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Induction and Catabolite Repression of Cellulase in *Penicillium funiculosum*†

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ABSTRACT

The regulation of endoglucanase synthesis in *Penicillium funiculosum* is investigated using a method based on the viscosity lowering effect on carboxy methyl cellulose (CMC) by endoglucanase. Cellobiose (1 mg/L) causes induction, whereas glucose (5 g/L) does not repress the enzyme formation. Lactose (5 g/L) has no effect on the synthesis of cellulase. Avicel and cellulose powder (CP) are the best inducers of cellulase and xylanase activity. Both endoglucanase and xylanase activity were induced by CMC, whereas xylan induced only xylanase activity. The effect of protease on induction of cellulase activity is discussed.

Index Entries: Cellulase; *Penicillium funiculosum;* regulation of enzyme synthesis; induction and repression; viscometric assay.

INTRODUCTION

Studies on the regulation of enzyme synthesis have practical significance in production and are helpful in understanding the biological processes such as gene expression. Although several reports (*1,2*) have dealt with the production and purification of the enzymes involved in cellulose degradation, the information available on synthesis and regulation of cellulase is limited. Cellulases are inducible by cellulose, its derivatives, and

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low molecular weight carbohydrates, but they are repressed by readily metabolizable substrates like glucose. It has been proposed (*3,4*) that the cellulases are induced by the product of hydrolysis of cellulose formed by the action of base levels of constitutive enzyme already present in cells. The regulation of cellulases in *Trichoderma viride* and *Sporotrichum pulverulentum* has been studied (*5,6*). Sophorose and cellobiose were found to be the inducers of cellulase synthesis in *T. viride* and *S. pulverulentum* respectively, whereas glucose caused repression of enzyme synthesis (*7,8*).

The synthesis of cellulases and xylanases was shown to be under separate control by analyzing the enzymes induced by specific inducers (*9*). The studies on regulation of cellulase biosynthesis would be important in optimizing the cellulase production. For the commercial utilization of cellulose, it is necessary to produce cellulase at low cost. Hence, it is advantageous to have a cellulase system induced by low levels of soluble inducer and resistant to high levels of glucose which is an end product of cellulose metabolism.

Penicillium funiculosum produces a complete cellulase with high β-glucosidase activity. The hydrolysis of various cellulosic substrates by the enzyme has been studied (*10,11*). Hydrolysis of alkali treated bagasse by this enzyme yielded 70% saccharification with glucose as the major end product. This paper deals with induction of celllulase in *P. funiculosum* in response to specific inducers and their repression by glucose and glycerol.

MATERIALS AND METHODS

Organism

P. funiculosum was obtained from National Collection of Industrial Microorganisms, Pune, India.

Chemicals

All chemicals used were of analytical grade. CMC (7 H 3 5 X F) for the viscometric assay of endoglucanase was obtained from Hercules Inc. (Wilmington, DE). The carbon sources used were Avicel (Honeywill and Stein, U.K.), Larchwood xylan (Fluka, FRG), and cellulose powder (V.P. Chest Institute, New Delhi). Glucose, cellobiose, CMC (for reducing sugar assay), phenyl methyl sulfonyl fluoride (PMSF), and dinitroalicyclic acid were obtained from Sigma Chemical Co., USA.

Fermentation Conditions

P. funiculosum was grown in Reese's medium (*12*) containing glucose (0.2%) and Avicel, xylan, CMC, lactose, or wheat bran (0.5% each) for 48 h and inoculated (10%) into experimental flasks containing Reese's medium with five times the nitrogen source and 2% of the respective carbon sources. Samples were removed periodically and estimated for the enzyme activity.

The effect of glucose, glycerol, cellobiose, casein, and PMSF on cellulase production was studied in modified Reese's medium using 2% cellulose powder as the carbon source.

Activity towards CMC and xylan were determined by incubating 0.5 mL of CMC or xylan (1%) with a suitably diluted enzyme in a final vol of 1 mL containing 0.05 *M* acetate buffer, pH 4.8, at 50°C for 30 min. Filter paper activity was estimated by incubating 25 mg of filter paper and enzyme in 1 mL of reaction mixture containing 0.05 *M* acetate buffer pH 4.8, at 50°C for 60 min. The reducing sugar formed was estimated by the dinitro salicyclic acid method (*12*). The β-glucosidase activity was determined according to Berghem and Pettersson (*13*). Endoglucanase activity was determined by viscometric method as described by Eriksson and Hamp (*8*). Protease activity was estimated by Kunitz method (*14*). Soluble protein was determined according to Lowry et. al. (*15*).

RESULTS AND DISCUSSION

Production of Cellulase Using Different Carbon Sources

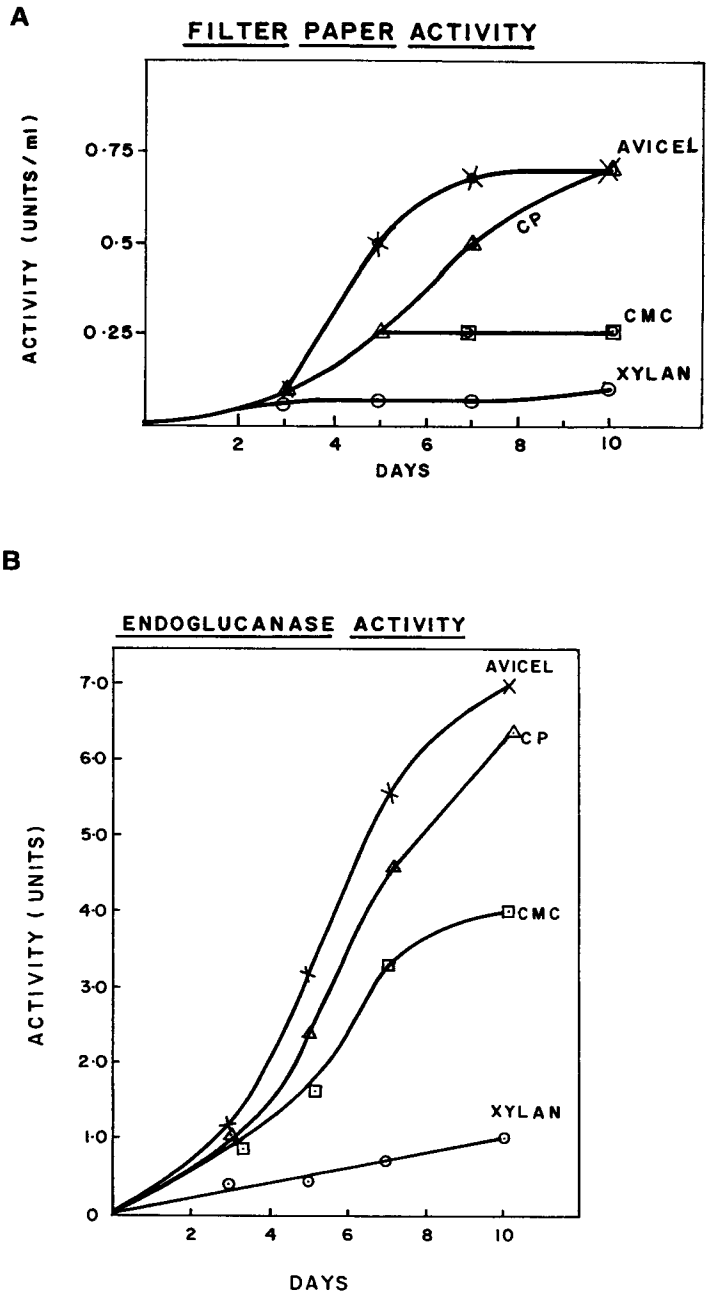
Fig. 1 (a, b and c) shows the production of filter paper activity, endoglucanase, and xylanase activity respectively by *P. funiculosum* during its growth on synthetic medium using different inducers. Avicel and CP induced all the components of the cellulase complex. When CMC was used as an inducer both endoglucanase and xylanase were produced to the same extent, whereas xylan induced xylanase activity selectively.

Effect of Casein on the Production of Cellulase

When cellulose powder was supplemented with casein, there was no significant increase in the endoglucanase (Fig. 2a) and filter paper activity (data not shown), although there was 50% increase in the β-glucosidase (Fig. 2b) and protease activity (data not shown). The addition of PMSF decreased the protease and the extracellular protein levels, but had no effect on the cellulase synthesis (Table 1).

Regulation of Cellulase Synthesis

The effect of glucose, cellobiose, and glycerol on cellulase synthesis in the presence of CP was studied. The synthesis was completely repressed in the early stages of fermentation (3 d) by 0.5% glucose or glycerol. However, when the fermentation was continued, the repression in synthesis of endoglucanase and exoglucanase activity was overcome by 70 and 50% by glycerol and 85 and 75% by glucose, respectively (Figs. 3a and b). Cellobiose was the best inducer of β-glucosidase, which was detected in the culture broth later than the cellulase (Fig. 3c). Glycerol and glucose (5%) completely repressed the enzyme synthesis. On the other hand, cellobiose, as low as 0.5 mg/L, showed a 10–15% increase in cellulase synthesis.


A
FILTER PAPER ACTIVITY
ACTIVITY (UNITS/ml)
0·75
0·5
0·25
AVICEL
CP
CMC
XYLAN
2
4
6
8
10
DAYS
B
ENDOGLUCANASE ACTIVITY
ACTIVITY (UNITS)
7·0
6·0
5·0
4·0
3·0
2·0
1·0
0
AVICEL
CP
CMC
XYLAN
2
4
6
8
10
DAYS

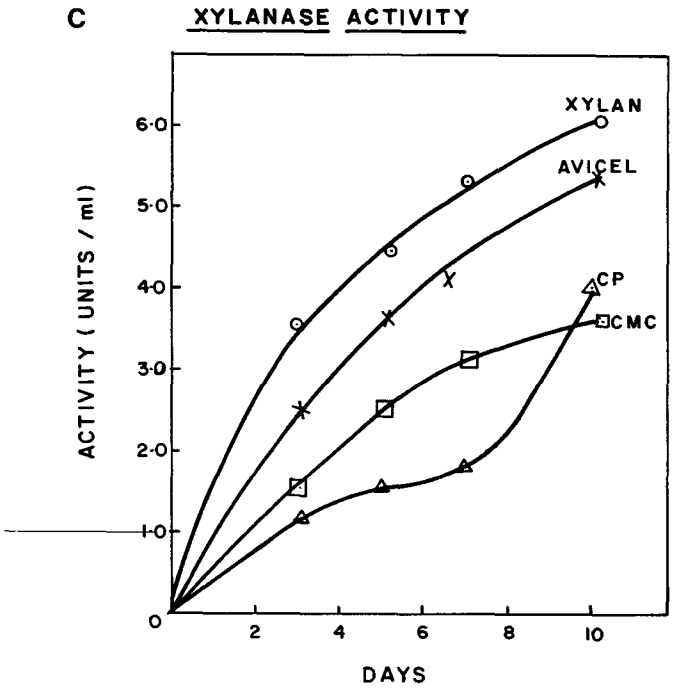


Fig. 1. The production of cellulase by *P. funiculosum* using different inducers.

Table 1
Synthesis of Extracellular Protein and Protease by *P. funiculosum*

Fermentation condition[a]	Protease activity, U/mL	Protein, mg/mL
Cellulose powder	2.8	3.1
Cellulose powder + casein	6.6	3.6
Cellulose powder + PMSF	1.2	1.92

[a]The fermentation was carried out for 10 d and the activity and protein were estimated as described in Materials and Methods.

Induction and Repression of Endoglucanase Activity

The effect of glucose and cellobiose on the regulation of endoglucanase activity was studied by viscosity lowering effect of CMC, using induction time as a function of their concentration. CMC alone was found to induce endoglucanase production in *P. funiculosum* as also observed for *S. pulverulentum* (*8*). However, CMC did not induce the endoglucanase activity

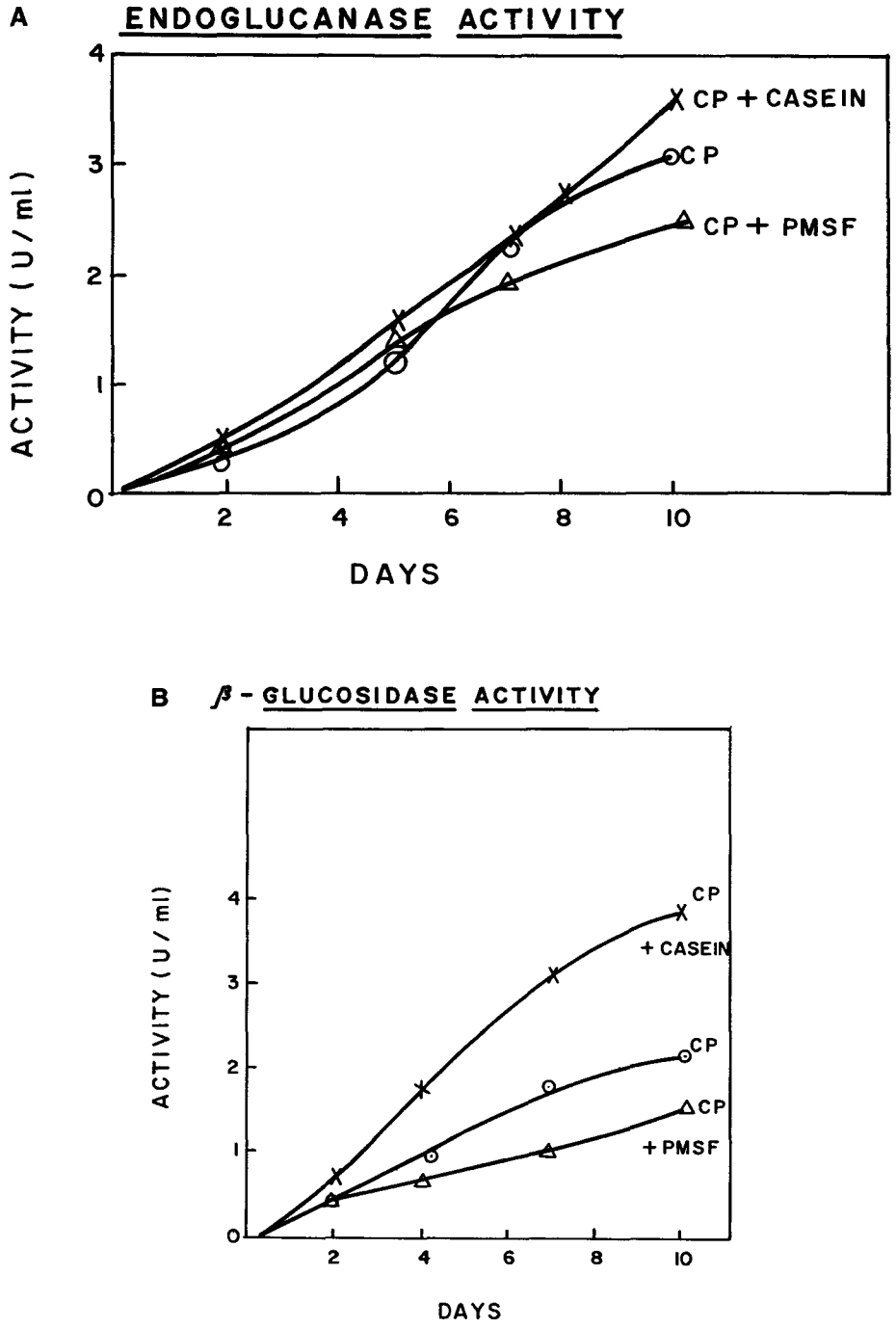
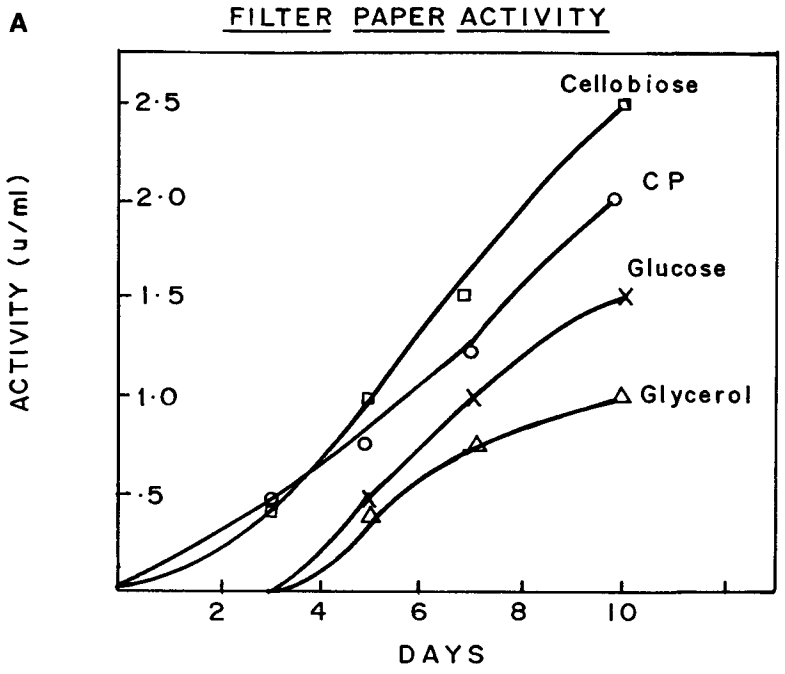
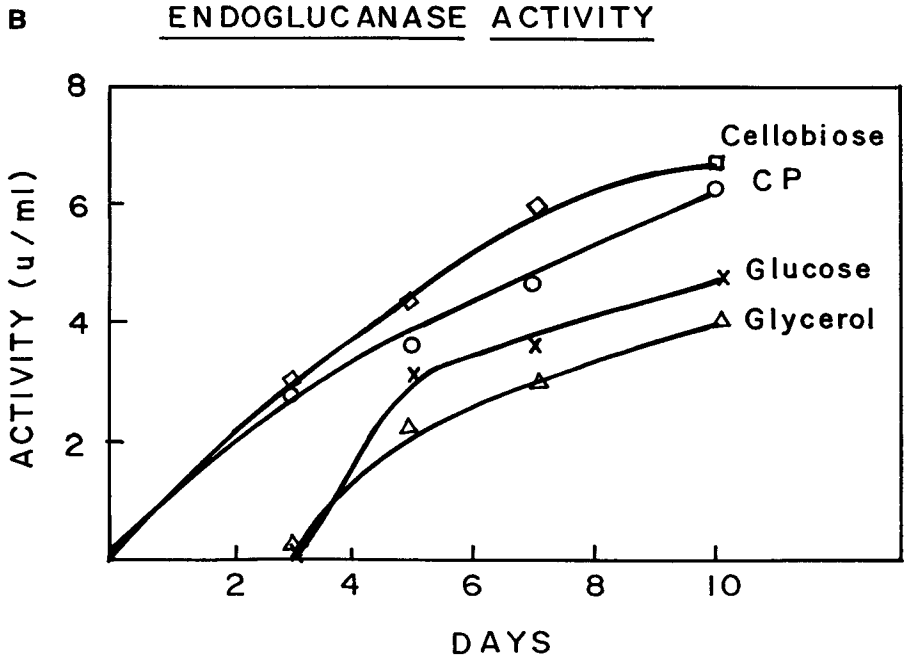


Fig. 2. Effect of casein on the production of cellulase.


A
FILTER PAPER ACTIVITY
ACTIVITY (u/ml)
2·5
2·0
1·5
1·0
·5
Cellobiose
CP
Glucose
Glycerol
2
4
6
8
10
DAYS


B
ENDOGLUCANASE ACTIVITY
ACTIVITY (u/ml)
8
6
4
2
Cellobiose
CP
Glucose
Glycerol
2
4
6
8
10
DAYS

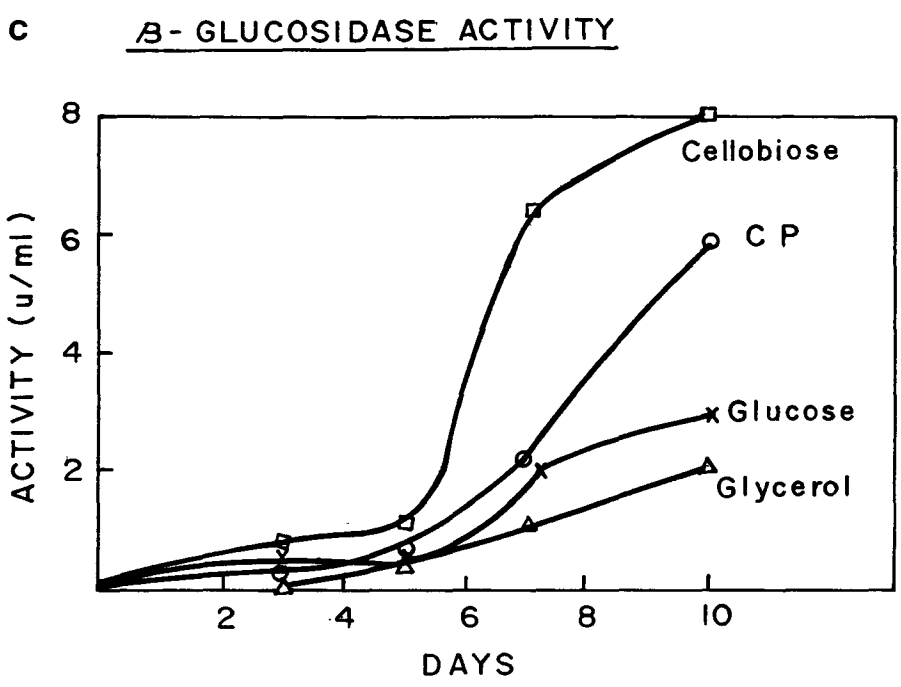


Fig. 3. Regulation of cellulase synthesis during fermentation.

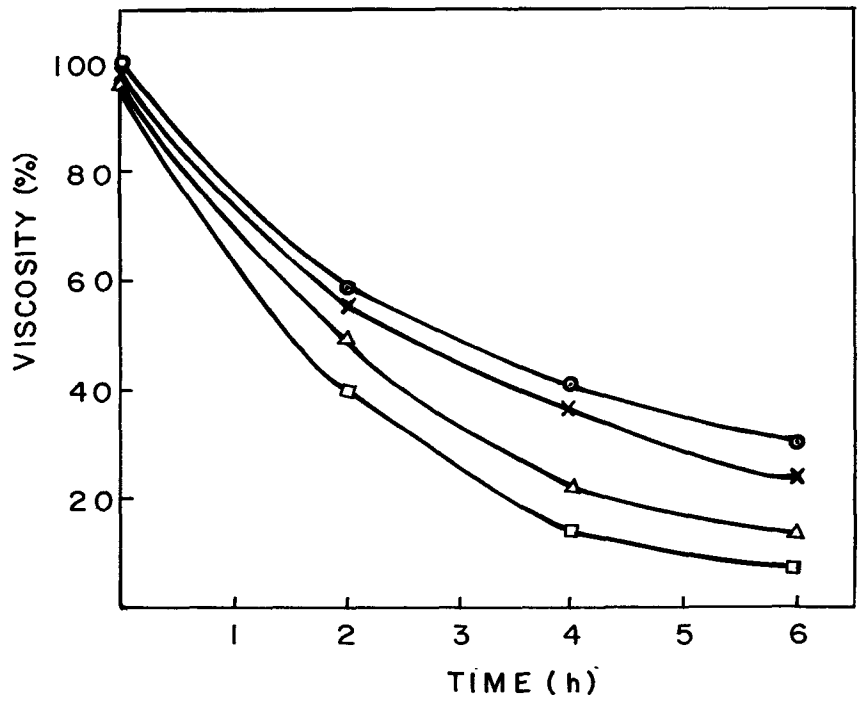


Fig. 4. Effect of cellobiose on the induction of endoglucanase activity. The fermentation was carried out in the presence of CMC (○) and with addition of cellobiose, 1 mg/L (x), 10 mg/L (△), and 50 mg/L (□).

in *T. viride* (*8*). Lactose (5 g/L) had no effect on cellulase production. The inducing effect of cellobiose in *P. funiculosum* was observed at the concentration of 1 mg/L. Increasing concentration of cellobiose (up to 50 mg/L) caused increase in induction, but did not influence the induction time significantly (Fig. 4). Induction of endoglucanase in *S. pulverulentum* was also observed at a cellobiose concentration of 1 mg/L, whereas it was a poor inducer of cellulase in *T. viride* (*8*).

The endoglucanase activity of *P. funiculosum* was not repressed by high concentrations of glucose (5 g/L), suggesting that its production is not subjected to catabolite repression in this organism. However, the other widely studied cellulolytic fungi like *T. viride* and *S. pulverulentum* showed repression by glucose concentrations of 50 mg/L and 1.8 g/L, respectively.

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