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REGULATION OF CELLULOLYTIC ACTIVITY IN *CELLULOMONAS* SP. IIBC

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Abstract

The effect of glucose, cellobiose and xylose on the regulation of cellulolytic activity in Cellulomonas *sp. IIbc was investigated. The addition of 1% glucose, cellobiose or xylose to actively growing bagasse pith cultures repressed the cellulose system. No enzyme inactivation by sugar concentrations up to 1·5% was detected. Both cellobiose and xylose, at concentrations ranging from 0·005 to 0·02%, served as inducers of the cellulolytic complex in resting cell experiments, while a basal level of constitutive synthesis occurred without any cellulosic substrate or derivative in the culture medium. Copyright © 1996.*

Key words: Cellulomonas, cellulase, enzyme regulation, bagasse, endoglucanase.

INTRODUCTION

The regulation of cellulolytic enzymes in bacteria has been studied in different species. Gong and Tsao (1979) have proposed a general model for cellulose regulation which suggests that a basal constitutive level of cellulase hydrolyses cellulose, providing small amounts of cellobiose. This cellobiose would then act as an inducer, while high levels of intracellular glucose could either inhibit cellulase activity or repress its synthesis. Alternatively, Hulme and Stranks (1971) suggested that catabolic repression is the only regulatory mechanism for cellulase regulation in *Myrothecium verrucaria*. In *Clostridium thermocellum*, constitutive cellulases have been reported (Golovchenko *et al.*, 1986), but induction of the cellulolytic system by cellobiose has also been demonstrated for different strains (Bhat *et al.*, 1993). In this paper, the mechanism of cellulase regulation for a strain of *Cellulomonas* used in single-cell-protein production studies is investigated.

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METHODS

Strain and cultivation conditions

Cellulomonas sp. IIbc (Enríquez, 1981) was used throughout this study. The inoculum was prepared from 48 h incubated nutrient agar slants. Cultures were grown in a rotary shaker at 100 rpm and 32°C, in MO medium, except for demonstration of constitutive synthesis, where nutrient broth medium was used. MO medium is composed of (g/l) $Na_2HPO_4 \cdot 12H_2O$, 10·0; KH_2PO_4, 2·5; NH_4Cl, 2·0; NaCl, 0·25; $MgSO_4 \cdot 7H_2O$, 0·25; thiamine, 0·001 (Rodríguez *et al.*, 1983). Nutrient agar was the Oxoid product, containing, in g/l: 'Lab-Lemco' powder, 1·0; yeast extract, 2·0; peptone, 5·0; NaCl, 5·0; agar No. 3, 15·0. Nutrient broth medium has the same composition as nutrient agar, but lacks agar. For cultivation, alkali-treated bagasse pith (Rodríguez *et al.*, 1993), glucose, cellobiose and xylose were used at 1%, and cellulose (CC-41 Whatman) was employed at 2%.

Analysis

CMC-activity and FP-activity were determined in the total culture sample as previously described (Rodríguez & Volfová, 1984), by measuring the reducing sugars formation from carboxymethylcellulose (CMC; BDH, degree of substitution 0·7–0·8) and filter paper strips (Whatman No. 1), respectively. International units (IU) represent μmoles of glucose released per minute, and specific activity was reported as IU per optical density unit of the culture (IU/OD). The concentration of sugars was determined by the dinitrosalicylic acid method (Miller, 1959). Growth was monitored by measurement of the absorbance at 600 nm.

Induction experiment

To investigate the effect of glucose, cellobiose and xylose on the induction of cellulolytic enzymes, an 18 h glucose culture was centrifuged, washed and resuspended in MO medium without nitrogen, in the

Table 1. CMC-activity of *Cellulomonas* sp. IIbc cultivated in MO medium with different carbon sources (IU/OD $\times 10^{-2}$)[a]

Culture time (h)	CMC-activity			
	Cellulose	Glucose	Cellobiose	Xylose
0	0	1·3	2·3	1·0
5	0·24	0·08	0·6	0·5
12	2·62	0	0	0
24	7·74	0·5	0·1	0·2
36	5·40	0·2	0·2	0
48	2·40	nd	nd	nd
69	2·16	nd	nd	nd

[a]Average values from triplicated experiments.
nd: Not determined.

presence of different concentrations of glucose, cellobiose or xylose. The suspensions were incubated for 4 h at 32°C and 100 rpm and dialysed overnight against 0·9% NaCl, prior to enzymatic activity measurements.

RESULTS AND DISCUSSION

Some cellulolytic bacteria, like *Clostridium thermocellum*, are able to produce cellulases in soluble carbon sources, such as fructose, cellobiose and glucose (Nochur *et al.*, 1993). In our strain, the activity per unit of biomass (IU/OD) sharply decreased with cultures grown on soluble sugars during the first hours of growth, in contrast to that of cellulose-grown cultures (Table 1). This indicates that no cellulase is synthesized in the presence of the sugars, suggesting repression, inhibition or inactivation of the cellulolytic enzymes.

Inhibition of enzyme activity by the sugars can be disregarded, since the samples were dialysed before the activity determination. In order to investigate the possibility of inactivation by glucose, cellobiose or xylose, the supernatant from a bagasse pith culture at stationary phase was mixed separately with 0·5, 1 and 1·5% of each sugar, and incubated on a shaker at 30°C and 100 rpm. Samples were taken at different times from the incubated mixtures, dialysed overnight against 0·9% NaCl, and evaluated for CMC- and FP-activity. In all cases, the CMC-activity and the FP-activity of the samples incubated with the sugars were similar to those of the control (samples incubated without sugars, data not shown), indicating that inactivation of the enzymes by any of the three sugars did not take place during the incu-

Table 2. Effect of cellobiose and xylose on the induction of cellulolytic activity[a]

Sugar	Concentration (%)	Enzymatic activity (IU/ml $\times 10^{-2}$)	
		CMC	FP
Cellobiose	0	4·1	0·4
	0·001	4·0	0·4
	0·005	4·5	1·0
	0·01	21·8	3·9
	0·02	41·0	5·9
Xylose	0	6·9	0·20
	0·001	7·0	0·18
	0·005	6·6	0·23
	0·01	7·1	0·97
	0·02	15·0	1·2

[a]Washed cells were incubated in MO medium without nitrogen with the different concentrations of each sugar. Figures represent mean values of triplicated experiments.

Table 3. Cellulolytic activity of *Cellulomonas* sp. IIbc grown in nutrient broth in comparison with cultures on MO medium plus 1% bagasse pith[a]

Culture medium	Culture time (h)	Growth phase	Enzymatic activity (IU/ml $\times 10^{-3}$)	
			CMC	FP
Nutrient broth	0	lag	0·92	0·43
	7	log	1·5	1·0
	21	stat.	11·0	1·4
Bagasse pith	0	lag	1·5	0·14
	24	log	71·0	1·4
	48	stat.	140·0	14·0

[a]Average values of triplicated experiments.

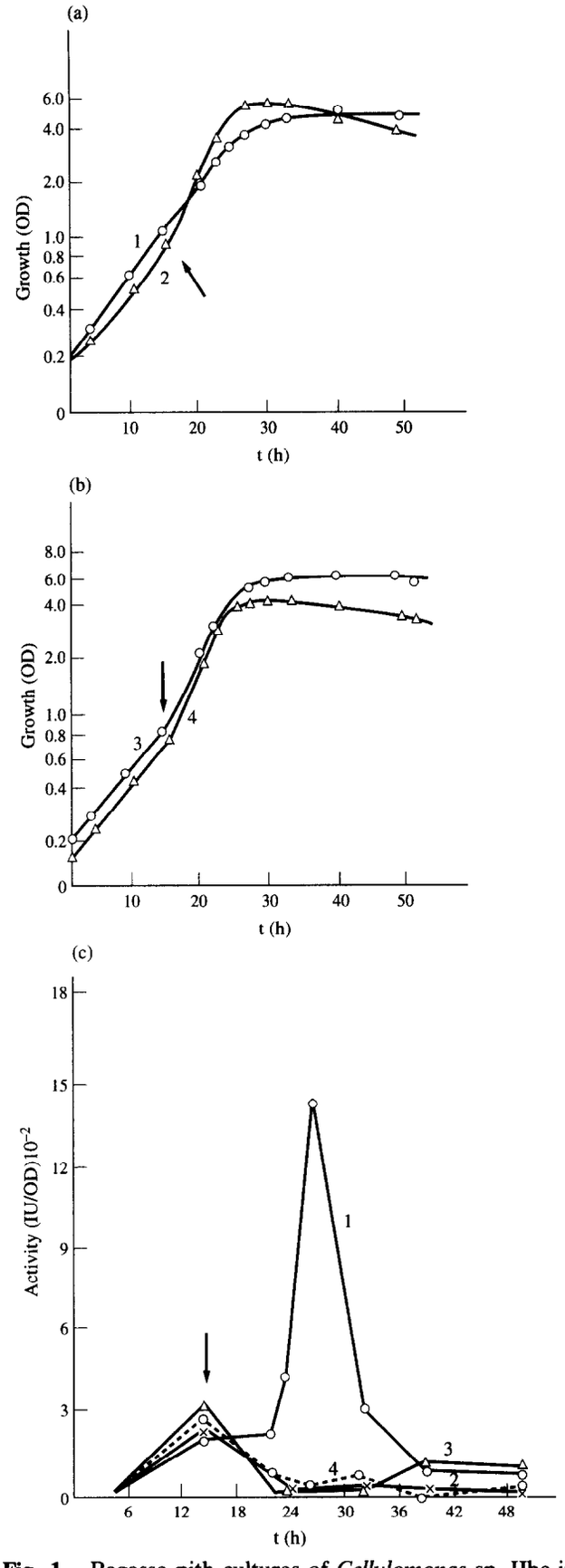

Fig. 1. Bagasse pith cultures of *Cellulomonas* sp. IIbc in MO medium with addition of sugars. (a), (b) Growth curves. (c) CMC-activity. 1: Control (no sugar addition); 2: addition of 1% glucose; 3: addition of 1% cellobiose; 4: addition of 1% xylose. Arrows indicate the time of addition.

bation period. Therefore, the negative effect on the cellulolytic activity by the three sugars seems to be due to a repression effect.

In order to confirm the existence of a repressive effect of these sugars on the cellulolytic system of *Cellulomonas* sp. IIbc, another type of experiment was performed. One percent glucose, cellobiose and xylose was added separately to actively growing bagasse pith cultures at 15 h of cultivation, when the cellulolytic system of this strain was being actively produced (Rodríguez & Volfová, 1984). The results are shown in Fig. 1. An increase in the growth rate was observed immediately after the addition of the sugars, indicating that the bacteria started growing at their expense. A drop in the cellulase activity per unit of cells was observed after the sugar addition in all three cases, in contrast to the activity in the control. This indicates that 1% glucose, cellobiose or xylose repressed the synthesis of the cellulolytic enzymes.

The observed repression of cellulases by high glucose and cellobiose concentrations agrees with that found for other cellulolytic bacteria (Robson & Chambliss, 1989). However, the xylose repression has not been previously reported. Importantly, xylose (the end-product of hemicellulose degradation) repressed the cellulolytic activity of the bagasse pith cultures. This might be an indication of some inter-relationship between the systems regulating cellulases and xylanases, which would be particularly important in the degradation of complex lignocellulosic materials.

The results of the induction experiment are summarized in Table 2. A basal enzymatic level was detected in the control samples. When the cells were incubated with 0·005, 0·01 and 0·02% cellobiose, an increase in CMC-activity and the FP-activity took place in comparison with the control (cells incubated without sugars), indicating the induction of new enzymes by the resting cells. The maximum effect was at 0·02%, where a 10-fold increase in both activities was achieved. Glucose, as expected, did not produce any inductive effect (data not shown). The presence of 0·01 and 0·02% xylose induced FP-activity (Table 2). At 0·02% xylose, CMC-activity was also produced. In *Streptomyces*, xylan-containing materials also induced cellulolytic enzymes (MacKenzie *et al.*, 1987).

The induction and repression of cellulases by xylose reported here is interesting. In the degradation of lignocellulosic substrates by *Cellulomonas*, it has been established that the first growth phase is developed at the expense of hemicellulose (Enríquez, 1981) and that the cellulase system is developed in a second stage (Rodríguez & Volfová, 1984). It would be feasible that the product of hemicellulose degradation (xylose) could act as a repressor of the cellulolytic system at high concentrations (Fig. 1), and as an inducer when its concentration drops to low levels (Table 2), thus

explaining the diauxic pattern of growth and enzyme production. This fact also supports the idea of an inter-relationship between the systems regulating cellulases and xylanases in this bacterium.

In order to investigate the possibility of constitutive synthesis, the strain was cultivated in nutrient broth, and the CMC-activity and the FP-activity determined at different times of cultivation. The results are shown in Table 3, in comparison with the activity in a bagasse pith culture. Both CMC-activity and FP-activity increased along the cultivation time, although the maximum values were from 10 to 15 times lower than the activity in the bagasse pith cultures. The increase of CMC-activity in nutrient-broth stationary cultures could be explained by the release of intracellular enzymes due to cell lysis, as it occurs in the bagasse pith cultures (Rodríguez & Volfová, 1984). This production of cellulolytic activity in a medium without any cellulosic substrate or derivative indicates constitutive basal synthesis of cellulases in *Cellulomonas* sp. IIbc. Similar results were reported by Vladut-Talor *et al.* (1986) for another *Cellulomonas* strain.

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