Asian Jr. of Microbiol. Biotech. & Env. Sc. Vol. 3, No. (4) : 2000 : 311-314
© Global Science Publications

EFFECT OF CELLULOSE (A POLYSACCHARIDE) ON SECRETION OF PECTOLYTIC AND CELLULOLYTIC ENZYMES BY BLIGHT PATHOGENS

M.P.N. SINGH, J. AHMED[1] AND M.P. SINHA[2]

Centre for Biotechnology, Marwari College, Ranchi - 834 001, India

Key words : Cellulose, Tomato, Wheat, A. solani, A. triticina.

Abstract–Experiments were performed to study the effect of cellulose (Polysaccharide) on secretion of pectolytic enzymes such as Polygalacturonase, (endo-PG), Poly methyl galacturonase (endo-PMG), Polygalacturonase transeliminase (endo PGTE), Pectin methyl transeliminase (endo-PMTE), Pectin methyl esterase (PME) and Cellulolytic enzyme (Cx) in the inoculum of two blight pathogens namely *Alternaria solani* and *Alternaria triticina* on modified Richard's Culture medium with tomato fruit extract of Pusa ruby variety in different concentrations ranging from 0.10% to 1.00%. The inhibition of pectolytic enzymes was higher with cellulose than the other carbohydrates, however the cellulolytic enzyme was stimulated more with cellulose by both pathogens under similar conditions.

INTRODUCTION

The blight pathogens *Alternaria solani* (Jones and Grout) and *Alternaria triticina* (Prabhu and Prasad) produce leaf blight disease on tomato and wheat. The pectic and cellulolytic enzymes are responsible for both maceration and killing effects during the disease development and are secreted in a routine manner as a feature of host-parasite interaction. Mehta (1977) reported that starch was found to be one of the most unfavourable compound for the production of pectic enzymes in *A. solani* and *A. tenuis*, but starch was found to favour maximum growth of the fungus. Mehta (1984) reported that inhibitory effect of some plant growth regulators, phenolics and fungicides on the production of pectin methyl galacturonase by *Alternaria solani* and *A. tenuis*. Pectic and Cellulolytic enzymes were repressed by *Corticium solani* in the disease process (Martin 1967). Cellulase was influenced by the different carbon sources and incubation period in *Alternaria solani* and *A. tenuis* (Mehta *et al.*, 1974). Wide variety of carbon sources help to grow cellulolytic fungi but Cx is produced only in the presence of cellulose (Mandels and Reese 1957, 1960). Similarly Olutiola (1978) reported that the growth, sporulation and production of pectic and cellulolytic enzymes in *Fusarium oxysporium* and suggested that the fungus released pectic and cellulolytic enzymes during growth in liquid media containing pectic or cellulosic C-source.

The present investigations deal the effect of cellulose and tomato fruit extract of Pusa ruby variety on Richard's culture medium in different concentrations (0.10% - 1.0%) in the secretion of pectolytic and cellulolytic enzymes by *Alternaria solani* and *A. triticina* under similar conditions.

MATERIALS AND METHODS

Isolation of *Alternaria solani* and *A. triticina* from leaf blight of tomato and wheat were employed in the investigations. The pathogens were grown on modified Richard's culture medium with phosphate buffer (0.1 M) and pH 6.0 was adjusted. Tomato fruit extract of Pusa ruby variety with cellulose was used in different concentration (0.10 - 1.0%) with the culture medium. Culture methods and enzyme assays were used as employed (Gupta 1973, Singh and Ram 1990, 1991, 1992). Without cellulose served as control. Each experiment was repeated thrice and maintained at 24 + 1°C in the dark for a fortnight.

RESULTS

The pathogens *Alternaria solani* and *A. triticina* were grown on the Richard's culture medium containing extract of Pusa ruby variety of tomato fruit supple-

[1]Principal, Marwari College, Ranchi - 834 001, and [2]Department of Zoology, Ranchi University, Ranchi - 834 008, India

Table 1. Secretion of Pectolytic and Cellulolytic enzymes with carbohydrates and tomato fruit extract by *A. solani*.

Carbohydrates %	Enzyme Unit in %											
	PME		Endo-PMTE		Endo-PGTE		Endo-PMG		Endo-PG		Cx	
Cellulose	0.10	1.0	0.10	1.0	0.10	1.0	0.10	1.0	0.10	1.0	0.10	1.0
Secretion of Enzyme unit	-0.5	-12.0	-0.0	-25.0	-0.0	-21.0	-0.0	-18.7	-0.0	14.0	+16.0	-45.6
Starch	0.10	1.0	0.10	1.0	0.10	1.0	0.10	1.0	0.10	1.0	0.10	1.0
Secretion of Enzyme unit	-7.0	-17.7	-8.0	-40.0	-6.3	-25.0	-7.1	-35.0	-9.5	-24.0	-46.0	-100.0
Maltose	0.10	1.0	0.10	1.0	0.10	1.0	0.10	1.0	0.10	1.0	0.10	1.0
Secretion of Enzyme unit	+22.2	+65.0	+14.4	+70.0	+16.7	+68.0	+13.3	+68.4	+9.5	+62.0	-51.4	-100.0
Glucose	0.10	1.0	0.10	1.0	0.10	1.0	0.10	1.0	0.10	1.0	0.10	1.0
Secretion of Enzyme unit	+22.2	-50.3	+7.7	+59.0	+13.7	+53.0	+13.3	57.0	+16.0	+49.0	-59.4	-100.0

Inhibition (-), Stimulation (+)

Fig. 1. *A. solani* grown on culture media containing extract of pusa ruby cultivar.

mented with cellulose in different concentrations (0.10 - 1.0%). When cellulose was supplemented with Richard's culture medium (pH 6.0) containing extract of Pusa ruby variety of tomato fruit then was observed that the cellulose repressed the production of endo-PG and endo-PMG, which enhance the viscosity of polypectate and pectin respectively in buffered solution pH 5.0 at 30°C within an hour. After enzyme assay the reaction mixture was treated with thiobarbuturic acid and maximum absorbance was recorded at 510 nm on scanning. Cellulose also repressed the production of endo-PGTE and endo-PMTE, which also enhance the viscosity of polypectate and pectin in the solution adjusted with buffer pH 8.5 at 30° C within an hour. After enzyme assay the reaction mixture was treated with thiobarbuturic acid and maximum absorbance was recorded at 550 nm. Cellulose stimulated the secretion of cellulolytic enzymes (Cx) which reduce the viscosity of CMC in the solution adjusted with buffer pH 5.0 at 30°C within an hour.

The secretion of pectolytic and cellulolytic enzymes were repressed and stimulated variably in different concentrations of cellulose. The endo-PG was repressed 0.0 - 14.0% and endo-PMG was 0.0 - 18.7% whereas endo-PGTE 0.0 - 21.0%, endo-PMTE 0.0 - 25.0%, PME 0.0 - 12.5% respectively. Cx was stimulated 16.0 - 45.6% by the addition of 0.10 - 1.0% cellulose which was inoculated by *A. solani*. (Fig. 1 and Table 1). *A. triticina* also repressed the secretion of endo-PG 6.7 - 20.0%, endo-PMG 9.1-27.2%, endo-PGTE 7.7 - 23.0%, endo-PMTE 5.0 - 30.0% and PME 0.0 - 20.0%. Cx was stimulated 13.0 - 58.0% by the addition of 0.10 - 1.0% cellulose (Fig. 2 and Table 2).

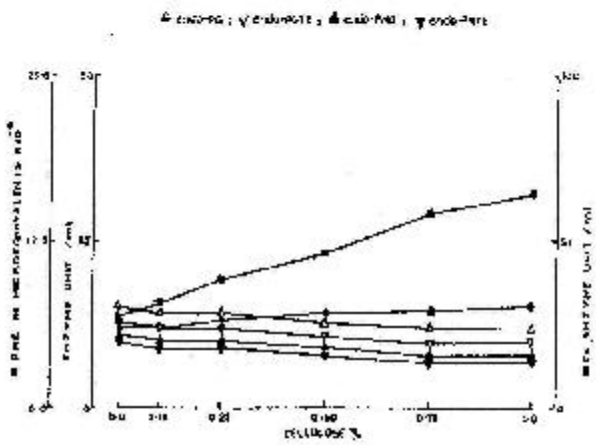

Fig. 2. *A. triticina* grown on culture media containing extract of pusa ruby cultivar.

Ruby). The starch recorded next to cellulose for the repression of pectic and cellulolytic enzymes. Mehta and Mehta (1979) observed that the starch was the only carbohydrates which could check completely the production of PGTE in *A. solani* while substantial decrease was noticed in case of *A. tenuis* with this compound. On the otherhand, Martin and Grossman (1972, 1972A) observed that the large quantity of organic and inorganic compounds inhibit the secretion of poly galacturonase and a few on pectin methyl esterase in vitro by *Rhizoctonia solani*. Mandels and Reese (1965) recorded that wide variety of carbon sources help to grow cellulolytic fungi but Cx is produced only in the presence of cellulose.

Table 2. Secretion of Pectolytic and Cellulolytic enzymes with carbohydrates and tomato fruit extract by *A. triticina*

Carbohydrates %	Enzyme Unit in %											
	PME		Endo-PMTE		Endo-PGTE		Endo-PMG		Endo-PG		Cx	
Cellulose	0.10	1.0	0.10	1.0	0.10	1.0	0.10	1.0	0.10	1.0	0.10	1.0
Secretion of Enzyme unit	-0.0	-20.0	-5.0	-30.0	7.7	23.0	-9.1	-27.2	-6.7	-20.0	+13.0	+58.0
Starch	0.10	1.0	0.10	1.0	0.10	1.0	0.10	1.0	0.10	1.0	0.10	1.0
Secretion of Enzyme unit	-0.0	-16.2	-0.0	-30.0	-7.7	-23.0	-9.0	-9.0	-7.0	-20.0	-18.6	100.0
Maltose	0.10	1.0	0.10	1.0	0.10	1.0	0.10	1.0	0.10	1.0	0.10	1.0
Secretion of Enzyme unit	+25.0	+63.0	+9.1	+61.5	+19.0	+60.0	+16.0	+62.0	-12.0	-34.6	-11.7	-93.0
Glucose	0.10	1.0	0.10	1.0	0.10	1.0	0.10	1.0	0.10	1.0	0.10	1.0
Secretion of Enzyme unit	+14.3	+45.5	+9.0	+33.4	+13.4	+31.6	+15.4	+35.3	-12.0	-32.0	-63.0	-100.0

Inhibition (-), Stimulation (+)

DISCUSSION

The production of Cx is adaptive in many fungi and the cellulosic substrates in medium induced the production of Cx enzyme (Gupta and Heale 1970; Mandels *et al.*, 1962). Extracellular cellulase activity has been found higher and appeared earlier in large propagules with carboxy methyl cellulose in culture medium of *Rhizoctonia solani* (Lisker *et al.*, 1975).

The above investigations deal the results of cellulose (Tables 1 and 2), compared with the results of starch, maltose and glucose used as inducer molecule in the same concentration. The cellulose repressed the pectic enzymes and stimulated the cellulase enzyme in all carbohydrates by *Alternaria solani* and *A. triticina* on Richard's culture medium supplemented with tomato fruit extract (Pusa

From the above investigations it is therefore, concluded that the cellulose consisting cellobiose units in long chain is the only carbohydrate which stimulates the cellulolytic enzyme and repressed the production of pectic enzymes in all carbohydrates with the extract of tomato fruit. The stimulation and repression of cellulolytic and pectolytic enzymes depends upon the interaction of the pathogen as well as the host and also depends on the structure of carbohydrate which was produced by the enzymatic degradation of the host constituents.

ACKNOWLEDGEMENTS

The authors are thankful to Professor D.P. Gupta, P.G. Department of Botany, University of Karachi for valuable suggestion, kind-co-operation and con-

tinuous encouragement during the research work.

REFERENCES

Gupta, D.P. and Heale, J.B. 1970. Induction of cellulase in *Verticillium albo-atrum*. R. & B.J. gen. Microbiol. **62** : 163.

Gupta, D.P. 1973. Endo polygalacturonase stimulation in *Verticillium albo-atrum*, Ind. Phytopath. **26** : 90-106.

Lisker, N. Katan, J. and Henis, Y. 1975. Sequential production of polygalacturonase, cellulase and pectin lyase by *Rhizoctonia solani* Can. J. Microbiol. **21** : 1298-1304.

Mandels, M. and Reese, E.T. 1957. Induction of cellulase in *Trichoderma viride*. J. Bacterio. **73** : 269-278.

Mandels, M. and Reese, E.T. 1960. Induction of cellulase in fungi by cellobiose. J. Bacteriol. **79** : 816-826.

Mandels, M., Parrish, F.W. and Reese, E.T. 1962. Sophorose as an inducer of cellulase in *Trichoderma viride* J. Bacteriol. **83** : 400-408.

Mandels, M. and Reese, E.T. 1965. Inhibition of cellulases. Ann Rev. Phytopath. **3** : 85-102.

Martin, J. 1967. On the inhibition of pectic and cellulolytic enzymes of *Corticium solani*. Dissertation Univ. Giessen. pp. 101.

Martin, J. and Grossman, F. 1972. On the inhibition of pectic and cellulolytic enzymes of *Rhizoctonia solani*. Phytopath. Z. **75** : 38-51.

Martin, J. and Grossman, F. 1972A. On the inhibition of pectic and cellulolytic enzymes of *Rhizoctonia solani*. Phytopath. Z. **75** : 97-110.

Mehta, P., Vyas, K.M. and Saxena, S.B. 1974. Effect of native carbon sources and pH on the pectolytic enzymes of *A. solani* and *A. tenuis*. Proc. Ind. Nat. Sci. Acad. B. **40** : 433-439.

Mehta, P. 1977. Studies on Glycosidases I. Effect of carbohydrates and amino acids on production and activity of polygalacturonase of *Alternaria* species. Ind. Phytopath. **30 (3)** : 306-310.

Mehta, P. and Mehta, Archana 1979. Studies the effect of carbohydrates and amino acids on *Alternaria* species. Ind. Phytopath. **32** : 401.

Mehta P. 1984. Inhibitory effect of some plant growth regulators, phenolics and fungicides on the production of pectin Methyl galacturonase by *Alternaria solani* and *A. tenuis*. Folia Microbiol. **29** : 43-50.

Oluciola, P.O. 1978. Growth, sporulation and production of pectic and cellulolytic enzymes in *Fusarium oxysporium* Trans. of the British Mycological Society. **70 (1)** : 109-114.

Singh, M.P.N. and Ram, R.L. 1990. Secretion of Pectic enzymes by *Alternaria solani* and *A. triticina*. Biovulture **10(1 & 2)** : 23-26.

Singh M.P.N. and Ram, R.L. 1991. Studies on the secretion of Pectic enzymes from tomato (*Lycopersicum esculentum*, Mill) extracts. Adv. Plant. Sci. **4(2)** : 416-418.

Singh, M.P.N. and Ram, R.L. 1992. In vitro stimulation of pectolytic enzymes from Maltose and tomato fruits extract by *Alternaria solani* and *Alternaria triticina*. Adv. Plant Sci. **5 (Special Issue)** : 391-397.