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EFFECT OF CELLOBIOSE ON SECRETION OF PECTOLYTIC AND CELLULOLYTIC ENZYMES BY BLIGHT PATHOGENS

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ABSTRACT

The effect of cellobiose (disaccharides) on the secretion of pectolytic enzymes endo-polygalacturonase (PG); endo-polymethyl galacturonase (PMG); endo-polygalacturonase trans-eliminase (PGTE); endo-pectin methyl trans-eliminase (PMTE); pectin methyl esterase (PME) and cellulolytic enzyme (Cx) in the inoculum of blight pathogens, i.e., *Alternaria solani* on tomato plant and *Alternaria triticina* on wheat plant in modified Richard's culture medium has been studied. The stimulation of pectolytic and cellulolytic enzymes was found only with the cellobiose among the carbohydrates like glucose fructose, maltose and cellulose by both the pathogens under similar conditions. The stimulation of pectolytic and cellulolytic enzymes depends upon the source of carbon as well as the nature of the pathogen and the host.

INTRODUCTION

Alternaria solani (Jones & Grout) and *Alternaria triticina* (Prabhu & Prasad) develop leaf blight disease on tomato and wheat plants respectively. The disaccharide could act as highly specific factor for the induction and repression of enzyme synthesis as reported by Albersheim et al. (1969). Mehta & Mehta (1979) observed that the glucose act as the best carbohydrate for endo-polygalacturonase (PG) production by *A. solani* and *A. tenuis*. The induction of cellulose in fungi by cellobiose was observed by Mandels & Reese (1960). The sequential production of polygalacturonase, cellulase and pectinolyase by *Rhizoctonia solani* was reported by Lisker et al. (1975). Chan & Sakston (1972) observed the production of pectolytic and cellulolytic enzymes by virulent and avirulent isolates of *Sclerotium bataticola* during disease development in sunflower. Grossman (1968) reported the production of pectic and cellulolytic enzymes by *Phytophthora infestans*, culture of the fungus in a nutritive solution.

The present communication deals with the effect of cellobiose on the production of pectolytic and cellulolytic enzymes on Richard's culture medium with tomato fruit extract in different concentrations (0.1 mg - 1.0 g%) by the blight pathogens, *A. solani* and *A. triticina*.

MATERIAL AND METHODS

Alternaria solani and *Alternaria triticina* were isolated from the blighted leaves of tomato and wheat plants. The modified Richard's culture medium was used with phosphate buffer (0.1 M) and pH was adjusted to 6.0.

Extract of tomato fruit was obtained from 125 g of healthy tomato (variety Prince long) and squeezed through centrifuge. The supernatant was obtained (180-185ml) and then 65-70 ml phosphate buffer (0.1 M) was mixed to prepare 250 ml of extract solution. After this 250 ml of double strength medium

was added to the extract solution to protect the strength of nutrients and finally 500 ml solution was made. This was divided into 5 beakers, each measuring 100 ml, and to each 100mg, 250mg, 500mg, 750mg and 1.0 g cellobiose was added to get 0.10%; 0.25%; 0.50; 0.75% and 1.0% concentrations. Similar experiments were conducted with glucose, fructose, maltose and CM-cellulose. Thereafter, 25ml of different concentrations of cellobiose were dispensed into each 250 ml Erlenmeyer flask. The medium without cellobiose served as control. The flasks were plugged with nonabsorbent cotton wrapped in cheesecloth. The flasks were autoclaved at 1.06 kg/sq cm for 15 minutes.

Each experiment with a minimum of 20 cultures was repeated thrice and cultures were maintained in dark at 24° ± 1°C for a fortnight. Culture methods and enzyme assays were employed following Gupta (1973) and Singh & Ram (1991, 1992).

RESULTS AND DISCUSSION

The investigation reveals the effect of cellobiose (0.10 - 1.0%) in comparison to other carbohydrates like glucose, fructose maltose and CM-cellulose on the secretion of pectolytic and cellulolytic enzymes when the pathogen *A. solani* and *A. triticina* are grown on Richard's culture medium containing tomato fruit extract. The secretion of endo-PG and endo-PMG was stimulated to 10.5 - 45.0% and 15.4 - 46.3%. The reaction mixture reduced the viscosity of polypectate and pectin respectively in buffer solution of pH 5.0 at 30°C within an hour. The thiobarbituric acid test of the reaction mixture also confirmed the maximum absorbance of these enzymes at 510nm on scanning using spectrophoto colorimeter.

The secretion of endo-PGTE and endo-PMTE was also stimulated to 18.0 - 46.5 and 20.8 - 50.0%. The reaction mixture also reduced the viscosity of polypectate and pectin respectively in the solution adjusted with buffer to pH 8.5 at 30°C within an hour. After enzyme assay the reaction mixture was treated with thiobarbituric acid and maximum absorbance was recorded at 550 nm for confirmation. The secretion of PME, which removed the ester groups of pectin in buffered solution of pH 7.0 when it was treated with 0.005 N NaOH and expressed at micro-equivalents of ester groups hydrolysed per minute by 1 ml of enzyme preparation, was also stimulated to extent of 16.0 - 45.0%. The secretion of cellulolytic enzyme (Cx) was also stimulated by 2.3 - 20.4%, which degrades randomly the viscosity of CMC in the solution adjusted with buffer pH 5.0 at 30°C within an hour in the case of *A. solani* (Fig. 1 & Table 1). When *A. triticina*, grown on different concentrations of cellobiose, it also stimulated the secretion of endo-PG (5.3 - 49.0%), endo-PMG (17.0 - 50.0%), endo - PGTE (5.2 - 50.0%) endo - PMTE (19.0 - 52.0%) PME (5.0 - 40.0%) and Cx (3.2 - 23.0%) (Fig. 2 and Table 2).

The investigation shows the role of cellobiose in the stimulation of pectolytic and cellulolytic enzymes which are responsible for both maceration and killing effect during the disease development and are secreted routinely as a feature of the host-parasite interaction. The secretion of both pectolytic and cellulolytic enzymes were higher with cellobiose when used as inducer molecule in comparison to other carbohydrates. However, when the CM-cellulose, used as inducer molecule, it stimulated the cellulolytic enzyme and repressed the pectolytic enzymes. On the other hand, when maltose and others, used as inducer molecules, stimulated the pectolytic enzymes and repressed the cellulolytic enzymes.

The fungus released pectolytic and cellulolytic enzymes during growth in liquid media containing pectic or cellulosic carbon source in *Fusarium oxysporium* was observed by Olutiola (1978). Effect of native carbon sources and pH on the pectolytic enzymes and on the cellulase by *A. solani* and

A. tenuis was recorded by Mehta et al. (1974, 1975). The production of pectolytic and cellulolytic enzymes by *Rhizoctonia bataticola, in-vitro* and *in vivo*, was reported by Goel and Mehrotra (1974).

It is, therefore, concluded that the stimulation of pectolytic and cellulolytic enzymes depends not only upon the interactive effect between the host and the pathogen but also on the source of carbon.

Table 1: Secretion of pectolytic and cellulolytic enzymes in different concentrations of carbohydrates and tomato fruit extract by *A. solani*.

	Percentage of secretion of enzyme unit/ml/hour								Microequivalents × 10^4			
	Endo-PG		Endo-PG		Endo-PG		Endo-PMTE		Cx		PME	
Carbohydrates	0.10 - 1.0g		0.10 - 1.0g		0.10 - 1.0g		0.10 - 1.0g		0.10 - 1.0		0.10 - 1.0g	
Cellobiose	+10.54	+45.0	+15.4	+ 46.3	+18.0	+46.5	+20.8	+50.0	+2.3	+20.4	+16.0	+45.0
Maltose	+13.3	156.0	+15.4	−57.0	+18.0	+60.0	+14.0	+60.0	−11.0	−93.0	+15.5	+57.7
Glucose	+13.4	+36.6	+8.3	+29.0	+11.6	+34.3	+9.5	+35.0	−53.5	−100.0	+8.4	+35.3
Fructose	+7.1	+33.33	+4.4	+24.1	+3.0	+30.0	+9.5	+27.0	−9.3	−86.0	+8.3	+21.5
Cellulose	−4.0	−8.0	−4.3	−12.0	4.2	−11.6	−5.0	−10.0	+15.7	+45.0	−0.0	−15.7

Stimulation (+), inhibition ( )

Table 2: Secretion of pectolytic and cellulolytic enzymes in different concentration of carbohydrates and tomato fruit extract by *A. triticina*.

	Percentage of secretion f Enzyme unit/ml/hour								Microequivalents × 10^4			
	Endo-PG		Endo-PG		Endo-PG		Endo-PMTE		Cx		PME	
Carbohydrates	0.10 - 1.0g		0.10 - 1.0g		0.10 - 1.0g		0.10 - 1.0g		0.10 - 1.0		0.10 - 1.0g	
Cellobiose	+5.3	+49.0	+17.0	+50.0	+5.2	+50.0	+19.0	+52.0	+3.2	+23.0	+5.0	+40.0
Maltose	+20.0	+60.0	+21.5	+57.2	+16.0	+60.0	+13.3	+56.7	−27.0	−100.0	+25.0	+62.0
Glucose	+6.0	+40.0	+6.5	+40.0	+6.0	+43.0	+14.0	+43.0	−60.0	−100.0	+19.0	+30.8
Fructose	+14.3	−35.7	+6.3	+35.0	+11.2	+39.0	+8.0	+32.0	−6.7	−90.0	+0.0	+18.0
Cellulose	−0.0	- 5.5	−7.0	−13.3	−6.2	−12.5	−7.7	−15.4	+21.0	+58.3	−0.0	−5.5

Stimulation (+), inhibition (−)

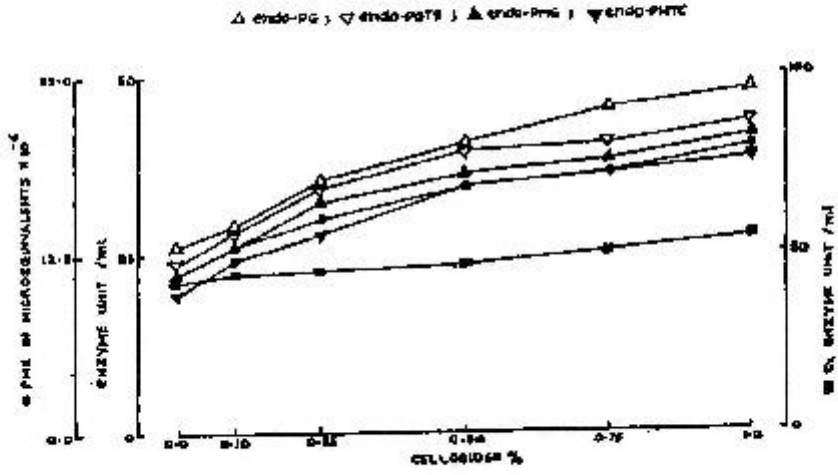

Fig. 1: *A. solani* grown on culture media containing extract of Prince long cultivar.

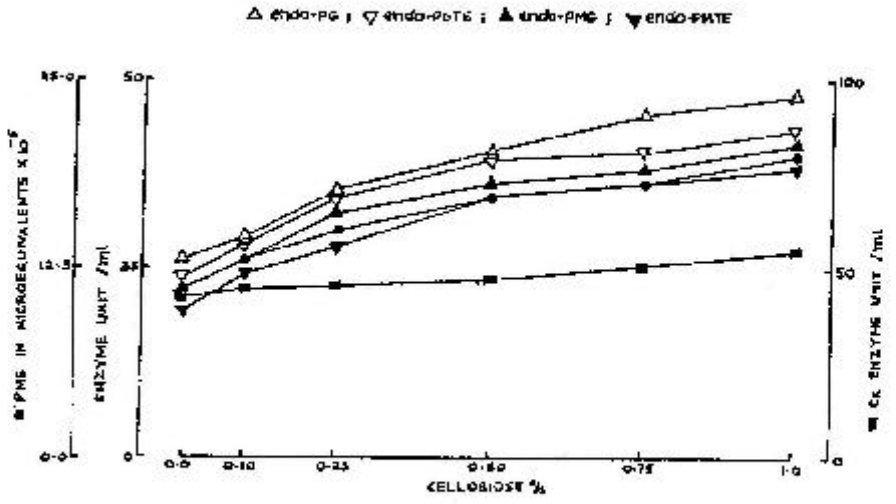

Fig. 2: *A. triticina* grown on culture media containing extract of Prince long cultivar.

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