INDUCTION AND CATABOLITE SUPPRESSION IN THERMOPHILIC FUNGAL CELLULASE SYNTHESIS

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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. Dialysed culture filtrates, after growth on cellobiose, wese also effective in degrading crystalline cellulose (Avicel). Cellulase, measured viscometrically with carboxymethylcellulose as substrate and therefore referred to as C M-cellulase, was induced by cellulose, glucomannan, cellobiose and, to a limited extent, by laminaribiose and cellobionic acid. Low CM-cellulase activities were also detectable when the organism was grown on other carbon sources. In culture media supplemented with readily metabolized non-inducing substrates, such as glucose, CM-cellulase activity did not increase after their exhaustion indicating that no derepression of constitutive CM-cellulase occurred. The use of glucose analogues gave some indications that cellobiose did not compete with glucose for the same transport carrier and that glucose catabolism was a prerequisite for the inhibition of CM-cellulase synthesis.

Key Words: Induction, Catabolite Suppression, Cellobionic acid, CM-cellulase synthesis, Fungal Cellulase Synthesis

INTRODUCTION

Catabolite repression, is an important part of global control system of various bacteria and other microorganisms. Catabolite repression allows microorganisms to adapt quickly to a preferred (rapidly metabolizable) carbon and energy source first. This is usually achieved through inhibition of synthesis of enzymes involved in catabolism of carbon sources other than the preferred one. The catabolite repression was first shown to be initiated by glucose and therefore sometimes referred to as the glucose effect.

The term carbon catabolite repression (CCR) is currently in use to describe the general phenomenon in microorganisms whereby the presence of a carbon source in the medium can repress expression of certain genes and operons, whose gene products are often concerned with the utilization of alternative carbon sources. In the vast majority of documented cases, the preferred carbon source is glucose with the famous *Escherichia coli* glucose–lactose diauxie as the classical example.

Catabolite repression of tryptophanase was studied in detail under various conditions in several strains of *Escherichia coli* and was compared with catabolite repression of β-glactosidase. Induction of tryptophanase and β-galactosidase in cultures grown with various carbon sources including succinate, glycerol, pyruvate, glucose, gluconate, and arabinose is affected differently by the various carbon sources.

Many inducible enzymes are subject to repression by glucose or other cell metabolites, a process termed catabolite repression. This allows an additional control of the synthesis of such an inducible enzyme, since during growth it will be subject to induction by its substrate and repression by the product or products of catabolic pathways. Almost all the studies on catabolite repression of inducible enzymes have been made in batch culture, usually with exponentially growing bacteria synthesizing the enzyme under conditions of gratuity in the presence of non-substrate inducers.

Fungi are responsible for causing devastating diseases of fauna and flora. Pathogenic fungi have developed genetic mechanisms and molecular strategies to survive unpredictable scenarios and establish effective disease conditions in their hosts. Regulation of carbon metabolism is very important for disease establishment by filamentous fungi. Carbon Catabolite Repression helps microorganisms to precisely adapt their physiology to the environment. The importance of this fine tuning is illustrated by the finding that within populations there may be balancing selections for individuals with fast and slow rates of reprogramming. CCR switches off certain enzymes required to utilize less-favored carbon sources when a more readily available carbon source is present in the medium.

LITERATURE REVIEW

Xinyue Zhang, Bo Ma, Jiawen Liu, Xiehui Chen, Shanshan Li, Erlie Su, Liyuan Gao & Hongtao Li (2020) Cellulose degradation by cellulase is brought about by complex communities of interacting microorganisms, which significantly contribute to the cycling of carbon on a global scale. β-Glucosidase (BGL) is the rate-limiting enzyme in the cellulose degradation process. Thus, analyzing the expression of genes involved in cellulose degradation and regulation of BGL gene expression during composting will improve the understanding of the cellulose degradation mechanism. Based on our previous research, we hypothesized that BGL-producing microbial communities differentially regulate the expression of glucose-tolerant BGL and non-glucose-tolerant BGL to adapt to the changes in cellulose degradation conditions.

Liangcai Lin, Shanshan Wang, Xiaolin Li, Qun He, J. Philipp Benz, Chaoguang Tian (2019) Cellulolytic fungi have evolved a complex regulatory network to maintain the precise balance of nutrients required for growth and hydrolytic enzyme production. When fungi are exposed to cellulose, the transcript levels of cellulase genes rapidly increase and then decline. However, the mechanisms underlying this bell-shaped expression pattern are unclear. We systematically screened a protein kinase deletion set in the filamentous fungus *Neurospora crassa* to search for mutants exhibiting aberrant expression patterns of cellulase genes. We observed that the loss of *stk-12* (NCU07378) caused a dramatic increase in cellulase production and an extended period of high transcript abundance of major cellulase genes. These results suggested that *stk-12* plays a critical role as a brake to turn down the transcription of cellulase genes to repress the overexpression of hydrolytic enzymes and prevent energy wastage. Transcriptional profiling analyses revealed that cellulase gene expression levels were maintained at high levels for 56 h in the Δ*stk-12* mutant, compared to only 8 h in the wild-type (WT) strain. After growth on cellulose for 3 days, the transcript levels of cellulase genes in the Δ*stk-12* mutant were 3.3-fold over WT, and *clr-2* (encoding a transcriptional activator) was up-regulated in Δ*stk-12* while *res-1* and *rca-1* (encoding two cellulase repressors) were down-regulated. Consequently, total cellulase production in the Δ*stk-12* mutant was 7-fold higher than in the WT.

Bijender Singh (2014) Myceliophthora thermophila syn. Sporotrichum thermophile is a ubiquitous thermophilic mould with a strong ability to degrade organic matter during optimal growth at 45 °C. Both genome analysis and experimental data have suggested that the mould is capable of hydrolyzing all major polysaccharides found in biomass. The mould is able to secrete a large number of hydrolytic enzymes (cellulases, laccases, xylanases, pectinases, lipases, phytases and some other miscellaneous enzymes) employed in various biotechnological applications. Characterization of the biomass-hydrolyzing activity of wild and recombinant enzymes suggests that this mould is highly efficient in biomass decomposition at both moderate and high temperatures. The native enzymes produced by the mould are more efficient in activity than their mesophilic counterparts beside their low enzyme titers. The mould is able to synthesize various biomolecules, which are used in multifarious applications. Genome sequence data of M. thermophila also supported the physiological data. This review describes the biotechnological potential of thermophilic mould, M. thermophila supported by genomic and experimental evidences.

Neelam Gurung, Sumanta Ray, Sutapa Bose, and Vivek Rai (2013) Enzymes are the large biomolecules that are required for the numerous chemical interconversions that sustain life. They accelerate all the metabolic processes in the body and carry out a specific task. Enzymes are highly efficient, which can increase reaction rates by 100 million to 10 billion times faster than any normal chemical reaction. Due to development in recombinant technology and protein engineering, enzymes have evolved as an important molecule that has been widely used in different industrial and therapeutical purposes. Microbial enzymes are currently acquiring much attention with rapid development of enzyme technology. Microbial enzymes are preferred due to their economic feasibility, high yields, consistency, ease of product modification and optimization, regular supply

due to absence of seasonal fluctuations, rapid growth of microbes on inexpensive media, stability, and greater catalytic activity.

Robyn Peterson and Helena Nevalainen (2012) The hypersecreting mutant Trichoderma reesei RUT-C30 (ATCC 56765) is one of the most widely used strains of filamentous fungi for the production of cellulolytic enzymes and recombinant proteins, and for academic research. The strain was obtained after three rounds of random mutagenesis of the wild-type QM6a in a screening program focused on high cellulase production and catabolite derepression. Whereas RUT-C30 achieves outstanding levels of protein secretion and high cellulolytic activity in comparison to the wild-type QM6a, recombinant protein production has been less successful. Here, we bring together and discuss the results from biochemical-, microscopic-, genomic-, transcriptomic-, glycomic- and proteomic-based research on the RUTC30 strain published over the last 30 years.

METHODOLOGY

Organism, culture media and cultivation techniques. The organism used in this study was a strain of Sporotrichum thermophile (var. 2) isolated from decaying newspapers and identified by T. G. Barnes (for a detailed description of the organism, see Barnes, 1974). No clamp connections occur in the mycelium and since sporulation produces aleurospores, the organism can be considered identical with Chrysosporium tkermophile as suggested by von Klopotek (1974). The strain was maintained on Difco Sabouraud maltose agar slants and stored at 2^0 C.

The following liquid media were used for submerged cultures: medium I had the same composition as that of Eggins & Pugh (1962) but without asparagine and with the addition of trace elements (10 ml 1-1 of the SL6 solution of Pfennig & Lippert, 1966), CaC12.2H20 (100 mg 1-l) and iron(II1) ammonium citrate (10 mg 1-l); medium I1 had the same composition as medium I, but (NH&S04 was replaced by an equivalent amount of Difco Casamino acids. Organic substrates were sterilized separately and mixed aseptically with the medium to give a final concentration ranging from 0.01 to 1 % (w/v) (see individual experiments).

For inoculation. conidia (from a Difco Sabouraud maltose agar plate) were harvested in sterile 0-9 % (w/v) NaCl solution (50 mlj supplemented with Tween 80 (4 drops) and vigorously shaken on a gyratory shaker. The spore suspension was then filtered through a thin layer of cotton wool sandwiched between two layers of cotton gauze and washed by centrifugation with sterile saline. The number of conidia in the inoculum was estimated turbidimetrically using a calibration curve which gave a direct relationship between the absorbance (at 400 nm) of the diluted spore suspension and the spore number. Inoculations were carried out with different initial spore densities (see individual experiments).

Culture flasks (up to 1 1 bafled Erlenmeyer flasks, or up to 6 1 flat-bottomed round flasks) were incubated at 44 "C with shaking or with stirring. Under these conditions spore germination occurred about 7 h after inoculation.

The growth of the organism was measured by weighing a sample of previously washed mycelium dried on a filter paper.

Preparation of cell extracts. The mycelium in a sample of growing culture was harvested on filter paper by filtration under reduced pressure. After washing and resuspension in buffer (0.05 M-sodium acetate buffer at pH 5.0, or at pH 5.75 when necessary), the mycelium was homogenized in the cold by sonication (Branson, model S-110 set at maximum power).

Enzyme assays. CM-cellulase (endo-l.4-/3-glucanase; EC 3.2.1 .4) was determined viscometricallyaccording to Husemann & Werner (1963). A 0.5 % (w/v) solution of carboxymethylcellulose (CMC 7HF, DS = 0-57 to 0-87; Hercules, New York, U.S.A.) in 0.05 M-sodium acetate buffer (6 ml, pH 5.0) was pipetted into an Ostwald viscometer placed in a water-bath at 40 "C. Enzyme assays are laboratory methods for measuring enzymatic activity. They are vital for the study of enzyme kinetics and enzyme inhibition. Enzyme activity = moles of substrate converted per unit time = rate × reaction volume. Enzyme activity is a measure of the quantity of active enzyme present and is thus dependent on conditions, *which should be specified*. The SI unit is the katal, 1 katal = 1 mol s^{-1}, but this is an excessively large unit. A more practical and commonly used value is enzyme unit (U) = 1 μmol min^{-1}. 1 U corresponds to 16.67 nanokatals. Enzyme activity as given

in katal generally refers to that of the assumed natural target substrate of the enzyme. Enzyme activity can also be given as that of certain standardized substrates, such as gelatin, then measured in *gelatin digesting units* (GDU), or milk proteins, then measured in *milk clotting units* (MCU). The units GDU and MCU are based on how fast one gram of the enzyme will digest gelatin or milk proteins, respectively. 1 GDU equals approximately 1.5 MCU. An increased amount of substrate will increase the rate of reaction with enzymes, however once past a certain point, the rate of reaction will level out because the number of active sites available has stayed constant.

Cellulolytic activity of concentrated culture filtrates (concentrated on a rotating evaporator under reduced pressure at 30° C followed by dialysis against buffer at 2^0C) was demonstrated by analysing the reducing sugars released after reaction with Avicel (type SF, American Viscose Corp.; 6 %, w/v, final concentration) in 0.05 M-sodium acetate buffer, pH 5.0, at 40^0 C.

Colorimetric determinations. Reducing sugars in culture fluids were determined colorimetrically by the procedure of Somogyi (1952) and Nelson (1944) using the corresponding sugars as standards. Glucose was specifically determined by the glucose oxidase method (Biochemica Test Combination, Boehringer).

Pentoses, alone or in the presence of cellobiose, were determined by the orcinol method (Herbert et al., 197 1) using individual sugars as standards. Cellobiose interference was negligible. Thecellobiose concentration in media containing a mixture of cellobiose and sorbose or cellobiose and fructose was determined by the o-toluidine method (Dawes et al., 1971).

Ammonia in the culture filtrates was assayed by the phenol-hypochlorite method described by Bernt & Bergmeyer (1970).

The protein content of cell extracts and concentrated culture filtrates was measured by the method of Lowry et al. (1951) using bovine serum albumin as a standard. Protein was precipitated with 4 % (w/v, final concentration) trichloroacetic acid in the cold.

Chemicals. All reagents used were of analytical grade. 2-Deoxyglucose, 6-deoxyglucose and 3-0-methylglucose were from Calbiochem. ~-[U-^{14}C]Glucose (198 mCi mmol-[1]) was from The Radiochemical Centre, Amersham.

Cellobiitol was prepared by reduction of cellobiose with NaBH, by the method of Lewis et al. (1963). The product was crystallized in the cold in ethanol/water and its purity was checked by descending paper chromatography with the solvent system ethyl acetate/pyridine/water (8 : 2: 1, by vol.) and with the alkaline silver nitrate spray reagent according to Trevelyan et al. (1950).

1,4-β-D-Xylopyranosyl-~-xylose (xylobiose) was prepared according to Sieber (1972) by partial acid hydrolysis of an acidic xylan obtained by alkaline extraction of beech holocellulose. The disaccharide was isolated from the mixture by chromatography on a charcoal/celite column (elution with a linear gradient of water/20 :< ethanol) and purified by preparative paper chromatography.

Glucomannan was extracted from dried tubers of Orchis sp. according to Buchala et al. (1974) and freeze-dried.

Laminaribiose was isolated from an enzymic hydrolysate of pachyman obtained by the action of endo1,3-/3-glucanase (EC 3.2.1.6) from Rhizopus arrhizus ~~6789 (obtained by courtesy of Dr E. T. Reese). Amorphous cellulose was prepared as follows. Avicel (5 g) was evenly dispersed in water (10 ml) and dissolved at room temperature by slowly adding concentrated phosphoric acid (85 %, 100 ml). The dissolved cellulose was immediately reprecipitated in cold water, washed with running tap water to neutrality and finally homogenized in distilled water with an Ultra-Turrax homogenizer.

Cellobionic acid was synthesized by chlorite oxidation of cellobiose (Westermark & Eriksson, 1974).

Medium I1 (3 1) was inoculated with conidia to an initial density of 3 x lo6 ml-I and stirred magnetically at 44 "C for 7 h in order for germination to occur. The washed, young mycelium was used to inoculate medium IT (300 ml in 500 ml baffled Erlenmeyer flasks; 0.225 mg mycelium ml-l) supplemented with glucose (0.2 %,

w/v) or cellobiose (0-2 %, w/v) or different kinds of cellulose: Avicel, I % (w/v); reprecipitated Avicel, about 1 % (w/v); CF 1, fibrous cellulose powder (Whatman), 1 % (w/v}; CC 31, microgranular cellulose powder (Whatman), 1 % (w/v); CM 32, insoluble carboxymethylcellulose (Whatman), 1 % (w/v) ; CMC 7HF, soluble carboxymethylcellulose (Hercules), 0.2 % (w/v). 'The cultures were swirled in a water-bath at 250 rev. min-l at 44 "C. At intervals between 2 and 10.5 h after inoculation, samples were withdrawn and the culture filtrates were analysed for CM-cellulase activity.

TABLE 1 shows the cellulolytic activity of the culture filtrates when the mycelium was grown in submerged cultures in the presence of different cellulosic substrates. A few hours after inoculation, there was already a considerable amount of CM-cellulase activity present in the filtrates of all the cultures on crystalline cellulose and on cellobiose. In the culture on Avicel, the increase in CM-cellulase activity was paralleled by the degradation of the crystalline substrate, as deduced from the decrease (40 yo after 10 h) in total dry substance (biomass produced plus residual cellulose). In the culture on amorphous cellulose (reprecipitated Avicel), despite the fact that CMcellulase activity did not rise significantly during growth, there was an even more rapid degradation of the substrate, no cellulose being left after 6 h incubation.

TABLE 1 CelIidoIytic activity of Sporotrichwn thermophile grown on diflerent types of cellulose (and on glucose and cellobiose as control)

Substrate	CM-cellulase activity (U ml-^{1})				
	2 h	4 h	6h	8 h	10-5 h
Avicel	20	1350	4160	3200	5300
Reprecipitated Avicel	25	180	425	840	2365
CC31	44	1900	8000	6600	14500
CF 1	35	2750	6800	7040	11600
CMC 7HF	300	820	2300	1100	900
CM32	19	220	680	400	580
Cellobiose	250	4400	-	-	-
Glucose	15	50	-	-	-

CM-cellulase activity (U ml-^{1}) were collected and those conta.ining glucose (no. 61 to 107) and cellobiose (no. 159 to 188) were pooled. The total glucose yielded 143 x lo6 c.p.m. and the cellobiose 281 x lo6 c.p.m. as measured in Instagel (Packard) in a liquid scintillation spectrometer.

RESULT

Sporotrichum thermoph ile is truly cellulolytic

Sporotrichum (Chrysosporium) thermophile is a thermophilic deuteromycete, having its temperature optimum for growth between 35 and 50 "C. When grown in a medium supplemented with cellobiose and in the absence of any cellulose, the organism also produced extracellular enzymes capable of degrading Avicel. A 10-fold concentrated and dialysed filtrate from a culture grown on cellobiose, whose CM-cellulase activity was 52 500 U ml-l, released 0.97 pmol reducing sugar equivalents min-l (mg protein)-l when incubated with crystalline cellulose (Avicel) at 40 "C and at pH 5.0.

Conditions for CM-cellulase synthesis

Although CM-cellulase was produced in the presence of cellulose, other polysaccharides, with the exception of glucomannan, failed to induce CM-cellulase synthesis (TABLE 2). Culture filtrates from the cultures listed in TABLE 2 were also tested for their ability to degrade xylan, starch, pectin and glucomannan. No xylanase, amylase or endo-polygalacturonase activities were found in any of them, but the two filtrates showing high CM-cellulase activity.

Erlenmeyer flasks (500 ml) containing 250 ml medium I1 and the organic substrate were inoculated with conidia (initial density 2 x lo6 ml-l) and shaken on a rotary shaker (200 rev. min-l) in a waterbath at 45 "C. The extracellular CM-cellulase activity was determined between 9 and 12 h after inoculation.

TABLE 2. CM-cellulase synthesis in the presence of non-cellulosic polysaccharides as carbon sources (and with cellobiose as control)

Organic substrate	Concn (%, w/v)	CM-cellulase* (U ml ')
Cellobiose	0-2	6800
Orchis glucomannan (soluble)	0 05	5300
Beechwood xylan (only partly soluble)	approx. 1	500
Solublestarch	0 2	168

Maximum value obtained during growth.

Erlenmeyer flasks (250 ml) containing 100 ml medium I and 0.5 % (w/v) organic substrate were inoculated with conidia to an initial density of 4 x lo5 ml-I. The cultures were shaken (reciprocal shaker, 60 strokes min-I) in a water-bath at 35 "C for 12 h, in order for germination to occur, before raising the temperature to 48 "C. The growth was checked visually and the cultures were harvested between 4 and 10 h after germination.

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 3 indicate that in cultivation on basal medium in the absence of carbon source the fungi accumulated 0.7e0.8 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 0.4e0.8 and 1.6e2 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 source (TABLE 3).

TABLE 3 Effect of carbon source on the basidiomycete's growth and enzyme production.

Carbon source	Biomass (mg/ml)	pH on the day 10	CMCase (U/ml)	(U/mg)	Xylanase (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.

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 (0.7e0.8 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 Avicelcontaining 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.

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 19e29% 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 3e4 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 (FIGURE1). 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 Avicelcontaining culture. As a result, already after 9e10 days of submerged cultivation of P. lecometei in media with dual carbon sources the fungus volumetric enzyme activity exceeded that in avicel-containing medium.
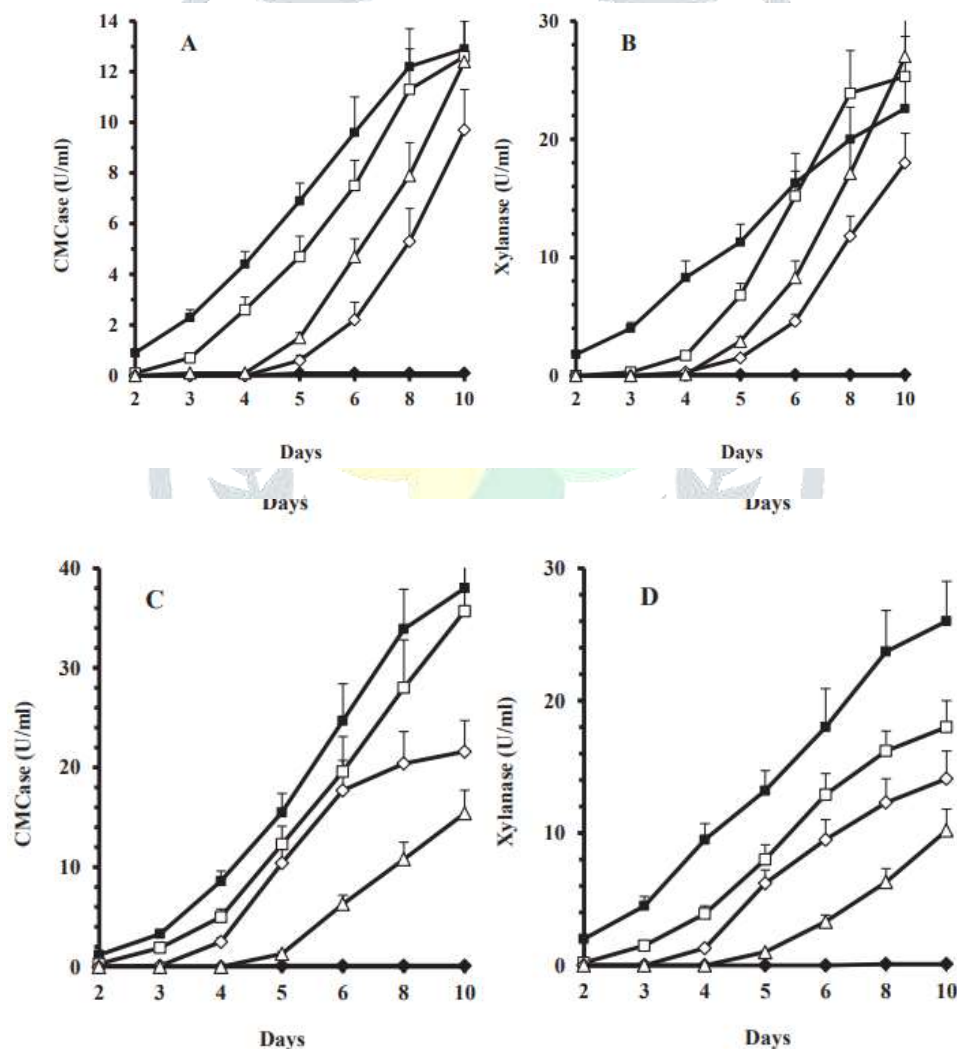

FIGURE 1 Induction of Panus lecometei (A, B) and Pseudotrametes gibbosa (C, D) endoglucanase and xylanase synthesis. The fungi were grown in media containing 1% Avicel (▪), 0.3% or 0.6% glycerol (A), 0.3% glycerolþ1% Avicel (▫), 0.6% glycerolþ1% Avicel (◊), 0.6% glycerolþ1% Avicel on day 4 (▴).

TIn addition to the catabolite repression, reduction f 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.

CONCLUSION

In many cellulolytic organisms the synthesis of cellulases is adaptive, i.e. the enzymes are induced only in the presence of cellulosic materials. Low levels of cellulase are probably always constitutively formed, supporting the idea that the true cellulase inducer is a soluble product of cellulose degradation which can be readily taken up by the cells. This view has been challenged by some authors who claim that cellulase synthesis is constitutive, being regulated by catabolite repression alone. The results presented here show that cellulase synthesis in Sporotrichum thermophile is an adaptive phenomenon, CM-cellulase being formed, in significant amounts, only when the organism is grown in the presence of cellulose or of a few other substances like glucomannan, glucosylmannose, cellobiose, larninaribiose and cellobionic acid, of which cellobiose is the most effective. Both, crystalline and ' amorphous ' reprecipitated cellulose were degraded efficiently during growth of S. thermophile on these insoluble substrates. Extracellular enzymes formed in cultures on cellobiose were also capable of attacking crystalline cellulose (Avicel) indicating that all the enzymes forming the cellulase complex (exo- and endo-cellulases) were formed. 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.

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