REGULATION OF CELLULASE SYNTHESIS IN *Chaetomium erraticum*

Sanjeev K. Soni [a*] and Raman Soni [b]

Chaetomium erraticum was capable of producing all the three components of a cellulase enzyme system including exoglucanase, endoglucanases, and β-glucosidase extracellularly. However, the cultivation conditions and the medium composition markedly affected the ability of microorganism to synthesize various enzymes. Exoglucanase was highest under static conditions, while endoglucanase and β-glucosidase were maximized under shake conditions. Among the various defined substrates, CMC proved to be the best inducer for exoglucanase under static conditions and β-glucosidase under shake conditions. MCC induced maximum endoglucanase under shake conditions. The biosynthesis of all three components of cellulases was repressed with different concentrations of glucose, puromycin, actinomycin, and actidione, while the supplementation of exogenous cyclic-AMP was fully capable of releasing the catabolite repression for production of all three components.

Keywords: Cellulases; Endoglucanase; Exoglucanase; β-glucosidase; Regulation; Catabolite repression

*Contact information: a: Department of Microbiology, Panjab University, Chandigarh 160014 INDIA; b: Department of Biotechnology, D.A.V. College, Sector 10, Chandigarh 160011 INDIA; *Corresponding author: sonisk@pu.ac.in*

INTRODUCTION

In view of depleting petroleum reserves and the rising prices of crude oil, the need for alternative sources of bioenergy is expected to increase sharply during the 21st century. Among potential alternative resources, cellulosic biomass has been identified as the prime source of biofuels and other value-added products. Lignocellulosics as agricultural, industrial, and forest residues account for the majority of the total biomass present in the world; it does not compete with human food and thus can be a cheaper source for biofuel production. To initiate the production of industrially important products from cellulosic biomass, bioconversion of the cellulosic components into fermentable sugars is necessary and is brought about by the synergistic action of three types of cellulases, viz. endoglucanases (EC 3.2.1.4), exoglucanases (EC 3.2.1.91), and β-glucosidases (EC 3.2.1.21) (Thongekkaew et al. 2008; Li et al. 2009).

Fungi are the main cellulase-producing microorganisms, although a few bacteria and actinomycetes have also been reported to yield cellulase activity. Fungal cellulases have been the major subjects of investigation over the years, as these catalyze the decay of lignocellulosic material in our ecosystem, and some fungi produce extracellular cellulases in significant amounts. Although several microorganisms have been evaluated for their ability to degrade cellulosic substrates, relatively few belonging to the genera

Trichoderma and *Aspergillus* have been studied in detail for their cellulase production potential (Kumar et al. 2008). Besides *Trichoderma viride*, the other mesophilic strains producing cellulases are *Fusarium oxysporium*, *Piptoporus betulinus*, *Penicillium echinulatum*, *P. purpurogenum*, *Aspergillus niger*, and *A. fumigatus.* (Kumar et al. 2008; Martins et al. 2008). The cellulases from *Aspergillus* usually have high β-glucosidase activity but lower endoglucanase levels, whereas *Trichoderma* has high endoglucanase and exoglucanase but lower β-glucosidase levels, and hence has limited efficiency in cellulose hydrolysis. Thermophillic fungi such as *Sporotrichum thermophile*, *Scytalidium thermophillum, Clostridium straminisolvens*, and *Thermonospora curvata* also produce the cellulase complex and can degrade native cellulose (Kato et al. 2004). Such thermophilic organisms may be valuable sources of thermostable cellulases. *Chaetomium* species are well known among the cellulolytic fungi for their potential to degrade cellulosic waste and for single cell protein (SCP) production. A few studies have been conducted earlier with a few species of *Chaetomium cellulolyticum*, *C. erraticum*, *C. fusisporale*, *C. globosum*, and *C. thermophile* to investigate their cellulolytic ability, localization, multiplicity, and characteristics of cellulase components (Fanhrich and Irrgang 1982; Sandhu and Puri 1989; Ganju et al. 1990; Lakshmikant and Mathur 1990; Soni et al. 1999), but still not much information is available regarding the regulation of cellulase biosynthesis in this genus. In this paper an attempt is made to investigate the possible mechanism of regulation of cellulases and their existing polymorphism in *Chaetomium erraticum*.

EXPERIMENTAL

Microorganism and Inoculum

Chaetomium erraticum, deposited with ATCC (No. 647740) and used in the present study was isolated from local soil and identified on the basis of cultural and morphological characteristics. The stock culture was maintained in soil and in sterilized paraffin oil at 4^{o}C. To revive the culture, sub-culturing was done on slants containing Vogel's glucose medium whenever required (Vogel 1956).

Inoculum

The discs (7mm diameter) cut from the periphery of the 72h-old cultures growing on Vogel's medium plates, incubated at 37^{o}C, were used as inoculum.

Cellulase Production on Various Substrates

This was studied by cultivating *C. erraticum* on different defined and insoluble delignified lignocellulosic carbon substrates, supplemented separately, in Vogel's complete medium (Vogel 1956). Fifty ml of Vogel's complete medium, pH 6.0, dispensed in 250 ml Erlenmeyer flasks contained 1% either of the various defined (carboxy-methyl cellulose (CMC), microcrystalline cellulose (MCC), cellobiose, galactose, pectin, glucose, and glycerol) and insoluble delignified lignocellulosic residues (wheat-straw, paddy-straw, sugarcane bagasse, dewaxed cotton, and wheat bran). The straws and bagasse were milled to fine mesh and delignified by the sodium

chlorite method (Sidhu and Sandhu 1980) before use. The flasks, in duplicate, were inoculated with five discs (7mm dia) cut from the periphery of actively growing colonies of *C. erraticum* on Vogel's glucose agar plates and incubated at 37°C in two different sets, one as static and the other as shake cultures (200 rpm). Each set was analyzed at regular intervals of 24 h, for 16 days.

The contents of each flask were centrifuged at 22,000 × g for 30 min at 4°C after decanting these free from the mycelium. The supernatant, thus obtained, was used as the extracellular enzyme and assayed for different components of cellulase enzyme complex viz. Exo-β-1,4-glucanase (EC 3.2.1.91), Endo-β-1,4-glucanase (EC 3.2.1.4), and β-1,4-glucosidase (EC 3.2.1.21), as discussed earlier (Soni et al. 1999), and stored at -20°C until further use.

Detection of Endoglucanase and β-glucosidase Isozymes

The polymorphic nature of endoglucanase and β-glucosidase in *C.erraticum* was revealed by polyacrylamide gel electrophoresis as reported earlier (Soni et al. 1999).

Catabolite Repression of Cellulase Production

The effect of readily utilizable carbon sources on cellulase production in *C. erraticum* was studied by dispensing varying concentrations (1-5%) of glucose to the cultures growing on 1% CMC during the exponential phase (i.e. 4th day of cultivation). The samples were later withdrawn at regular intervals of 24 h for 12 days, and processed as mentioned above. The supernatant obtained after dialysis against 0.1M sodium acetate buffer at pH 5.0 was assayed for cellulase components along with acidic, neutral, and alkaline proteases.

Proteases Assay

The extracellular proteases were estimated by the method of Makonnen and Porath (1968) using casein as the substrate at three different pH ranges of acidic, neutral, and alkaline, using sodium acetate (pH 5.0), Tris-acetate (pH 7.0), and sodium phosphate (pH 8.0) buffers, respectively. The enzyme activity was expressed in International units (IU) as the amount of enzyme required to release 1 μM of tyrosine per minute at 37°C.

Efffect of Exogenous Adenosine-3',5'-monophosphate (cAMP) on Catabolite Repression of Cellulase Production

C. erraticum, which was grown in Vogel's complete medium containing 1% CMC at 37°C, was dispensed with 5% glucose on the 4th day of cultivation, followed by the addition of 1mM cAMP 24h after the addition of glucose. The samples were withdrawn at regular intervals of 24 h for 12 days. The supernatant obtained was used for the determination of cellulases along with alkaline proteases.

Effect of Different Antibiotics on Cellulase Regulation

To various sets of CMC-grown static cultures of *C. erraticum*, different concentrations of puromycin (100 and 200 μg/ml), actinomycin D (10 and 20 μg/ml), and actidione (10, 20, and 40 μg/ml) were supplemented, separately, on the 4th day of cultivation at 37°C. In another set, 30 mM glucose was added simultaneously, and the

contents were analyzed for cellulases until 8 h at regular intervals of 1h for puromycin and actinomycin D and for actidione at regular intervals of 24 h for 6 days.

RESULTS AND DISCUSSION

Production of Cellulases on Different Substrates

Medium composition markedly affects the cellulolytic ability of micro-organisms. The production of cellulase enzymes is induced only in the presence of cellulosic substrate (or products there of), but it is suppressed when readily utilizable sugars, such as glucose, are available (Carle-Urioste et al. 1997). Among the cellulosic materials, sulfite pulp, printed papers, mixed waste paper, wheat straw, paddy straw, sugarcane bagasse, jute- stick, carboxy-methyl cellulose, corncobs, groundnut shells, cotton, ball milled barley straw, delignified ball milled oat spelt xylan, larch wood xylan, etc. have been evaluated as substrates for cellulase production (Lynd et al. 2002). Menon et al. (1994) also demonstrated that CMC or cereal straw (1%, w/w) would be the best carbon source compared to sawdust for endoglucanase and β-glucosidase production using *C. globosum* as the cellulolytic agent. Induction of cellulases by MCC has been reported in *C . thermophile* (Ganju et al. 1990) and *T. koningii* (Sidhu et al. 1984).

The different components of cellulase complex in *C. erraticum* showed variation in their levels on different cellulosic substrates used as carbon sources in the present study, with better yields on defined substrates. CMC appeared to be a good inducer for all the three components of cellulases. The peaks for different components were achieved with different substrates. MCC induced maximum endoglucanase under both static and shake conditions, while exoglucanase and β-glucosidase were induced maximally under shake conditions only.

The production of exoglucanase was highest with CMC, inducing the yield of 0.44 IU/ml on the 8th day of cultivation under static conditions (Table 1). On the other hand, except for wheat bran, which produced 0.15 IU/ml on the 10^{th} day under shake conditions, all other delignified substrates proved to be poor inducers as compared to defined substrates. On the contrary, the induction of endoglucanase was higher under shake conditions on defined and delignified lignocellulosic substrates. Highest levels were induced by MCC after the 8th day (1.30 IU/ml) of incubation. Among the various delignified lignocellulosic substrates, sugarcane bagasse proved to be the best one, producing 1.25 IU/ml after the 12th day of incubation under shake conditions (Table 1). The induction of β-glucosidase was good in presence of defined substrates, with CMC and MCC producing the highest levels of 0.09 and 0.09 IU/ml after 16 and 14 days of cultivation as shake cultures, respectively. Delignified sugarcane bagasse also induced comparable yield of 0.09 IU/ ml after 10 days under shake conditions of growth.

Table 1. Effect of Various Carbon Sources on the Production of Cellulases by *Chaetomium erraticum*

Days	Exoglucanase		Endoglucanase		β-glucosidase	
	Static	Shake	Static	Shake	Static	Shake
CMC						
2	0.07	0.04	0.06	0.39	0.00	0.00
4	0.09	0.03	0.06	0.32	0.00	0.00
6	0.34	0.06	0.15	0.35	0.01	0.01
8	0.44	0.02	0.38	0.62	0.05	0.05
10	0.34	0.01	0.28	0.68	0.05	0.02
12	0.26	0.04	0.78	0.82	0.04	0.01
14	0.16	0.02	0.69	0.72	0.05	0.03
16	0.08	0.01	0.70	0.60	0.05	0.01
MCC						
2	0.00	0.00	0.30	0.80	0.03	0.00
4	0.01	0.00	0.10	0.99	0.03	0.02
6	0.00	0.01	0.50	1.10	0.00	0.01
8	0.00	0.00	0.45	1.30	0.03	0.01
10	0.01	0.00	0.55	0.60	0.00	0.05
12	0.00	0.06	1.02	0.20	0.01	0.07
14	0.00	0.10	0.30	0.19	0.00	0.09
16	0.00	0.07	0.25	0.19	0.01	0.07
Glucose						
2	0.00	0.00	0.00	0.00	0.00	0.00
4	0.00	0.00	0.00	0.00	0.00	0.00
6	0.06	0.01	0.10	0.00	0.00	0.00
8	0.05	0.01	0.11	0.01	0.00	0.00
10	0.02	0.04	0.10	0.01	0.00	0.00
12	0.02	0.05	0.26	0.00	0.00	0.00
14	0.01	0.00	0.14	0.00	0.00	0.00
16	0.00	0.00	0.12	0.00	0.00	0.00
Glycerol						
2	0.00	0.00	0.00	0.00	0.00	0.00
4	0.01	0.00	0.01	0.00	0.00	0.00
6	0.06	0.00	0.13	0.00	0.00	0.00
8	0.07	0.01	0.26	0.01	0.00	0.00
10	0.02	0.01	0.28	0.01	0.02	0.00
12	0.02	0.01	0.20	0.12	0.02	0.00
14	0.01	0.01	0.20	0.06	0.00	0.00
16	0.01	0.00	0.12	0.02	0.00	0.00

Table 1, Continued.						
Days	Exoglucanase		Endoglucanase		β-glucosidase	
	Static	Shake	Static	Shake	Static	Shake
Cellobiose						
2	0.01	0.00	0.30	0.35	0.02	0.00
4	0.00	0.00	0.34	0.40	0.03	0.02
6	0.00	0.01	0.30	0.60	0.00	0.01
8	0.02	0.00	0.32	0.68	0.03	0.01
10	0.01	0.00	0.54	0.61	0.00	0.05
12	0.00	0.06	0.68	0.19	0.01	0.07
14	0.00	0.10	0.36	0.19	0.00	0.09
16	0.00	0.07	0.25	0.17	0.01	0.08
Galactose						
2	0.00	0.00	0.00	0.00	0.00	0.00
4	0.00	0.00	0.01	0.00	0.00	0.00
6	0.00	0.00	0.02	0.02	0.01	0.00
8	0.00	0.00	0.02	0.03	0.01	0.00
10	0.00	0.00	0.02	0.01	0.01	0.01
12	0.00	0.00	0.03	0.00	0.00	0.01
14	0.00	0.00	0.03	0.00	0.00	0.01
16	0.00	0.00	0.01	0.00	0.00	0.01
Pectin						
2	0.05	0.21	0.05	0.21	0.01	0.01
4	0.23	0.02	0.23	0.02	0.00	0.01
6	0.06	0.02	0.06	0.02	0.01	0.01
8	0.02	0.00	0.02	0.00	0.01	0.03
10	0.00	0.00	0.00	0.00	0.01	0.02
12	0.00	0.00	0.00	0.00	0.01	0.06
14	0.08	0.00	0.08	0.00	0.02	0.03
16	0.06	0.00	0.06	0.00	0.02	0.04
Paddy straw						
2	0.00	0.00	0.03	0.05	0.00	0.00
4	0.01	0.01	0.04	0.05	0.00	0.00
6	0.02	0.03	0.04	0.05	0.00	0.00
8	0.02	0.04	0.05	0.06	0.00	0.00
10	0.03	0.03	0.05	0.04	0.00	0.01
12	0.02	0.02	0.04	0.04	0.00	0.01
14	0.02	0.02	0.04	0.03	0.00	0.01
16	0.02	0.01	0.02	0.02	0.00	0.01
Sugarcane bagasse						
2	0.08	0.05	0.09	0.17	0.01	0.01
4	0.07	0.08	0.20	0.92	0.01	0.06
6	0.01	0.08	0.17	0.98	0.01	0.05
8	0.06	0.07	0.25	1.20	0.01	0.06
10	0.07	0.08	0.13	1.23	0.00	0.09
12	0.01	0.08	0.61	1.25	0.04	0.06
14	0.03	0.08	0.29	0.88	0.02	0.05
16	0.03	0.08	0.36	0.92	0.02	0.04

Table 1, Continued.						
Days	Exoglucanase		Endoglucanase		β-glucosidase	
	Static	Shake	Static	Shake	Static	Shake
Dewaxed cotton						
2	0.03	0.05	0.28	0.35	0.00	0.00
4	0.02	0.04	0.29	0.32	0.00	0.00
6	0.03	0.04	0.29	0.38	0.00	0.01
8	0.04	0.05	0.35	0.42	0.00	0.01
10	0.05	0.06	0.36	0.88	0.00	0.03
12	0.05	0.04	0.72	0.78	0.00	0.04
14	0.07	0.04	0.80	0.78	0.00	0.05
16	0.06	0.02	0.76	0.72	0.00	0.04
Wheat straw						
2	0.00	0.00	0.01	0.03	0.00	0.00
4	0.00	0.00	0.02	0.04	0.00	0.00
6	0.00	0.00	0.02	0.04	0.00	0.00
8	0.00	0.01	0.02	0.04	0.00	0.01
10	0.00	0.03	0.03	0.04	0.00	0.01
12	0.00	0.03	0.03	0.03	0.00	0.01
14	0.00	0.01	0.01	0.02	0.00	0.01
16	0.00	0.00	0.00	0.02	0.00	0.01
Wheat bran						
2	0.04	0.01	0.11	0.18	0.00	0.01
4	0.07	0.03	0.25	0.38	0.00	0.02
6	0.07	0.13	0.31	0.27	0.00	0.03
8	0.06	0.04	0.28	0.28	0.00	0.01
10	0.09	0.15	0.21	0.38	0.00	0.02
12	0.06	0.04	0.27	0.62	0.01	0.06
14	0.05	0.06	0.16	0.62	0.01	0.07
16	0.01	0.02	0.11	0.22	0.01	0.02

Low enzyme production on insoluble substrates might have resulted due to slow metabolism of the fungus on these substrates, which are not known to be hydrolyzed easily, or due their exclusion along with the unutilized substrates, since they are known to be more strongly absorbed on cellulose particles (Bagga and Sandhu 1987). Ghose and Bisaria (1979) reported absorption of cellulase to be a widely occurring phenomenon amongst cellulolytic fungi, and it is a prerequisite for cellulose hydrolysis (Manning and Wood 1983). Although most of the substrates used were capable of inducing all three of the components, except for glucose, the levels of enzyme produced varied greatly, depending upon the nature of the substrate and the conditions of cultivation. From this differential response of the fungus shown towards induction, it appears that synthesis of the individual components of cellulases in *C. erraticum* may not be regulated in a strictly coordinated manner. The appearance of peak levels with different substrates at different intervals indicates that these enzymes are not secreted into the extracellular medium simultaneously. Such reports are available in the literature where different cellulolytic components were released sequentially. In *Sporotrichum thermophile* (Coutts and Smith 1976) and *A. nidulans* (Trivedi and Rao 1980), endocellulases were released first into the medium, followed by exoglucanases.

Isozymes of Endoglucanase and β-glucosidase

The use of electrophoretic technique revealed the existence of multiple forms of endoglucanase and β-glucosidase in *C. erraticum*, indicating the polymorphic nature of cellulase enzymes in this fungus. The multiplicity of cellulases as observed in the present study has also been observed in different fungi and bacteria (Sandhu et al. 1991; Soni et al. 1999; 2005; Kumar et al. 2008). Cellulolytic enzyme systems from the filamentous fungi, especially *T. reesei*, contain two exoglucanases or cellobiohydrolases (CBH1 and CBH2), at least four endoglucanases (EG1, EG2, EG3, EG5), and one β-glucosidase (Kumar et al. 2008). The differential effect of different carbon sources has also been proven by electrophoretic studies carried out with the *C. erraticum* culture filtrates grown on these substrates. This revealed two forms of endoglucanase after induction with various defined carbon substrates (Fig.1), but the cellulases induced by insoluble delignified lignocellulosic substrates revealed an additional form of endoglucanase, i.e. EG III, on the polyacrylamide gel. Perhaps this additional form is required for rapid hydrolysis of cellulose by *C. erraticum.* On the contrary, only two forms of β-glucosidase were observed in the different culture filtrates, irrespective of the substrate used for induction (Fig. 2). Corresponding to the different peak levels at different intervals, the intensities of the isozymes were variable. The bands remained undetected on the gels when culture filtrates having low activity of endoglucanases were subjected to electrophoresis. This differential appearance of different multiple forms at different intervals and under different conditions has also been reported in *A.nidulans* (Bagga et al. 1989), *C. fusisporale* (Sandhu and Puri 1989), and *C. erraticum* (Soni et al. 1999).

Catabolite Repression of Cellulase Production

With the addition of varying concentrations of glucose, the levels of exoglucanase, endoglucanases, and β-glucosidase decreased, indicating their repression, which was generally proportional to the concentrations of sugar added (Fig. 3, a-c). This kind of cellulase repression, in the presence of readily utilizable sugars, has already been shown by several workers (Manning and Wood 1983; Strauss et al. 1995; Ilmén et al. 1996; Lynd et al. 2002). Like other fungi, *C. erraticum* utilizes cellulose for growth and regulates cellulase biosynthesis by feedback control in the presence of soluble free sugars. Catabolite repression can cause decrease or cessation of enzyme biosynthesis but the reason for the decrease in presynthesized enzyme activities is hard to explain. Horton and Keen (1966) reported inactivation of cellulolytic enzymes due to readily metabolizable sugars, which was correlated with a drastic drop in pH of the medium and later described as a pH effect by Halliwell (1979). But as the pH in the present study never dropped below 7.0 (data not shown), the inactivation due to a pH effect is ruled out. Various strains of *Cellulomonas* have been reported to produce high yields of cellulase on cellulosic substrates and lower yields on xylan, galactomannan, starch, and sugars (Poulsen and Petersen 1988). The production of cellulases by *T. reesei* is also repressed in the presence of glucose, but a basal level of production occurs in the absence of glucose (Carle-Urioste et al. 1997).

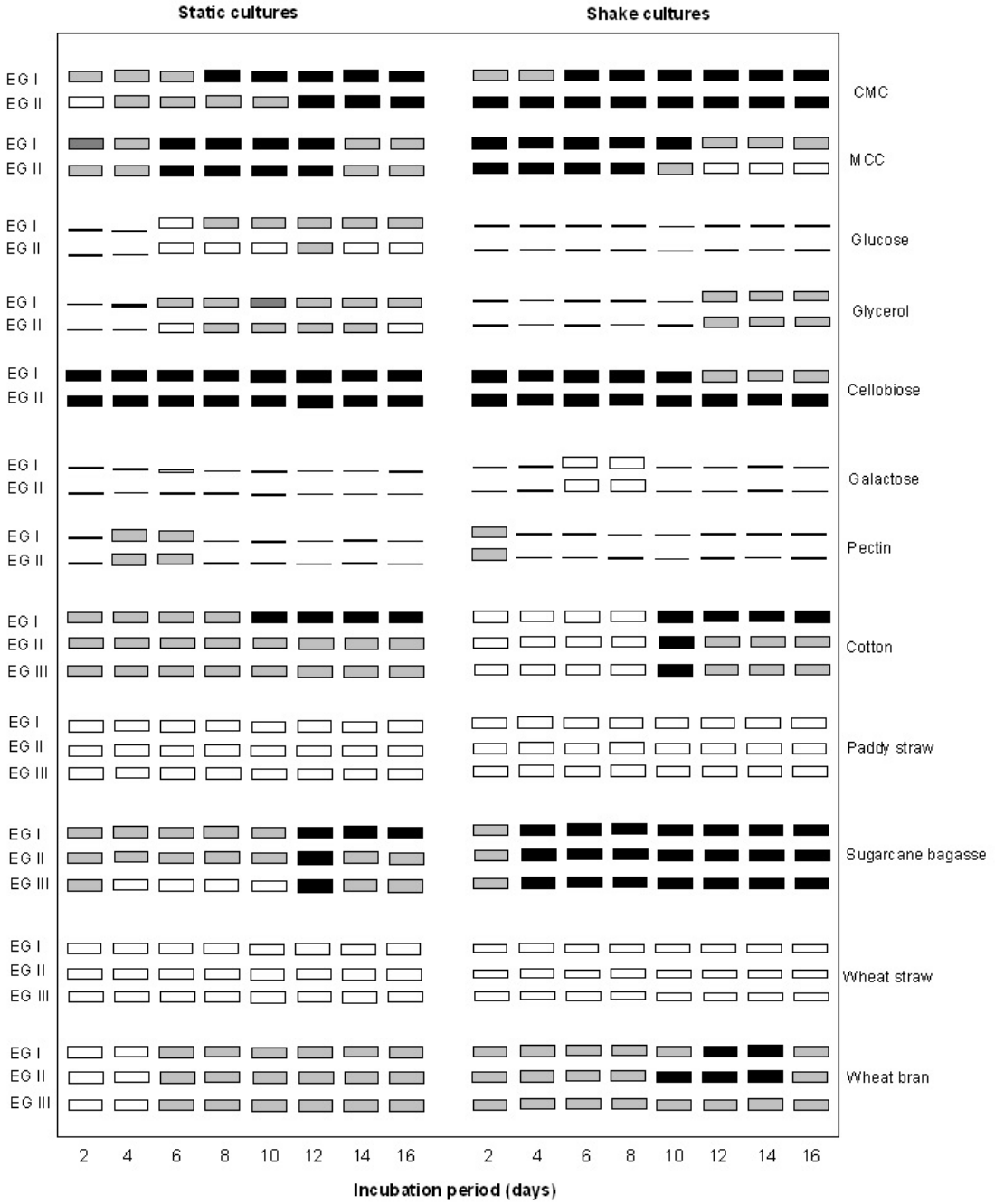

Fig. 1. Zymograms showing isozymes of endoglucanase from *C. erraticum* at various intervals when grown on various defined and delignified lignocellulosic substrates under static and shake conditions of growth

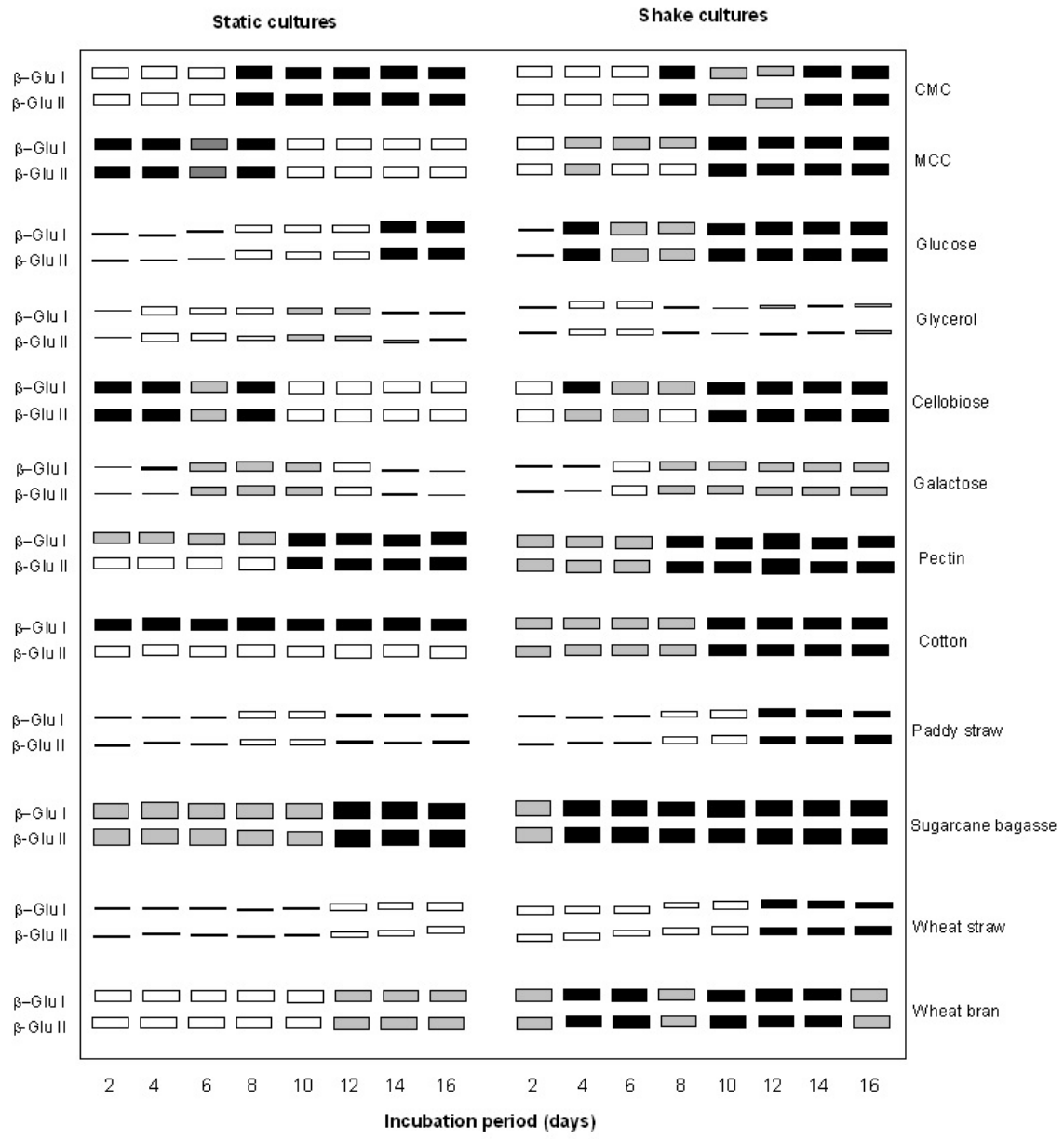

Fig. 2. Zymograms showing isozymes of β-glucosidase from *C. erraticum* at various intervals when grown on various defined and delignified lignocellulosic substrates under static and shake conditions of growth

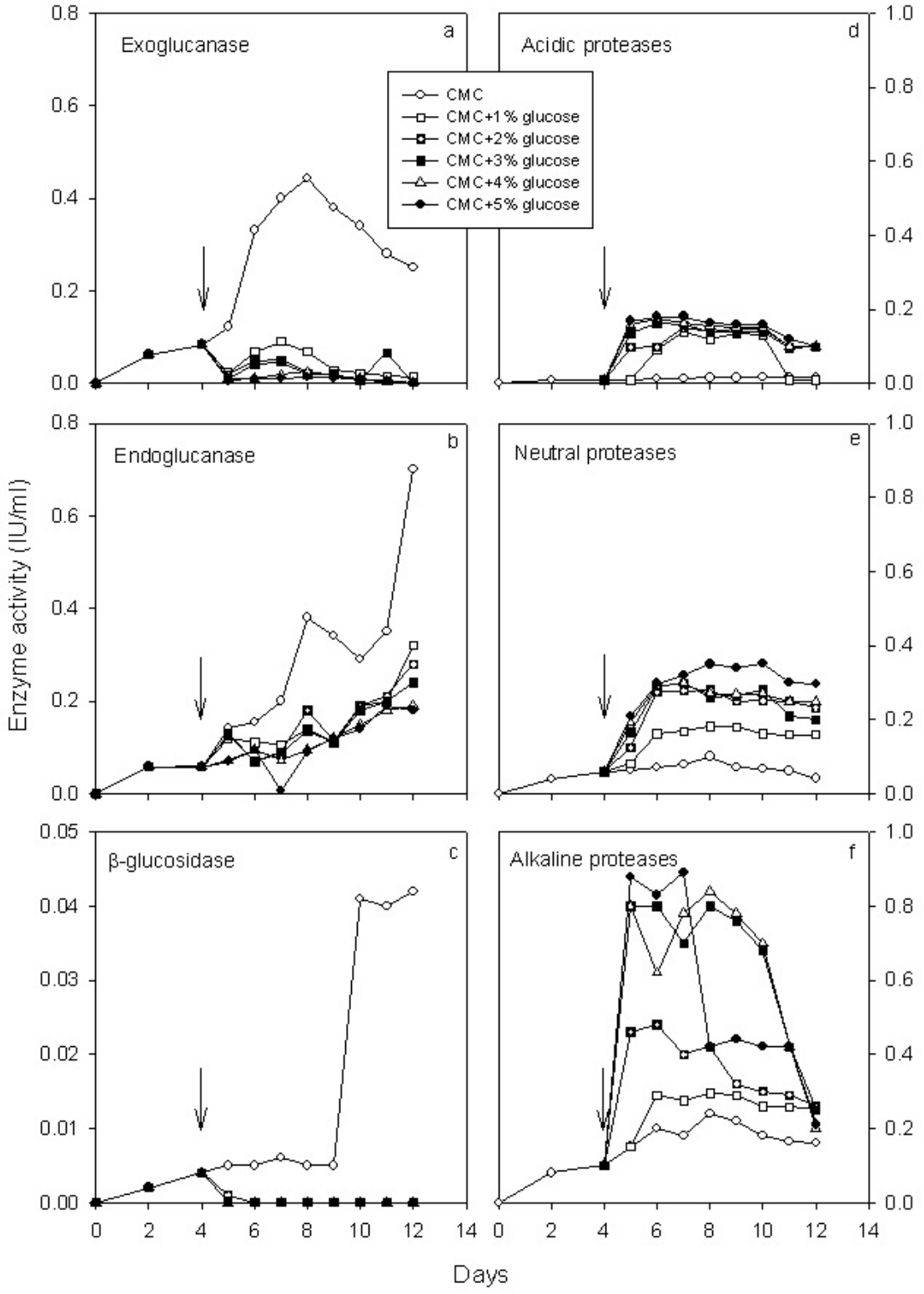

Fig. 3. Effect of addition of varying concentrations of glucose on the production of cellulases and proteases in CMC induced cultures of *C. erraticum*. Arrows indicate when glucose was added.

The addition of glucose also brought about the synthesis of extracellular acidic, neutral, and alkaline proteases in the culture. Highest levels of all the proteases were observed in the presence of 5% glucose, and among them the levels of alkaline proteases were highest, followed by neutral and acidic proteases (Fig. 3, d-f). Earlier studies conducted with *Trichoderma* spp. and *Aspergillus* spp. support the present results, indicating that protease levels get enhanced during catabolite repression (Kalra et al. 1986). Not only in these fungi, but in yeast also the proteolytic enzymes synthesized in the state of catabolite repression are known to degrade malate dehydrogenase (Ferguson et al. 1967), β-galactosidase, phosphoenol pyruvate, carboxykinase, and fructose-1,6-biphosphatase (Holzer 1976).

Effect of Exogenous Adenosine-3'5'-monophosphate on Catabolite Repression of Cellulase

In general, repression seems to occur under various conditions in which catabolism exceeds the requirements of cells for biosynthetic process. However, neither the real chemical identity of the catabolite repressor nor the mechanism that underlies this phenomenon has been identified.

Catabolite repression of cellulase genes occurs in the presence of glucose and may be regulated by cAMP levels, as indicated by studies done with *Thermobifida curvata* (Wilson and Irwin 1999). The addition of exogenous 1mM cAMP to the CMC grown *C. erraticum*, although it could not induce cellulases, was fully capable of releasing repression caused due to 5 % glucose. The *C. erraticum* cultures grown on CMC alone presented a regular increase in exoglucanase, endoglucanase, and β-glucosidase activities. The production profiles, though slightly higher, were very similar to the above when 1mM cAMP was added to CMC-grown cultures (Fig. 4, a-d). The addition of 5% glucose inactivated and repressed all the three cellulolytic activities. The addition of 1mM cAMP to this repressed culture not only lifted repression, but it also increased production of the three cellulases. The pH of the culture filtrate remained alkaline throughout (data not shown); therefore, only the production profile of alkaline proteases in the filtrates was determined. The production profile of proteases showed an increase up to the 8th day of incubation, recording a maximum of 0.26 IU/ml, and followed a decline thereafter until the termination of the experiment (Fig. 4, d). The addition of glucose on the 4th day of cultivation led to a pronounced increase in protease production, being maximum on the 5th day of the experiment. The addition of cAMP to the cultures slightly increased the protease production, but the production profile pattern was similar to the control. The addition of exogenous cAMP to the repressed cultures caused a drop in the protease levels.

Bagga et al. (1991) and Wang et al. (1996) also reported that cAMP plays a role in derepression of enzyme synthesis in mycelial fungi. Regarding the mode of action of cAMP, which is a hydrophobic and negatively charged compound, it is not known exactly whether it enters the fungal cells or just binds to their surface to trigger a secondary signal. Terenzi et al. (1976), working with adenyl-cyclase deficient mutants of *Neurospora*, suggested that cAMP itself enters the cells and binds to some cAMP receptors and thereby produces a response similar to that of the endogenous cAMP. On

the other hand, studies on *Coprinus macrorhizus* suggest that exogenous cAMP acts after binding to an extracellular receptor (Uno and Ishikawa 1971).

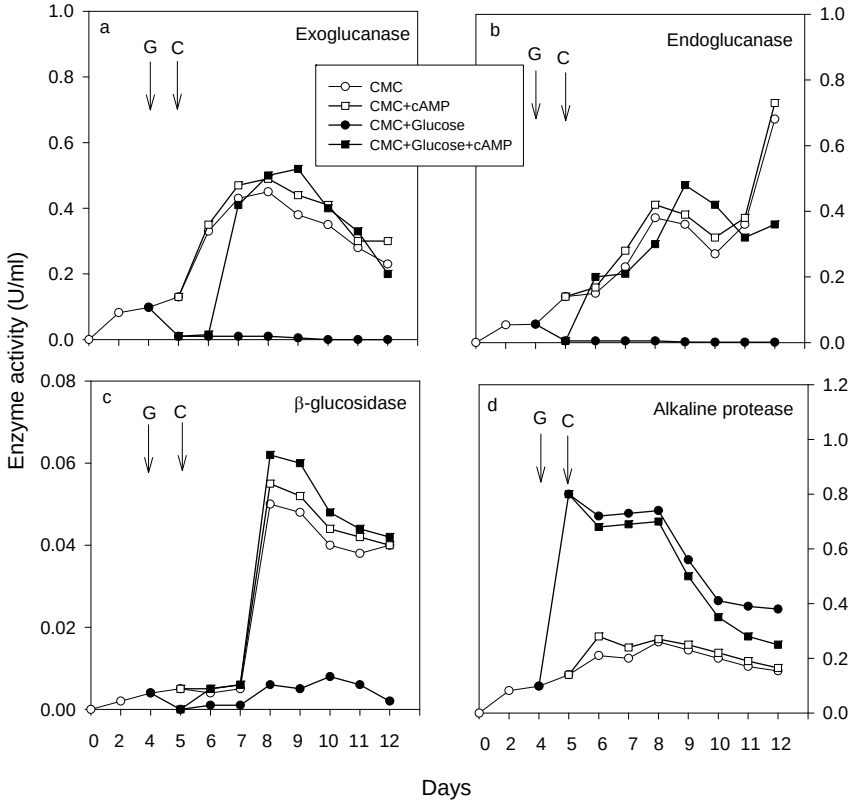

Fig. 4. Effect of addition of exogenous cAMP on CMC and CMC+glucose grown cultures of *C. erraticum.* Arrows indicate when glucose and cAMP were added.

The Effect of Antibiotics on Catabolite Repression

The antibiotics actinomycin-D, actidione, and puromycin are known to inhibit protein synthesis at transcriptional and translational levels, respectively. These are also capable of inhibiting the inductive formation of enzymes due to their specific inhibitory actions.

Puromycin

The supplementation of various concentrations of puromycin (data not shown for all concentrations) led to a decrease in enzyme production just after 1h of addition. The cellulase components were completely lost in the culture within 5 to 8h of incubation in the presence of puromycin. Similarly, with the addition of 30mM glucose, the inactivation started just after 1h of addition and caused complete inactivation of exo- and endo-glucanase after 7h of incubation and β-glucosidase after 5 h (Fig. 5, a-c). The cellulase inactivation observed just after the addition of glucose and puromycin may be due to inactivation of mRNA, which codes for cellulases, and thus both appear to act at a translational level. Similar studies conducted by Nisizawa et al. (1972), in *T. viride*

have shown that catabolite repression is due to glucose, and puromycin seems to act at a translational level.

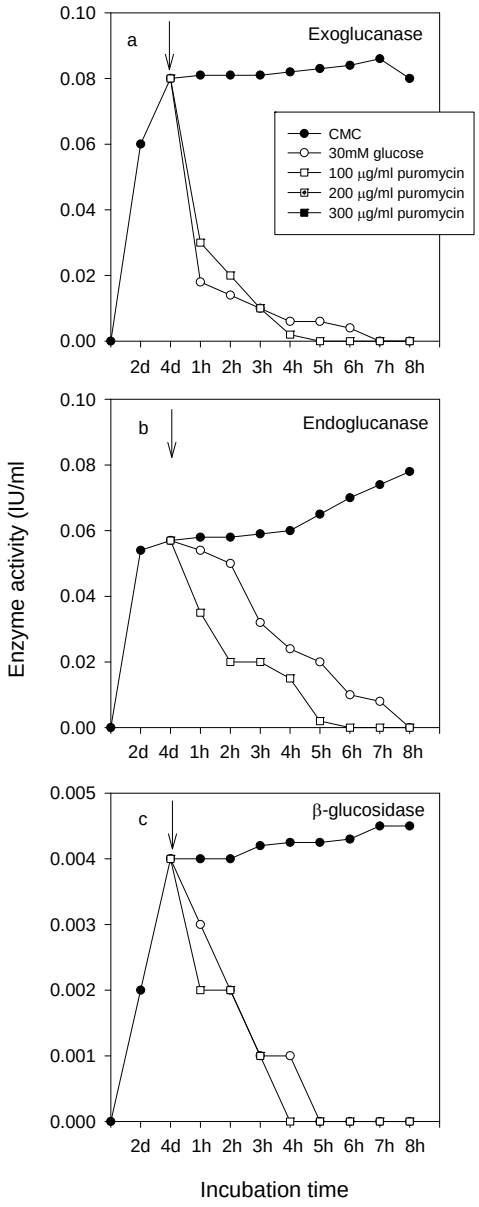

Fig. 5. Effect of puromycin and glucose on the production of cellulases in CMC induced cultures of *C. erraticum.* Arrows indicate when glucose and puromycin were added

Actinomycin-D

The addition of actinomycin-D to the cellulase-induced culture led to a decrease in the levels of cellulases after 3-4 h of the addition of actinomycin-D, whereas the glucose (30mM) caused inactivation, just after 1h of its addition. (Figs. 6 a-c). Actinomycin-D did not affect production of cellulases, as the mRNA already produced

doesn't get affected, and it is the DNA that is affected. New mRNA is not synthesized further, while already-synthesized mRNA continued to produce cellulases in the medium.

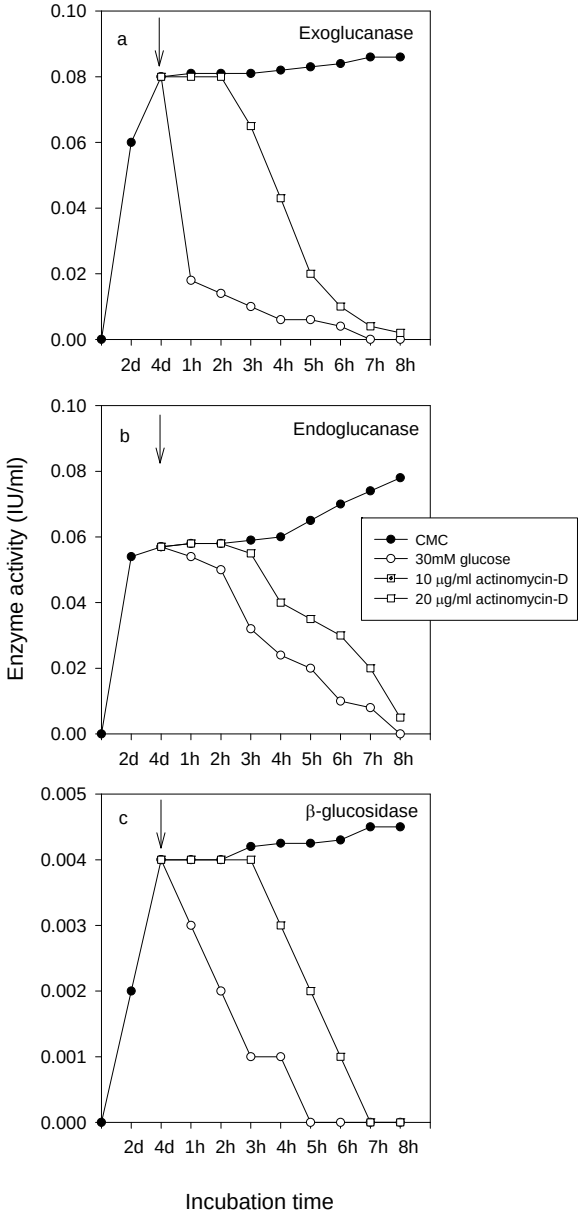

Fig. 6. Effect of actinomycin-D and glucose on the production of cellulases in CMC induced cultures of *C. erraticum.* Arrows indicate when glucose and actinomycin-D were added.

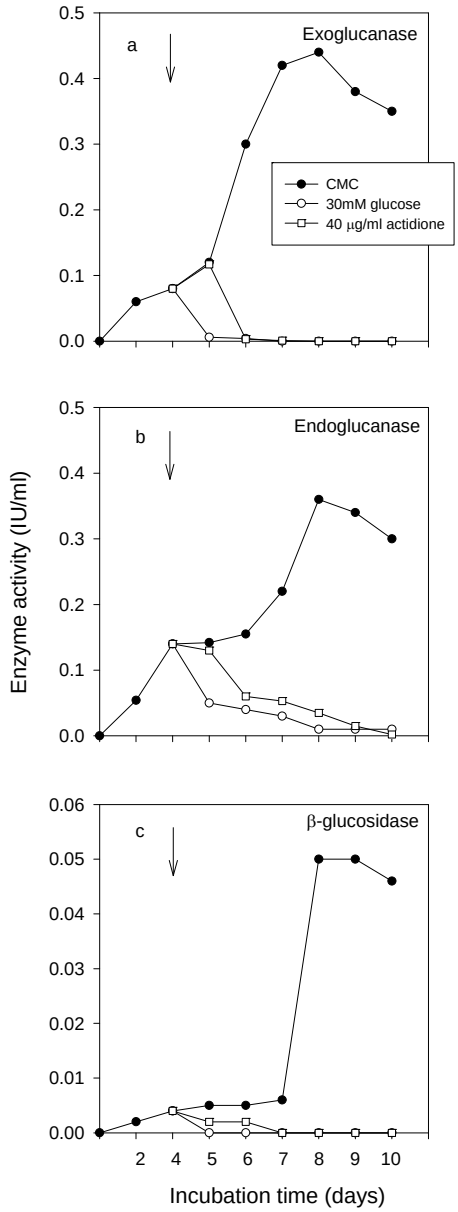

Fig. 7. Effect of actidione and glucose on the production of cellulases in CMC induced cultures of *C. erraticum*. Arrows indicate when glucose and actidione were added.

Actidione

The addition of actidione in the cellulase-induced cultures resulted in the inactivation of different components of cellulase after 2 to 3 days of incubation, while the repression of cellulases by addition of glucose was initiated just after a day of its addition (Fig. 7a-c). Actidione appeared to affect the whole system of fungus and thus inhibited cellulases.

CONCLUSIONS

C. erraticum appears to have potential for the production of a complete cellulase enzyme complex and therefore can be exploited for hydrolysis of cellulosic waste. The regulation mechanism including catabolite repression and presence of multiple molecular forms of endoglunase and β-glucosidase suggests a level of complexity for the synthesis of cellulases in this fungus and are probably regulated by separate genes. Although extracellular synthesis of cellulases proved to be an inducible system, as it has been affected by the nature and diversity of substrates used, the inactivation of cellulolytic components due to the presence of antibiotics further suggests that all the components are probably also regulated at the transcriptional and translational levels.

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Article submitted: Aug. 29, 2009; Peer review completed: Oct. 4, 2009; Revised version received and accepted: Nov. 9, 2009; Published: Nov. 13, 2009.