycerol, 1% Avicel, 0.3% glycerol + 1% Avicel, 0.6% glycerol + 1% Avicel, and 0.6% glycerol + 1% Avicel added on day 4. To study the catabolite repression of enzyme synthesis, the fungi cultivation was performed in nutrient media containing 1% Avicel, 1% Avicel + 0.4% glycerol on day 3 or 4, and 1% Avicel + 0.4% glucose on day 3 or 4. The flasks were inoculated with 3 mL of mycelial homogenate after 1 h of preliminary starvation. To monitor the time course of enzyme accumulation, 1 ml samples were taken from flasks at 24 h interval and the solids were separated by centrifugation at 10000 *g* for 10 min at 4 °C. The experiments were performed twice using 3 replicates. The data presented in the tables correspond to mean values with a standard error less than 16%.

Biomass estimation

The separated biomasses were dried to constant weight at 60 °C. To determine protein concentration, the fungal biomasses were first treated with 0.5% trichloroacetic acid for 15 min on a boiling water bath to remove none protein nitrogen, then centrifuged at 10000 *g* for 10 min, washed with 96% ethanol, and dried at 60 °C to constant mass. The nitrogen content in biomasses was estimated by the Kjeldahl procedure with a Nessler reagent using the coefficient of 4.38.

Enzyme activity assays

The supernatants obtained after biomass separation were analyzed for the target enzyme activity. Endoglucanase (CMCase, EC 3.2.1.4) activity was assayed according to IUPAC recommendations by mixing 70 μl appropriately diluted samples with 630 μl of low-viscosity carboxymethyl cellulose (1% w/v) in 50 mM citrate buffer (pH 5.0) at 50 °C for 10 min [10]. Xylanase (EC 3.2.1.8) activity was determined in the same conditions using birch wood xylan (Roth 7500) (1% w/v) as an enzyme substrate [11]. Glucose and xylose standard curves were used to calculate the cellulase and xylanase activities. In all assays the release of reducing sugars was measured using the dinitrosalicylic acid reagent method [12]. One unit of enzyme activity was defined as the amount of enzyme, releasing 1 μmol of reducing sugars per minute.

Results

Effect of carbon source

To obtain an insight into the induction of endoglucanase and xylanase activities in four WRB species, various mono-, di-, and polysaccharides were tested along with lignocellulosic substrate. Results given in Table 1 indicate that in cultivation on basal medium in the absence of carbon source the fungi accumulated 0.7–0.8 g/l mycelial biomass. The fungi were capable of utilizing the selected carbon sources; however the biomass yield differed significantly. Carboxymethyl cellulose followed by xylan appeared to be very poor growth substrates providing the lowest yields of all basidiomycetes biomass (biomass gains were only 0.4–0.8 and 1.6–2 g/l, respectively, as compared with the control medium). The carbon source yielding a maximum growth of fungi differed from species to species. Highest mycelial biomass production occurred when *F. fomentarius* and *T. versicolor* were cultivated in presence of glucose; lactose ensured the highest yield of *P. lecometei* biomass accumulation, while cellobiose was favorable for the growth of *P. gibbosa*. With the exception of Avicel, the fungal growth in control and polysaccharide-containing media accompanied with increase of initial pH. In presence of other carbohydrates, all fungal cultures with the exception of *P. lecometei* had rather decreased or maintained the initial medium pH.

The results obtained show that the production of hydrolytic enzymes strongly depends on the nature of carbon

Table 1 – Effect of carbon source on the basidiomycete's growth and enzyme production.

Carbon source	Biomass (mg/ml)	pH on the day 10	CMCase		Xylanase	
			(U/ml)	(U/mg)	(U/ml)	(U/mg)
Fomes fomentarius 38						
Control	0.8 ± 0.1	7.1 ± 0.1	0.1 ± 0	0.12	0.1 ± 0.01	0.12
Avicel	3.0 ± 0.3[a]	5.7 ± 0.1	3.9 ± 0.42	1.30	7.4 ± 0.93	2.47
CMC	1.5 ± 0.1	7.5 ± 0.1	0.5 ± 0.04	0.33	0.6 ± 0.06	0.40
Xylan	2.5 ± 0.2	6.3 ± 0.1	0.7 ± 0.11	0.27	0.4 ± 0.03	0.16
Glucose	5.4 ± 0.3	4.6 ± 0.2	0.2 ± 0.04	0.04	0.2 ± 0.03	0.04
Cellobiose	5.1 ± 0.3	4.7 ± 0.2	0.3 ± 0.04	0.06	0.3 ± 0.05	0.06
Lactose	3.0 ± 0.2	5.3 ± 0.1	0.8 ± 0.13	0.27	0.4 ± 0.05	0.13
Glycerol	3.6 ± 0.2	4.7 ± 0.1	0.1 ± 0.01	0.03	0.1 ± 0.02	0.03
EPR	4.1 ± 0.2	5.8 ± 0.2	4.6 ± 0.54	1.12	8.5 ± 1.28	2.07
Panus lecometei 903						
Control	0.8 ± 0.1	7.8 ± 0.1	0.1 ± 0	0.12	0.1 ± 0	0.12
Avicel	3.8 ± 0.2	5.5 ± 0.2	12.8 ± 1.78	3.37	25.2 ± 2.93	6.63
CMC	1.6 ± 0.1	7.6 ± 0.1	0.9 ± 0.11	0.56	1.1 ± 0.16	0.69
Xylan	2.7 ± 0.2	7.3 ± 0.2	2.8 ± 0.50	1.04	0.8 ± 0.11	0.30
Glucose	5.0 ± 0.2	6.9 ± 0.1	0.2 ± 0.03	0.04	0.3 ± 0.05	0.06
Cellobiose	5.8 ± 0.3	5.4 ± 0.1	0.5 ± 0.06	0.09	0.3 ± 0.05	0.05
Lactose	6.0 ± 0.2	6.6 ± 0.1	0.7 ± 0.11	0.12	0.2 ± 0.03	0.03
Glycerol	5.4 ± 0.2	6.6 ± 0.2	0.1 ± 0.01	0.02	0.2 ± 0.02	0.04
EPR	4.4 ± 0.1	5.5 ± 0.1	10.9 ± 1.87	2.48	11.8 ± 1.75	2.68
Pseudotrametes gibbosa 17						
Control	0.7 ± 0.1	6.5 ± 0.1	0.1 ± 0.02	0.14	0.1 ± 0.02	0.14
Avicel	4.0 ± 0.3	5.9 ± 0.1	34.2 ± 5.02	8.55	29.5 ± 3.90	7.38
CMC	1.2 ± 0.1	7.5 ± 0.1	1.3 ± 0.20	1.08	0.8 ± 0.14	0.67
Xylan	2.6 ± 0.1	6.3 ± 0.2	1.0 ± 0.15	0.38	0.6 ± 0.10	0.23
Glucose	4.7 ± 0.2	6.9 ± 0.1	0.1 ± 0.02	0.02	0.1 ± 0.02	0.02
Cellobiose	5.1 ± 0.2	6.9 ± 0.1	0.1 ± 0.01	0.02	0.4 ± 0.08	0.08
Lactose	4.6 ± 0.2	5.2 ± 0.2	0.4 ± 0.05	0.09	0.4 ± 0.06	0.09
Glycerol	5.0 ± 0.2	6.8 ± 0.1	0.1 ± 0.02	0.02	0.1 ± 0.02	0.02
EPR	4.7 ± 0.2	5.9 ± 0.1	17.0 ± 2.04	3.62	15.6 ± 2.10	3.32
Trametes versicolor 13						
Control	0.7 ± 0.1	7.7 ± 0.1	0.1 ± 0.02	0.14	0.2 ± 0.03	0.29
Avicel	3.6 ± 0.2	6.4 ± 0.1	10.2 ± 1.46	2.83	11.5 ± 1.10	3.19
CMC	1.1 ± 0.1	7.4 ± 0.1	1.0 ± 0.12	0.91	1.1 ± 0.16	1.00
Xylan	2.5 ± 0.2	7.3 ± 0.2	0.3 ± 0.04	0.12	0.6 ± 0.09	0.24
Glucose	4.9 ± 0.3	6.2 ± 0.2	0.1 ± 0.02	0.02	0.1 ± 0.01	0.02
Cellobiose	4.5 ± 0.2	4.5 ± 0.1	0.1 ± 0.01	0.02	0.2 ± 0.04	0.04
Lactose	4.4 ± 0.3	4.5 ± 0.1	0.1 ± 0.01	0.02	0.1 ± 0.01	0.02
Glycerol	4.3 ± 0.1	4.4 ± 0.1	0.1 ± 0.02	0.02	0.1 ± 0.01	0.02
EPR	4.2 ± 0.1	5.3 ± 0.1	7.4 ± 1.13	1.76	8.2 ± 1.33	1.95

Samples were taken after 5, 7, 10, and 14 days of submerged cultivation.
Values presented are the means ± SD of two experiments with three replicates.
[a] In Avicel-containing media biomass was calculated from the protein content.

source (Table 1). When the fungi were grown in the presence of low-molecular-weight compounds (glucose, cellobiose, lactose, or glycerol) only traces or very low CMCase and xylanase activities were found. Even in the case of complete consumption of the carbon source from the nutrient medium no noticeable increase of enzyme activity was observed during fungi cultivation. Nevertheless, appreciable levels (0.7–0.8 U/ml) of endoglucanase activity were detected in cultivation of *F. fomentarius* and *P. lecometei* in lactose-based medium. Among polysaccharides, neither CMC nor xylan appeared to be appropriate substrates for both enzyme syntheses, whereas the Avicel-based medium exerted the highest levels of both hydrolases activities in all fungi when added at 1% to the cultures. However, the maximum endoglucanase and xylanase activities of tested basidiomycetes varied, respectively, from 3.8 U/ml and 7.4 U/ml (*F. fomentarius*) to 34.2 U/ml and 29.5 U/ml (*P. gibbosa*). The medium supplemented with EPR favored the hydrolytic enzyme secretion by tested WRB. However, only in cultivation of *F. fomentarius* their yields appeared to be rather higher than those in crystalline cellulose containing medium. The CMCase and xylanase activities of *P. lecometei*, *P. gibbosa*, and *T. versicolor* in Avicel-containing medium exceeded those in fermentation of EPR by 17 and 114%, 101 and 89%, 38% and 40%, respectively. Moreover, not only the values of individual hydrolases but also the ratios of both enzymes activity differed significantly depending on the fungus species. Thus, *F. fomentarius*, *P. lecometei*, and *T. versicolor* accumulated much higher xylanase activity, while *P. gibbosa* produced rather higher levels of endoglucanase.

Induction of cellulase and xylanase synthesis

The data received in this study indicate that endoglucanase and xylanase of the tested WRB are produced only under conditions in which they use cellulosic materials as a growth substrate. *P. lecometei* and *P. gibbosa* have been selected for further experiments to elucidate the inducible mechanism of these enzymes synthesis. On the base of data received, glycerol can be considered as a carbohydrate providing comparatively good growth of fungi and constitutive levels of hydrolases. Therefore, this compound has been used as a non inducing carbon source. Indeed, only traces of cellulase and xylanase could be detected at different stages of *P. lecometei* (Fig. 1A, B) and *P. gibbosa* growth in media containing 0.3 or 0.6% glycerol (Fig. 1C, D). In Avicel-containing medium the appreciable cellulase and xylanase activities were detected from the second day of cultivation and they gradually increased till the end of experiments achieving, respectively, 12.9 and 22.6 U/ml in *P. lecometei* culture and 38 and 26 U/ml in cultivation of *P. gibbosa*.

When the 0.3 and 0.6% glycerol-containing cultures were supplemented with the crystalline cellulose from the beginning of cultivation delay of cellulase and xylanase formation was observed, respectively, during 2 and 4 days of *P. lecometei* cultivation and during 2 and 3 days of *P. gibbosa* growth. By this time, only traces or no reducing sugars could be detected in the nutrient media. It is obviously that upon growth in the medium with two sources of carbon, the fungi first utilized glycerol. After its depletion during indicated time, the synthesis and secretion of both enzymes occurred very rapidly. Moreover, the CMCase activity of *P. lecometei* in this medium achieved that in Avicel-containing medium by the end of cultivation while the fungus xylanase activity exceeded that in the control medium already after 8 days cultivation. It is worth noting that the cellulose-based medium supplementation with 0.3 and 0.6% glycerol increased the fungus biomass yield by 28–50% (Table 2). When Avicel was added to the culture *P. lecometei* growing in the presence of 0.6% glycerol for 4 days, the cellulase and xylanase accumulation started within 24 h and on the day 10 of fungus growth their levels were comparable to those obtained in the cultivation on cellulose alone.

Somewhat different picture was revealed when *P. gibbosa* was cultivated under the same conditions although a similar response to the media composition and the same regularities in the cellulase and xylanase synthesis induction and accumulation were revealed (Fig. 1C, D). Unlike *P. lecometei*, in cultivation of *P. gibbosa* the levels of these enzymes activity in glycerol + Avicel cultures never exceeded those in cellulose-based medium. Moreover, the supplementation of 0.6% glycerol-containing medium with Avicel on day 4 approximately two-fold decreased the rate of enzyme accumulation by *P. gibbosa* as compared with control culture although the fungus biomass yield was increased by 29% (Table 2).

Catabolite repression of the cellulase and xylanase synthesis

To study the cellulase and xylanase synthesis catabolite repression in *P. gibbosa* and *P. lecometei*, glucose and glycerol at final concentration of 0.4% were added to the cultures growing in the presence of 1% Avicel after 3 and 4 days of fungi cultivation, respectively. Easily metabolizable sources of carbon together with crystalline cellulose stimulated fungal growth and increased the biomass yields by 19–29% versus the Avicel-containing medium. The production profiles shown in Fig. 2 indicate that in the cellulose-based medium appreciable levels of CMCase and xylanase activities were detected already after 3–4 days of *P. lecometei* and *P. gibbosa* cultivation and the enzyme activities gradually increased achieving maximum by the day 10.

Addition of glucose or glycerol to the induced *P. lecometei* culture accelerated the fungus growth but caused repression of endoglucanase and xylanase synthesis and even a partial inactivation of the existing enzymes (Fig. 2A, B). However, after 1 day of further cultivation the enzyme synthesis resumed, thus proving the reversibility of the repression mechanism of the hydrolases synthesis by easily metabolizable compounds. It is interesting that the rates of CMCase and xylanase accumulation after depletion of glycerol or glucose were significantly higher as compared to that in Avicel-containing culture. As a result, already after 9–10 days of submerged cultivation of *P. lecometei* in media with dual carbon sources the fungus volumetric enzyme activity exceeded that in avicel-containing medium.

In cultivation of *P. gibbosa* it was observed that the addition of glucose or glycerol to the induced culture in Avicel-based medium strongly repressed the fungus endoglucanase and xylanase production during 1 and 2 days cultivation (Fig. 2C, D). In addition to the catabolite repression, reduction

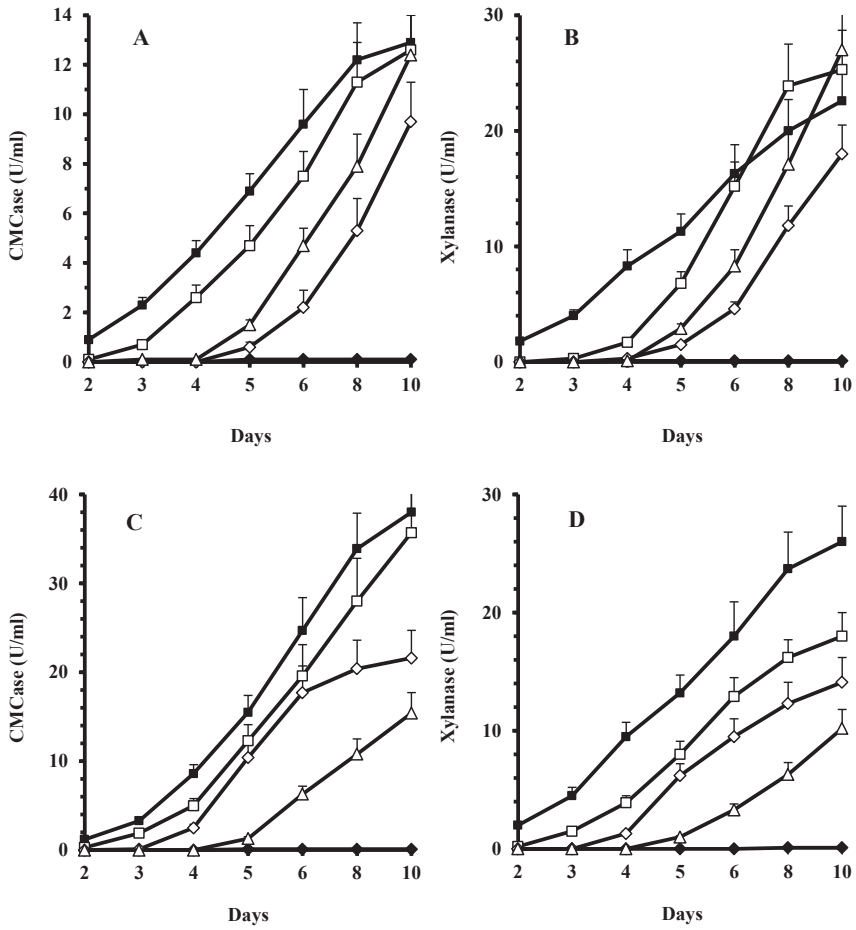

Fig. 1 – Induction of *Panus lecometei* (A, B) and *Pseudotrametes gibbosa* (C, D) endoglucanase and xylanase synthesis. The fungi were grown in media containing 1% Avicel (■), 0.3% or 0.6% glycerol (◆), 0.3% glycerol+1% Avicel (□), 0.6% glycerol+1% Avicel (◊), 0.6% glycerol+1% Avicel on day 4 (△).

of synthesized enzymes activity was observed, obviously because of inactivation during rapid acidification of the nutrient media. Subsequently, the secretion of cellulase and xylanase resumed and the rates of both enzymes production in these media achieved or rather exceeded those in Avicel-containing medium.

Discussion

The results presented in this paper indicate that the crystalline cellulose and natural cellulosic material had the great impact on cellulase and xylanase expression by four WRB. Best enzyme activities were received when the fungi were grown in the Avicel-containing medium (specific cellulase and xylanase activities varied from 1.30 to 2.47 U/mg in *F. fomentarius* to 8.55 and 7.38 U/mg in *P. gibbosa*, respectively) while the lignocellulosic substrate used as the carbon source provided a lower productivity (the specific CMCase activity was 1.12–3.40 U/mg and the specific xylanase activity was 1.95–3.11 U/mg) (Table 1). Unexpectedly, replacement of Avicel in the medium with CMC or xylan significantly lowered the enzyme yield of the tested WRB. Moreover, xylan did not ensure high xylanase activity of these fungi. The highest xylanase activity was produced during the fungi cultivation on cellulose testifying the presence of xylan does not seem necessary for induction of this enzyme. These results are in consensus with several earlier studies. Namely, the growth of *Schizophyllum commune* in xylan-based medium did not resulted in an increase of xylanase secretion; production of xylanase was strictly linked to the presence of cellulose [13]. The highest xylanase activity was produced by *Phanerochaete chrysosporium* in the corn stalk-containing medium, while the xylan-based fermentation resulted in the lowest induction [14]. At the same time, in the cultures of *Coriolus pubescens* and *Lentinus tigrinus*, *Cerrena unicolor*, *Funalia trogii*, and *Merulius tremellosus* xylan induced comparatively high levels of both endoglucanase and xylanase [15,16]. Moreover, in these cultures the xylanase activity appeared to be rather higher than that of CMCase. It is worth noting that in this study, WRB growth in CMC- or xylan-based media accompanied with significantly higher increase of pH compared to Avicel medium, which, obviously, hampered hydrolysis of polysaccharide and provision of fungi with compounds required for their growth and enzyme production (Table 1). Nevertheless, CMC and xylan provided as high as 0.33–1.08 and

Table 2 – *Panus lecometei* and *Pseudotrametes gibbosa* biomass yield and specific activities of endoglucanase and xylanase during enzyme synthesis induction and catabolite repression.

Media composition	Biomass (mg/ml)	Endoglucanase (U/mg)	Xylanase (U/mg)
Induction			
P. lecometei			
0.3% glycerol	1.4	0.07	0.07
1% Avicel	4.0	3.23	5.65
0.3% glycerol + 1% Avicel	5.1	2.47	4.96
0.6% glycerol + 1% Avicel	6.0	1.62	3.00
0.6% glycerol + 1% Avicel on day 4	5.9	2.10	4.58
P. gibbosa			
0.3% glycerol	1.5	0.06	0.06
1% Avicel	4.1	9.27	6.34
0.3% glycerol + 1% Avicel	4.8	7.44	3.75
0.6% glycerol + 1% Avicel	5.6	3.64	2.52
0.6% glycerol + 1% Avicel on day 4	5.3	2.91	1.92
Catabolite repression			
P. lecometei			
1% Avicel	4.1	3.48	5.61
1% Avicel + 0.4% glycerol on day 4	5.2	2.94	4.58
1% Avicel + 0.4% glucose on day 4	5.3	3.32	4.68
P. gibbosa			
1% Avicel	4.2	8.31	5.60
1% Avicel + 0.4% glycerol on day 3	5.0	6.60	4.20
1% Avicel + 0.4% glucose on day 3	5.2	7.10	3.65

0.12–1.04 U/mg enzyme specific activity of the studied WRB. These values are much higher than those detected in the cultures grown in media supplemented with glycerol or glucose. Moreover, the fungi cultivation in media lacking carbon source did not induce cellulase or xylanase synthesis during 10 days of their cultivation. These results suggest that carbon starvation is not sufficient to initiate cellulase expression and the levels of CMCase and xylanase obtained in control or glycerol-based medium indicate a constitutively formed enzyme activity.

The regulation of cellulases and xylanases synthesis by white and brown-rot fungi has been reported. The available data indicate that brown-rot fungi produce these hydrolases constitutively accumulating approximately the same levels of enzyme activity during cultivation in the presence of mono-, di-, and polysaccharides as well as in media containing various plant raw materials [15–17]. The only exception to this rule is *Piptoporus betulinus*. In this culture, cellobiose served as an inducer of the cellulase and xylanase synthesis [16]. Cellulases and xylanases of *Agaricus bisporus* [18], *Sporotrichum pulverulentum* [19], *S. commune* [13], *C. pubescens* [15], *C. unicolor* [16], *Polyporus arcularius* [20,21] and *P. chrysosporium* [22] are inducible enzymes. These macromycetes secrete cellulases and xylanases with high efficiency upon cultivation in presence of cellulose or lignocellulosic substrate.

In this study, we considered glycerol and Avicel as carbohydrates providing, respectively, constitutive and inducible levels of the target enzymes. Indeed, the fungi specific CMCase and xylanase activities varied from 0.02 to 0.04 U/mg in glycerol-based medium to 1.30–8.55 U/mg dry biomass in Avicel-containing medium (Table 1). Consequently, our calculations showed that the induction ratios for the *F. fomentarius* 38, *P. lecometei* 903, *P. gibbosa* 17, and *T. versicolor* 13 endoglucanase synthesis in the tested cultivation conditions were 43, 169, 428, and 142, while those for the xylanase synthesis were 82, 166, 369, and 160, respectively. Experiments with crystalline cellulose added to glycerol-containing medium also proved the existence of the induction mechanism of *P. lecometei* and *P. gibbosa* cellulases and xylanases synthesis after depletion of an easily metabolized source of carbon (only traces of reducing sugars could be detected in Avicel + glycerol containing media after 3–4 days of fungi cultivation). It is worth noting that the endoglucanase and xylanase specific activities of fungi grown in presence of both carbon sources appeared to be slightly lower as compared with those detected in Avicel-based medium (Table 2). This could be due to the higher biomass production owing glycerol utilization and shorter period of enzyme production. Thus, the literature data and the observations in the present study prove that the inducible synthesis of cellulases and xylanases is characteristic of basidiomycetes degrading wood through white rot. Few studies also indicate involvement of cellooligosaccharides in induction of the cellulases encoding genes [20–22].

Another mechanism of cell economy, namely, catabolite repression of cellulases and xylanases synthesis is widespread in WRB. Supplementation of the Avicel-containing cultures with 0.3 and 0.6% glycerol from the beginning of *P. gibbosa* and *P. lecometei* cultivation prevented cellulase and xylanase formation during 2 days and 3–4 days of fungi cultivation, respectively, even though the inducer was also present (Fig. 1). Duration of repression increased with an increase of glycerol concentration in the culture medium. When the most beneficial carbon source was completely utilized the synthesis of both enzymes started with gradual activity increase till day 10. Addition of glycerol or glucose to the induced cultures of *P. lecometei* and *P. gibbosa* immediately repressed enzyme secretion and the cellulase and xylanase synthesis resumed only after depletion of glucose and glycerol from the medium (Fig. 2). This peculiarity of WRB can be exploited in development of plant raw material biological delignification process. Supplementation of medium with easily metabolizable carbon source such glycerol can prevent polysaccharides degradation and metabolism during solid-state fermentation of ligno cellulose.

From the results received in this study and from the literature data, it can be speculated that not only glucose, but other readily metabolizable carbohydrates repress the synthesis of enzymes related to catabolism of polysaccharides. Finally, our data show that endoglucanase and xylanase are co-ordinately expressed under all the conditions studied indicating that both enzymes synthesis is under a common regulatory control mechanism. This finding is not unexpected keeping in mind a natural substrate of wood-rotting basidiomycetes. Similar results have also been reported in *S. commune* [13], *C. pubescens* [15], and *C. unicolor* [16].

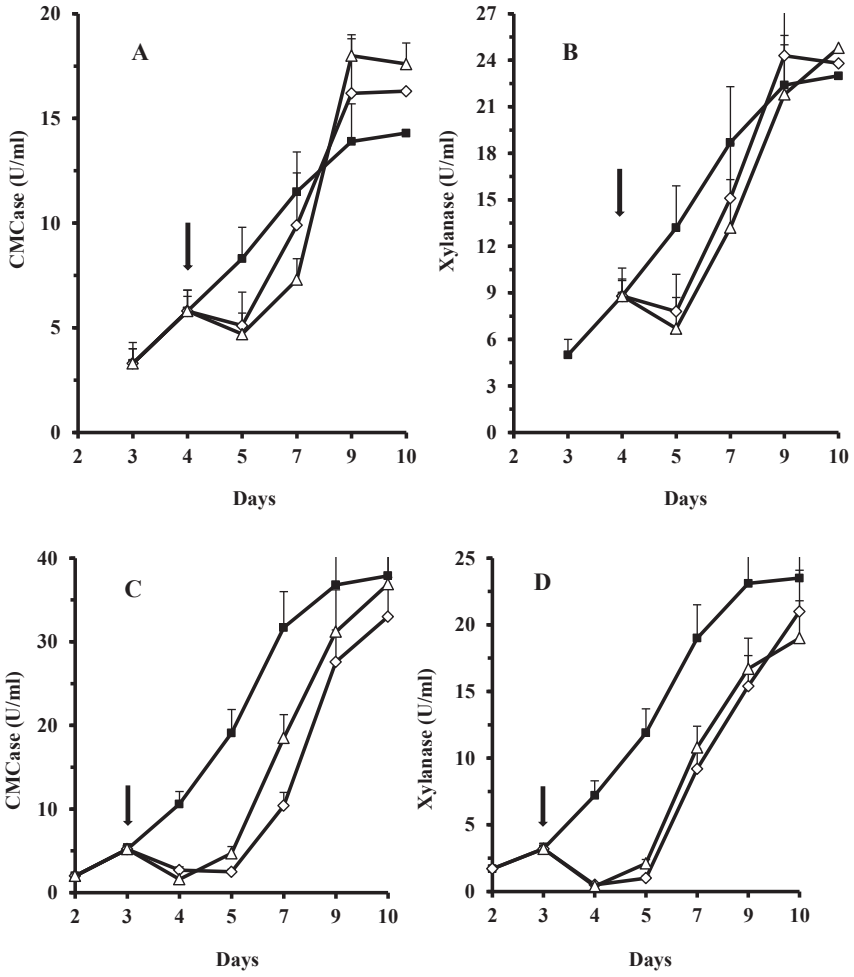

Fig. 2 – Catabolite repression of *Panus lecometei* (A, B) and *Pseudotrametes gibbosa* (C, D) endoglucanase and xylanase synthesis. The fungi were grown in media containing 1% Avicel (■), 1% Avicel + 0.4% glycerol on day 4 (◊), 1% Avicel + 0.4% glucose (△) on day 4 and 3 in cultivation of *P. lecometei* and *P. gibbosa*, respectively. Arrows indicate the days of glucose or glycerol addition.

Thus, *P. lecometei* and *P. gibbosa* as well as several other WRB, such as *Irpex lacteus* [23], *S. commune* [13], *Coprinellus disseminates* [24], and *A. gemina* [7] are promising producers of cellulase and xylanase with high lignocellulose saccharification potential. Recently, new endoglucanase from *Ganoderma lucidum* was isolated; it was tolerant to high temperature, metal ions, surfactants, and organic solvents, suggesting that it is appropriate for use in biomass conversion for biofuel production under harsh environmental conditions [25]. Moreover, crude enzyme complex obtained after solid-state fermentation of grass powder by *P. chrysosporium* was employed for efficient hydrolysis of untreated and mild acid pretreated rice husk while hydrolyzates were converted into bio-hydrogen with high yield [26]. The capacity of these basidiomycetes to produce high levels of cellulases and xylanase is of importance in supplying the growing cultures with a carbon source essential for their biosynthetic activity. Rapid recovery of enzyme synthesis after catabolite repression makes it possible to synthesize large quantities of cellulase and xylanase after first-stage growth on glucose to achieve a desired biomass. Therefore, a two-stage continuous process for cellulase production could be developed in the fed-batch fermentation in which the growth phase and production phase will be separated.

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