EFFECT OF NATIVE CARBON SOURCES AND *p*H ON THE PECTOLYTIC ENZYMES OF *ALTERNARIA SOLANI* AND *A. TENUIS*

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Effect of native carbon sources and *p*H on the production of various pectolytic enzymes by *Alternaria solani* and *A. tenuis* has been studied. Mycelial growth of the two test fungi was determined during these experimental conditions. On the basis of overall experimental results it could be concluded that both the species of *Alternaria* were capable of producing various pectic enzymes constitutively and the increased production in presence of certain native pectic substances, could be considered merely the stimulation of pectic enzyme synthesis rather than actual induction. None of the carbon sources, i.e. pectin and sodium polypectate supported mycelial growth better than glucose, in both the cases.

The production of pectic enzyme in the synthetic medium by both, *A. solani* and *A. tenuis* was found to be greately influenced by the *p*H of the medium. Both PG and PMG in both the organisms were actively produced in low *p*H range (3–6); the *p*H 4 was found to be optimum for both the enzymes. However, the alkaline *p*H (8–9) favoured the formation of PGTE and PMTE in both the organisms. With regard to the fungal growth, it was observed that *p*H 4 was optimum for both the fungi.

INTRODUCTION

Pectic enzymes play an important role in pathogenesis (Albersheim *et al.* 1969, Brown 1955; Bateman and Miller 1966; Wood 1967). Secretion of particular pectic enzyme by the test organism in culture and the amount of activity in the crude enzyme extract are largely influenced by various components in the culture media, the *p*H of the broth and the incubation periods (Mehta, 1973). Since it had already been proved by the experimental data, that pathogenesis of *A. solani* and *A. tenuis* was associated with the increased activity of various pectolytic enzymes and also that both the pathogens were capable of producing certain very highly active pectic enzymes in culture, it was thought desirable to determine the optimum cultural conditions for the secretion of these enzymes in culture. Therefore, in the present communication effect of native carbon sources and *p*H, on the production of various pectolytic enzymes by *A. solani* and *A. tenuis* has been studied; simultaneously the growth of two test fungi was also determined during these experimental conditions.

MATERIALS AND METHODS

Isolates of *A. solani* and *A. tenuis* were those obtained from the diseased tomato fruits. To see the effect of native carbon sources and *p*H on pectic enzymes activity the following basal synthetic medium was used; asparagine, 5g; glucose, 12 g;

KH_2PO_4, 6.8 g; $MgSO_4.7H_2O$, 5g; distilled water, 1000 ml. Glucose was replaced by the same amount of pectin or sodium polypectate. To see the effect of *p*H, the pathogens were grown in synthetic medium with *p*H range from 2.5 to 12. The *p*H of the medium was adjusted with 1N NaOH and 0.1N HCl and buffered with McIlvaines buffer of the desired *p*H for values upto *p*H 8 and with standard phosphate buffer for higher values. The pathogen was grown in 100 ml conical flasks containing 25 ml of various broth media. These flasks were sterilized at 15 lb/sq. in pressure for 10 min. The inoculum in each case consisted of a single disc of 8 mm diameter cut-out from the margin of a freshly grown colony of the pathogen on Czapek's agar medium. The flasks were incubated at 26±1°C. Two replicates were taken in each case. After the desired days of incubation the culture filtrate and the mycelial mat were harvested. The culture filtrates were centrifuged at 10,000 rpm for 20 min. The supernatant was used as crude enzyme preparation. The enzyme was assayed by standard viscometric method (Keen and Horton 1966; Hancock and Miller 1965). The reaction mixture in case of different pectolytic enzymes contained :

(*a*) *Polygalacturonase* (*PG*)—1.2 per cent sodium polypectate (substrate), 3.5cc; distilled water, 1.5cc; McIlvaines buffer (*p*H 4.5), 1.5cc and enzyme extract, 1.5cc.

TABLE I

Effect of carbon sources on PG, PMG, PGTE, and PMTE production by A. solani

Media	4 days			8 days			12 days			16 days		
	% loss in viscosity after minutes											
	15	45	90	15	45	90	15	45	90	15	45	90
	Polygalacturonase activity											
Glucose (Control)	48.2	78.9	88.0	78.7	91.3	95.2	76.0	91.5	96.4	58.3	85.4	89.2
Pectin	48.2	72.4	82.7	80.0	91.4	100.0	87.8	96.9	99.8	162.6	168.9	168.9
Sodium-polypectate	25.7	50.9	67.3	86.1	94.7	97.0	76.3	91.8	95.9	79.6	95.4	98.7
	Pectin methyl galacturonase activity											
Glucose (Control)	4.3	17.3	26.0	11.5	42.3	57.6	38.8	61.1	72.2	61.0	62.7	64.2
Pectin	48.6	72.4	86.2	46.1	80.7	100.0	77.2	90.9	100.0	70.2	88.0	90.6
Sodium-polypectate	15.4	23.0	26.9	23.0	57.6	76.9	18.5	25.9	70.0	43.7	65.5	75.0
	Polygalacturonate transeliminase activity											
Glucose (Control)	4.3	9.8	20.3	9.2	19.5	28.4	9.8	30.4	40.4	26.9	42.6	56.9
Pectin	15.1	23.4	34.8	41.8	77.0	85 1	64.2	82.1	89.4	63.5	85.1	91.8
Sodium polypectate	0.5	1.0	1.0	3.5	11.9	16.6	2.9	8.7	22.2	16.9	36.3	47.2
	Pectin methyl transeliminase activity											
Glucose (Control)	10.0	10.0	10.0	17.5	34.3	43.7	47.0	67.4	69.8	55.1	56.8	56.8
Pectin	40.6	59.3	68.7	74.5	81.6	86.4	17.2	41.3	51.7	10.5	23.6	44.7
Sodium-polypectate	–	–	3.3	8.8	17.6	23.5	40.0	53 3	63.3	9.0	22.7	31.7

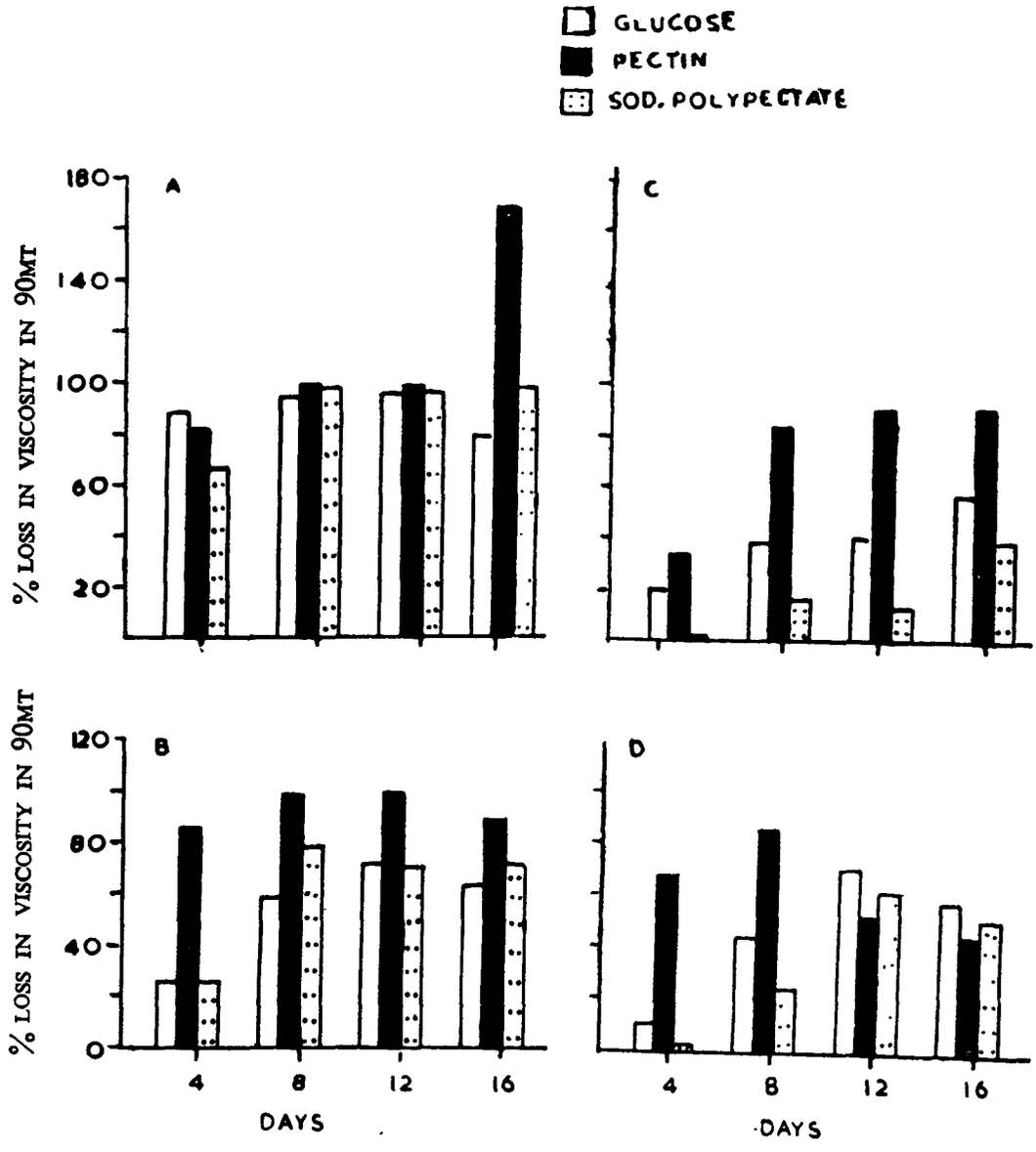

FIG. 1. A-D Pectic enzymes production by *A Solani* after 4 8,12,16 days of incubation period. *A*, Polygalacturonase; *B*, Pectin methyl galacturonase; *C*, Polygalacturonate transeliminase; *D*, Pectin methyl transeliminase.

(*b*) *Polygalacturonate transeliminase* (*PGTE*)—This enzyme was detected by using the McIlvaines buffer of *p*H 8 in place of *p*H 4.5, while other components were the same as mentioned for PG.

(*c*) *Pectin methyl galacturonase* (*PMG*)—1.2 per cent pectin solution (substrate), 3.5cc, distilled water, 1.5cc, McIlvaines buffer (*p*H 4.5), 1.5cc and the enzyme extract, 1.5cc.

(*d*) *Pectin methyl transeliminase* (*PMTE*)—The reaction mixture was similar to that, used for PMG; McIlvains buffer of *p*H 8 was used instead of *p*H 4.5.

The enzyme activity has been expressed as percentage reduction in viscosity after a given reaction time.

RESULTS

Alternaria solani was found to be capable of producing all four pectolytic enzymes i.e. PG, PMG, PGTE and PMTE in the culture media containing pectin and Sod.

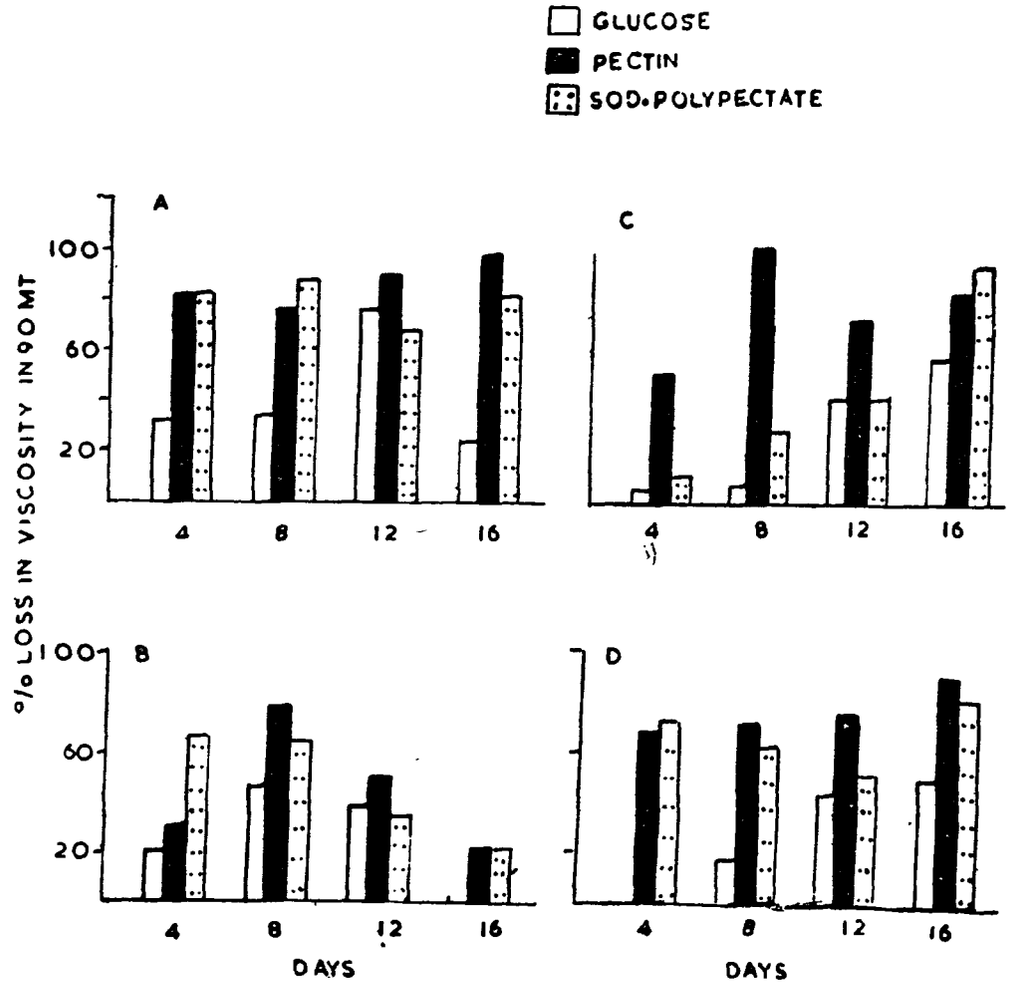

FIG. 2. Pectic enzymes production by *A. tenius* after 4,8,12,16 days of incubation period. *A*, Polygalacturonase; *B*. Pectin methyl galacturonase; *C*, Polygalacturonate transeliminase; *D*, Pectin methyl transeliminase.

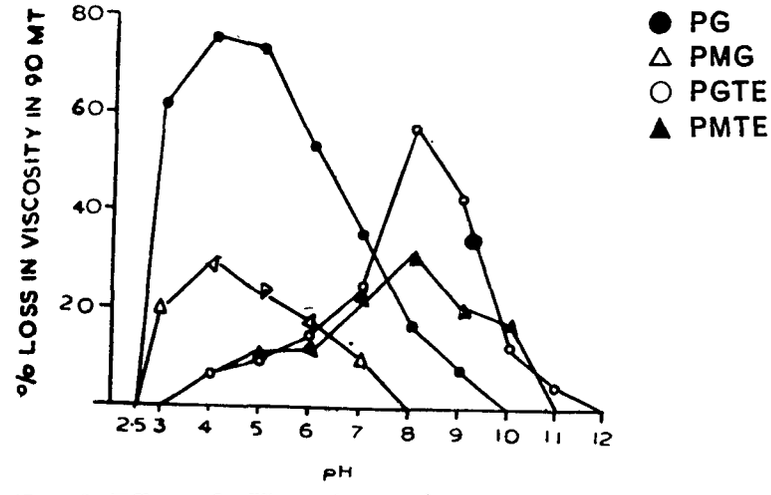

FIG. 3· Effect of *p*H on the pectic enzymes production *by A. Solani.*

polypectate as the sole source of carbon (Tables I). Pectin caused greater stimulation of both the glycosidases and transeliminases in comparison to control which contained only glucose as the sole source of carbon (Fig. 1). Sodium polypectate,

TABLE II

Effect of carbon sources on PG, PMG, PGTE and PMTE production by A. Tenuis

	4 days			8 days			12 days			16 days		
	% loss in viscosity after minutes											
	15	45	90	15	45	90	15	45	90	15	45	90
	Polygalacturonase activity											
Glucose (Control)	8.6	22.8	31.5	11.7	23.4	34.0	73.3	75.7	78.6	2.8	18.4	23.1
Pectin	58.4	81.5	81.5	66.6	75.7	75.7	65.5	83.3	90.0	66.1	93.8	98.4
Sodium-polypectate	51.5	62.2	82.2	33.3	72.2	88.8	24.0	50.6	68.0	24.2	69.6	81.8
	Pectin methyl galacturonase activity											
Glucose (Control)	6.6	20.0	20.0	19.6	33.6	46.9	8.2	26.9	38.5	3.3	3.3	3.3
Pectin	5.0	15.0	30.0	17.3	58.4	78.2	12.5	50.0	50.0	6.7	17.1	21.6
Sodium-polypectate	3.6	33.3	66.6	25.0	58.9	65.0	9.2	21.3	33.4	8.7	19.0	21.7
	Polygalacturonate transeliminase activity											
Glucose (Control)	4.6	5.3	6.1	3.2	6.5	6.5	34.5	39.2	42.8	22.7	35.4	48.2
Pectin	16.9	51.5	53.0	36.7	58.3	72.8	40.2	58.3	75.0	65.3	77.5	85.5
Sodium-polypectate	5.4	5.4	13.4	5.7	8.5	22.8	12.9	29.0	45.1	75.8	82.3	94.1
	Pectin methyl transeliminase activity											
Glucose (Control)	–	–	–	8.3	16.6	16.6	19.3	30.2	42.3	17.8	32.8	50.3
Pectin	31.2	43.7	68.7	35.7	57.1	71.4	38.4	61.5	76.9	29.0	77.4	90.3
Sodium-polypectate	28.5	57.1	71.4	30.7	61.5	61.5	23.5	44.5	52.9	22.7	57.5	81.8

however, was proved to be slightly inferior than glucose in this respect, for all the enzymes. None of these carbon sources supported mycelial growth better than control (synthetic medium containing glucose). It may however, be mentioned that the growth of *A. solani* was higher than *A. tenuis* in all the cases.

In *Alternaria tenuis*, the production of same four pectic enzymes was very much increased in presence of both the native carbon sources, i.e. pectin and sodium polypectate; the activity of both glycosidases (PG and PMG) and transeliminases was significantly greater in pectin and sodium polypectate containing media than the control (Fig. 2), which contained only glucose as a sole source of carbon (Table II).

Glycosidases (PG and PMG) of both the organisms were actively produced in low *p*H range (3–6). The maximum activity of both the enzymes was observed at *p*H 4 (Figs. 3 and 4). Comparatively PMG was more sensitive than PG to alkaline *p*H range, as no PMG activity in either case could be observed beyond *p*H 7. Transeliminases, however, were favoured only in the alkaline range of the medium. The maximum activity of both (PGTE and PMTE) was observed at *p*H 8–9, in

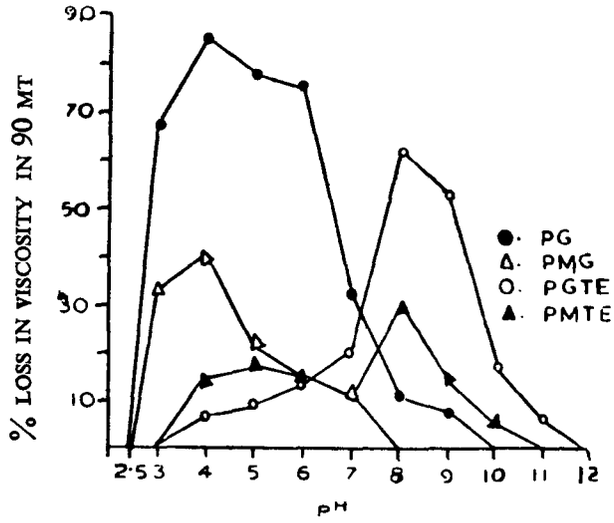

FIG. 4. Effect of *p*H on the pectic enzymes production by *A. teuius*.

both the cases (Figs. 3 and 4). The *p*H range below 8 and above 9 appeared to be very much unfavourable for these enzymes. Comparatively PGTE was more active than PMTE. However, the transeliminase as such showed greater activity in case of *A. solani* than *A. tenuis*. Relatively PMTE was more sensitive for very high *p*H (11 and 12) than PGTE. *p*H 4 was optimum for fungal growth of both *A. solani* and *A. tenuis*.

DISCUSSION

Pectin and sodium polypectate when used as a sole sources of carbon, stimulated the production of both glycosidases and transeliminases and the activity of these enzymes was greater than in the synthetic medium (control), which contained only glucose as a sole carbon source in case of *A. tenuis*. In *A. solani*, however, similar stimulation by sodium polypectate was not observed. It appears as if sodium polypectate was causing repression of pectic enzymes in *A. solani*. Differential control mechanisms have also been demonstrated for pectin degrading enzyme production in *Fusarium solani* f. *phaseoli* by Bateman (1966). Increased activity of pectic enzymes by pectin has also been reported by Waggoner and Dimond (1955) in case of *F. oxysporum* f. *lycopersici*. Ali (1970) in *Rhizoctonia* sp., and Rai (1971) in *Colletotrichum capsici* have also come to the same conclusion.

Both pectin and sodium polypectate, however, failed to stimulate the fungal growth of either species. Similar results showing slow growth of fangal pathogen in presence of pectic substances as sole carbon source have also been recorded by Rai (1971) in case of *Colletotrichum capsici*.

Pectic enzymes are secreted by pathogens constitutively (Sadasivan and Subramanian, 1963; Ayers and Papavizas 1965; Singh and Wood 1956). However, at times the production of these enzymes has been reported to be inductive as in case of *F. solani* f. *phaseoli* (Batemen 1966), *F. moniliforme* and *Sclerotium rolfsii* (Sadasivan and Subramanian 1963). However, the results obtained here indicated that *A. solani* and *A. tenuis*, produced various pectic enzymes constitutively and the increased production in presence of certain native substances may be

considered merely the stimulation of pectic enzyme synthesis rather than actual induction.

*p*H changes of the media play an important role in deciding the type of pectic enzymes to be secreted by the pathogen (Sheerwood 1966; Bateman 1966; Hancock 1966). Production of glycosidases was favoured by acidic range while only alkaline *p*H greatly supported the synthesis of transeliminases. Sheerwood (1966) has also reported that the association of high levels of PG produced with low *p*H and the association of high levels of pectin lyase production with alkaline conditions reflected a fundamental relation of *p*H to production of these enzymes. Similar results have also been reported by several other workers (Ali 1970; Rai 1971).

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