Regulation of Exoglucanase and Endoglucanase Biosynthesis in *Streptomyces* Species

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Abstract

Exoglucanase and endoglucanase synthesis in *Streptomyces* HM-15 was induced by cellobiose, maltose, amorphous and crystalline cellulose as well as by CM-cellulose. Low exoglucanase and endoglucanase activities were also detected when the organism was grown on glucose and lactose. In culture media supplemented with readily metabolizable substrates, exoglucanase and endoglucanase activity increased after the exhaustion of carbon source indicated that derepression of exoglucanase and endoglucanase synthesis has occurred. When glucose was added to a culture growing actively on cellulose, exoglucanase synthesis was repressed initially; however, the endoglucanase levels are not affected. This study showed that exoglucanase and endoglucanase synthesis in *Streptomyces* HM-15 is controlled by two regulatory mechanisms: an induction mechanism that increased the exoglucanase and endoglucanase by about 2 to 3-fold and a basal level expression of enzyme in the presence of low concentration of glucose. Besides these two mechanisms exoglucanase and endoglucanase synthesis in *Streptomyces* HM-15 was also subjected to catabolite repression by glucose.

Key words: Exoglucanase, endoglucanase, induction, repression, cellulose.

Introduction

Despite the current surplus of oil and the corresponding stability and even fall in energy-related prices, the conversion of wood or agricultural residues to fermentable sugars continues to be one of the most intensively studied areas of research[1,2]. The cellulases of microorganisms are enzyme complexes that consists of exoglucanases (1,4-β-D-glucan cellobiohydrolase, EC 3.2.1.91), endoglucanases (1,4-β-D-glucan glucanohydrolase, EC 3.2.1.4) and β-glucosidases (β-D-glucoside glucohydrolase, EC 3.2.2.21)[3]. Little is known about the molecular mechanism of the regulatory system of this complex in cellulolytic microorganisms. However, two different mechanisms have been proposed for the regulation of cellulose synthesis. Cellulose synthesis is induced by cellulose and its derivatives but their formation is repressed by readily metabolizable substrates such as glucose[4]. It is claimed that cellulases are constitutive, its synthesis being repressed by conditions favoring a rapid balanced growth[5]. Irrespective of their constitutive or inducible nature, cellulases from fungi have been reported to be catabolite sensitive[6]. This study was carried out to examine the production of enzyme and regulation of the exoglucanase and endoglucanase activity of *Streptomyces* HM-15 grown on media containing various soluble and insoluble carbon sources.

Experimental

Resources

Cellulose powder, Carboxymethyl cellulose (CMC)-Na salt, 3,5-dinitrosalicylic acid (DNS) and other chemicals used were of analytical grade.

Organism

A culture of *Streptomyces* HM-15 was isolated from compost and maintained on Bennett's agar medium[7], subcultured every fortnight and stored at 4°C.

Medium composition

The medium used initially for the cultivation of *Streptomyces* HM-15 contained the following ingredients (gL^{-1})- yeast extract, 0.01; beef extract, 0.01; casitone, 0.02 and cellulose, 10.0 (w/v); tween-80, 1.0 (v/v), the pH of the medium was adjusted to 7.2 and distributed as 100mL medium in 250mL Erlenmeyer flasks. Sterilization was carried out at 15psi for 15 minutes.

Inoculum preparation

The spore suspension ($1x10^8$ spores mL^{-1}) was prepared by harvesting the spores from seven day old Bennett's agar slant in sterile distilled water containing 0.1% (v/v) Tween-80. Spore suspension obtained was used to inoculate aforementioned medium and incubated for 48 hours on rotary shaker (200rpm) at

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room temperature.

Enzyme production

For induction studies, various carbon sources were added as 1% (w/v) to the liquid medium and inoculated with 5% (v/v) 48 hours old mycelia inoculum.

In order to study the effect of glucose on cellulose production, 1mL of a 20% (w/v) glucose solution was added to a set of flasks containing cellulose. Samples were withdrawn at regular intervals and centrifuged at 2500rpm for 20 minutes. The clear supernatant was used for the determination of different enzyme activities.

Enzyme assays

Exoglucanase and endoglucanase activity was assayed as described earlier[3]. Extracellular protein and reducing sugar was measured by standard methods using BSA (Bovine Serum Albumin) as standard[8,9].

Results and Discussion

The *Streptomyces* HM-15 was cultured on a range of insoluble and soluble sugars as sole carbon sources to determine whether extracellular cellulose formation was inducible or constitutive. Significant variations in growth rate with different carbon sources and even larger variations of exoglucanase and endoglucanase levels were observed. Of all the carbon sources tested, amorphous and crystalline cellulose, Whatman No. 1 and Avicel, as well as the soluble cellulose derivative, CM-cellulose gave high exoglucanase and endoglucanase activities. Thus cellulose seems to be an inducer of exoglucanase and endoglucanase synthesis. Exoglucanase synthesis in presence of CM-cellulose was maximum on 3rd day and endoglucanase synthesis reaching maximal level on 6th day of growth. The levels of exoglucanase and endoglucanase on CM-cellulose were higher than Whatman No. 1 and Avicel types of celluloses (Table 1). These observations are in contrast to those reported earlier[10] for the endoglucanases of *Sporotrichum thermophile*. They reported that highest cellulose synthesis occurred when highly crystalline cellulose such as Avicel was used as substrate while incubation on CM-cellulose resulted in lower yields. A comparison of exoglucanase and endoglucanase synthesis during growth on amorphous and crystalline cellulose shows that exoglucanase synthesis in the amorphous cellulose was higher than on crystalline cellulose, while endoglucanase synthesis in the crystalline cellulose was higher than on amorphous cellulose (Fig. 1).

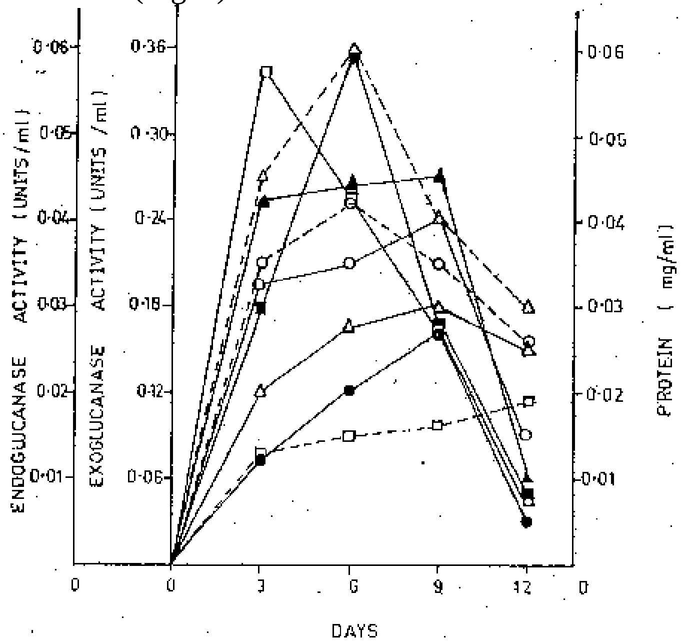

Fig. 1 : Exoglucanase production on amorphous (0-0), crystalline (△-△) & CM-cellulose (□-□), and endoglucanase production on amorphous (•-•), crystalline (▲-▲) & CM-cellulose (■-■) by *Streptomyces* HM 15. The broken line indicates protein levels in the broth.

In order to determine the ability of the more easily utilizable carbon sources to induce exoglucanase and endoglucanase synthesis, the organism was cultivated on cellobiose, lactose, maltose and glucose. Table 2 shows the maximum units of exoglucanase and endoglucanase obtained on each of these carbon sources. All the disaccharides tested as well as glucose showed exoglucanase and endoglucanase production (Fig. 2 and 3). The maximum activity of exoglucanase and endoglucanase on glucose was reached on 9th day.

It is being reported that the true inducer of cellulose synthesis is cellobiose and glucose represses enzyme production in *Trichoderma* sp11. In *Streptomyces* HM-15 we observed that cellobiose was effective in inducing exoglucanase and endoglucanase synthesis. Maltose and lactose had an inducing capacity to a lesser extent than that of cellobiose. In contrast to fungal cellulose, *Streptomyces* HM-15 showed about 42% and 47% less exoglucanase and endoglucanase respectively as compared to cellobiose in the medium containing glucose as sole carbon source. This shows that besides induction, the existence of another regulatory mechanism for exoglucanase and endoglucanase cannot be ruled out in *Streptomyces* sp.

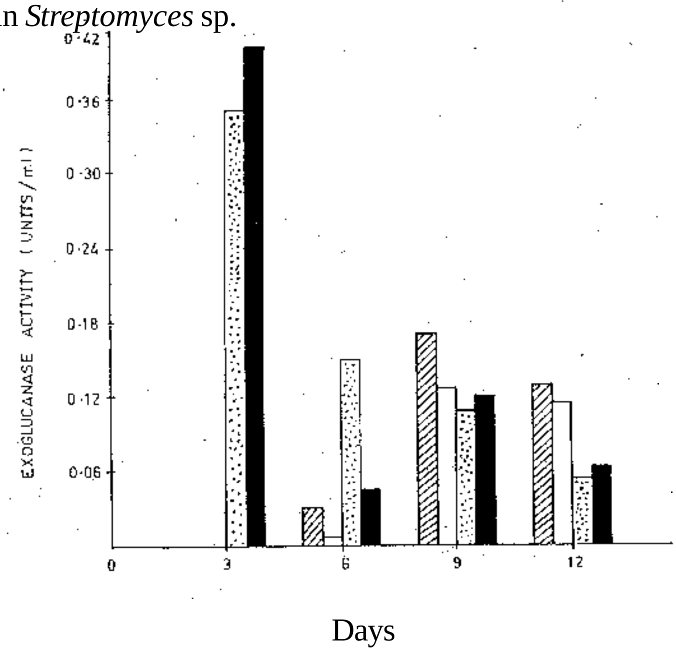

Fig. 2 Exoglucanase production by *Streptomyces* HM 15 on various soluble sugars. Glucose (▨), lactose, (□), maltose, (⊞) and cellobiose (■)

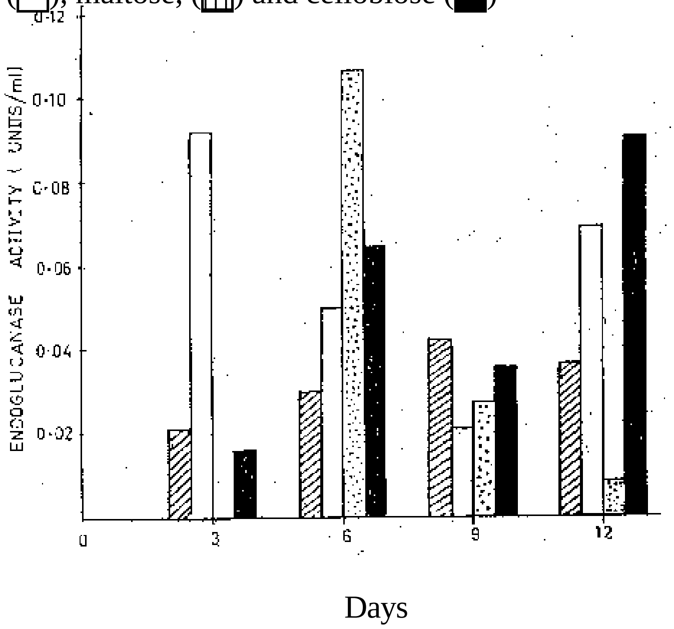

Fig. 3 Endoglucanase production by *Streptomyces* HM 15 on various soluble sugars. Glucose (▨), lactose, (□), maltose, (⊞) and cellobiose (■)

An experiment was carried out to test the concentration dependent effect of glucose on biosynthesis of exoglucanase and endoglucanase in cellulose containing medium. It was found that exoglucanase levels increase rapidly till 6th day of growth and endoglucanase levels increase till 9th day of growth when initial glucose concentration was less than 0.5% (w/v) (Fig. 4). Exoglucanase and endoglucanase was inhibited when initial glucose concentration increases beyond 0.5% (w/v). Maximum exoglucanase activity being obtained in the culture grown on 0.5% glucose concentration and endoglucanase activity on 0.2% glucose concentration.

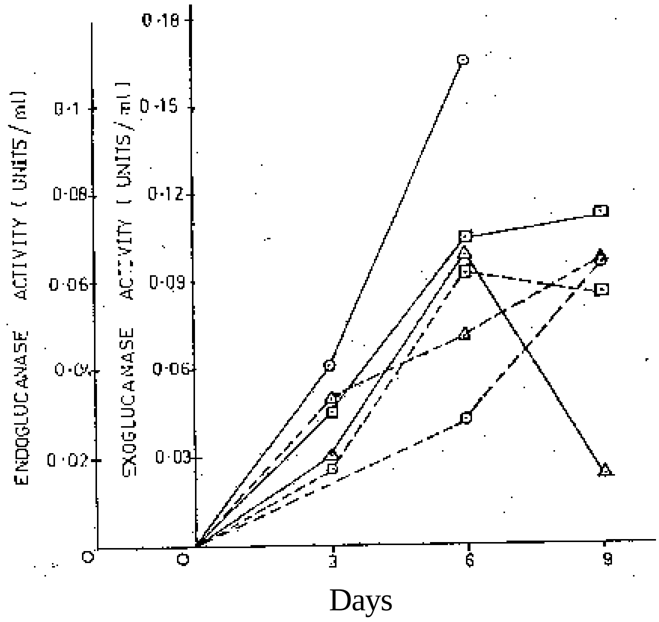

Fig. : 4 Induction of exoglucanase and endoglucanase synthesis in *Streptomyces* HM 15 by varying concentrations of glucose. 0.5% (⊙-⊙), 0.2% (▲-▲), and 0.1% (⊡ -⊡). The solid line indicates exoglucanase activity while broken line represents the endoglucanase activity.

The existence of a repression control was studied by adding glucose to an exponentially growing culture of the organism with cellulose as the carbon source (Fig. 5). The addition of glucose resulted in an immediate drop in exoglucanase levels; however, the endoglucanase levels are not affected. The enzyme levels rose to a level higher than those obtained in the control set to which no glucose was added. However, the specific activity of the enzyme dropped with the addition of glucose.

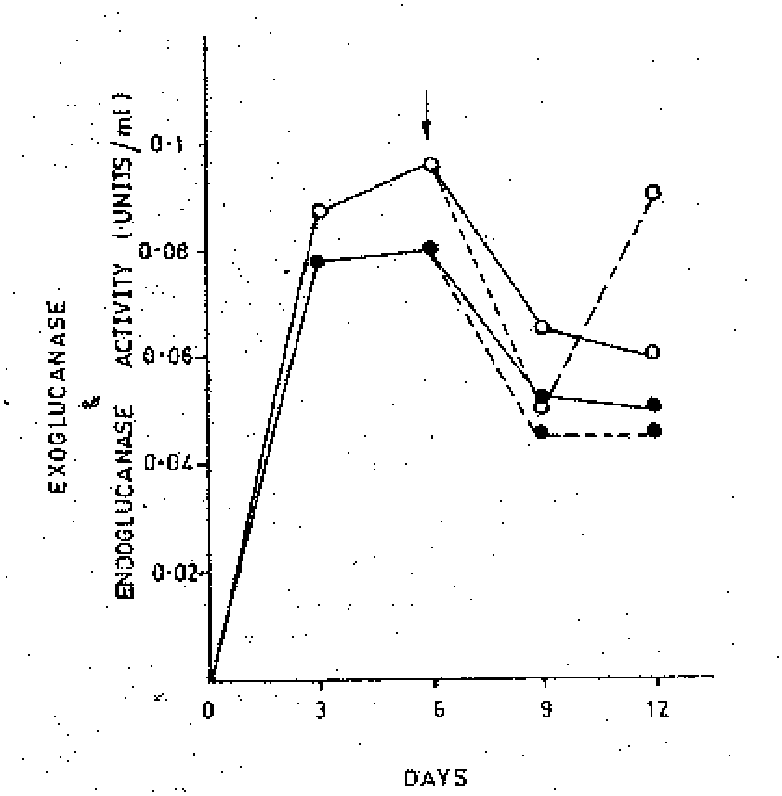

Fig. 5 Repression of exoglucanase and endoglucanase ↓ activity by glucose added to *Streptomyces* HM 15 growing actively on cellulose. The solid line indicates exoglucanase (○-○) and endoglucanase (●-●) activity in control while broken line represents the activity after adition of glucoase. The arrow ( ) indicates time of glucose adition.

These observations indicate that exoglucanase and endoglucanase production in *Streptomyces* HM-15 is constitutive, its synthes is being controlled by two independent mechanisms, induction and a growth-rate dependent repression mechanism and/or basal level expression. In addition the enzyme is also subject to catabolite repression like other metabolites and enzymes.

Table 1 Exoglucanase, endoglucanase and extracellular protein in *Streptomyces* HM-15

Inducer	Nature	Maximum enzyme activity (units per mL)		Total protein (mg mL^{-1})
		Exoglucanase	Endoglucanase	
Whatman No. 1	Amorphous	0.243	0.027	0.042
Avicel	Crystalline	0.186	0.0475	0.060
CM-cellulose	Soluble	0.345	0.059	0.019

Table 2 Exoglucanase, endoglucanase and extracellular protein in *Streptomyces* HM-15 grown on simple sugars

Inducer	Component	Glucosidic bond	Maximum enzyme activity (units per mL)		Total protein (mg mL^{-1})
			Exoglucanase	Exoglucanase	
Lactose	Glu-gal	1,4-	0.126	0.092	0.087
Cellobiose	Glu-glu	1,4-β	0.402	0.091	0.061
Maltose	Glu-glu	1,4-β	0.354	0.107	0.151
Glucose	-	-	0.171	0.043	0.081

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