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Role of Pectic and Cellulolytic Enzymes in Pathogenesis of *Pythium aphanidermatum* in Tomato Seedlings

BY

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ABSTRACT

An appreciable reduction in pectin and cellulose content was observed in *P. aphanidermatum* - infected tomato hypocotyls. Only in the infected tissues endo-PG, PGTE and macerating enzymes were detected while PME and Cx enzymes were found both in healthy and infected tissues. The pathogen produced endo - PG, PGTE, Cx and macerating enzymes *in vitro*.

INTRODUCTION

Pectic and cellulolytic enzymes produced by the pathogens have been attributed to be responsible for the development of damping-off diseases (Winstead and McCombs, 1961; Mellano *et al.*, 1970). The present study is to assess the role of pectic and cellulolytic enzymes produced by *Pythium aphanidermatum* (Edson) Fitz. in the development of damping-off of tomato seedlings.

MATERIALS AND METHODS

Tomato (Co. 1) seedlings were grown in sterilized soil and the freshly isolated virulent *P. aphanidermatum* culture which was multiplied in sand-maize (95:5) medium was incorporated in the soil when the seedlings were 7, 22 and 37 days old. After 3 days of inoculation, the seedlings were uprooted and the hypocotyls were analysed for their pectin and cellulose content. Comparable hypocotyls of healthy plants were also analysed and the analyses were triplicated. Pectic and cellulolytic enzyme extracts were obtained from the tomato hypocotyls and *in vitro* production of pectic and cellulolytic enzymes by the pathogen was assessed by growing the pathogen in a special medium (Gupta, 1956) for different days. Pectin methyl esterase (Hancock *et al.*, 1964), endopolygalacturonase (Uritani and Stahmann, 1961), exopolygalacturonase (Hancock and Miller, 1965), polygalacturonase transeliminase (Bateman, 1966), macerating enzyme (Mellano *et al.*, 1970), Cx (Barker and Walker, 1962) and C_1 enzyme (Norkrans, 1950) activities were assessed in both the tissue extracts and in the culture filtrates

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TABLE 1. Changes in pectin and cellulose content of tomato hypocotyls due to infection

Age of the hypocotyl tissue	Disease reaction	Pectin content in percentage on dry weight basis		Cellulose content in percentage on dry weight basis	
		Healthy tissues	Infected tissues	Healthy tissues	Infected tissues
10	Highly susceptible	7.5	4.5	19.9	14.8
25	Susceptible	9 5	6.5	20.9	17.6
40	Resistant	13.0	12.5	28.1	27.0

RESULTS AND DISCUSSION

The results in Table 1 reveal that in the severely infected young hypocotyls both pectin and cellulose contents decreased appreciably while in the slightly infected 40-day old hypocotyls the reduction in both pectin and cellulose contents was only negligible.

Both pectin methyl esterase (PME) and cellulase (Cx) enzymes were detected in the healthy tomato hypocotyls. In the infected hypocotyls, besides the two enzymes, endopolygalacturonase (endo PG), polygalacturonate trans-eliminase (PGTE) and macerating enzymes were also detected (Table 2). Exopolygalacturonase (exo PG) and C_1 enzymes could not be detected in both healthy and infected hypocotyls.

The pathogen produced endo PG, exo PG, PGTE, Cx and macerating enzymes in culture. Both PME and C_1 could not be detected in the culture filtrates. Maximum enzymes production was observed in 3-day old cultures (Table 3).

The reduction in soluble pectin and cellulose content in the severely infected tomato hypocotyls suggests the possible role of pectic and cellulolytic enzymes in the damping-off disease development. The detection of endo PG, PGTE, and macerating enzymes only in the infected hypocotyls provides further evidence for the possible involvement of these enzymes in the disease development. However, the detection of PME and Cx enzymes even in the healthy hypocotyls suggests that these enzymes may be of host origin (Chakravarty and Srivastava, 1967). The other enzymes detected only in the infected hypocotyls may also be of host origin and the pathogen would have induced the synthesis of these enzymes by the host (Vidhyasekaran *et al.*, 1971). But the pathogen, *P. ophanidermatum* was found to produce these enzymes *in vitro* also. *In*

TABLE 2. Pectic and cellulolytic enzymes in the healthy and infected tomato hypocotyls

1*	2**	3	4	5	6	7	8
Healthy	2.3	0.0	0.0	0.0	No maceration	8.2	0.0
Diseased	3.0	20.3	0.0	24.3	160	19.8	0.0

*1. Host tissue

TABLE 3. Production of pectic and cellulolytic enzymes by *P. aphanidermatum in vitro*

Days after Inoculation	2**	3	4	5	6	7	8
2	0.0	25.0	17.0	32.3	30.0	39.8	0.0
3	0.0	41.9	23.5	36.4	25.0	57.0	0.0
4	0.0	20.0	13.0	21.6	70.0	57.7	0.0
5	0.0	16.2	5.0	21.4	75.0	60.6	0.0

**2. Pectin methyl esterase (Micro-equivalent of NaOH required to maintain the original pH/hr/ml of enzyme,

3. Endopolygalacturonase (pet cent reduction in viscosity in 30 minutes)

4. Exopolygalacturonase (1 unit = Change in absorbance of 0.01/hr)

5. Polygalacturonate transeliminase (per cent reduction in viscosity in 30 minutes)

6. Macerating enzyme (Time taken to macerate potato discs in 30 minutes)

7. Cellulase Cx (per cent reduction in viscosity in 30 minutes)

8. Cellulase C_1 (1 unit = Change in absorbance of 0.01)

vitro production of endo PG and macerating enzyme by *P. aphanidermatum* has been reported (Chakravarty and Srivastava, 1967). Lin Reen (1971) found *P. ultimum* to produce PGTE *in vitro*. Winstead and McCombs (1961) could not detect exo PG in the culture filtrate of *P. aphanidermatum*. However, in the present studies *P aphanidermatum* was found to produce exo-PG *in vitro*; but *in vivo* production of this enzyme could not be detected.

The pathogen did not produce PME *in vitro* although it was detected *in vivo* suggesting that this enzyme was only of host origin (Vidhyasekaran and Kandasamy, 1971). Cx enzyme was detected both in healthy and infected host tissues and the pathogen produced this enzyme *in vitro* also. Hence the increased amount of Cx enzyme detected in the infected hypocotyls may be due to the activation of host enzyme by the pathogen or it may be of pathogen origin The *in vitro* production of these enzymes was detected even on the second day of inoculation. Similarly damping-off symptom appeared within 2-3 days (Muthusamy, 1972). Hence the pectic and cellulolytic enzymes seem to play an important role in the damping-off development in tomato.

REFERENCES

BARKER, K. R. nnd J. C. WALKER. 1962. Relationship of pectolytic and cellulolytic enzymes production by strains of *Pellicularia filamentosa* to the pathogenicity. *Phytopathology*. 52 : 1119 25.

BATEMAN, D. F. 1966. Hydrolytic and transeliminative degradation of pectic substances by extracelluar enzymes of *Fusarium solanif phaseoli*. *Phytopathology* 56 : 238–44.

CHAKRAVARTY, D. K. and D. N. SRIVASTAVA. 1967. Pectic enzymes of *Pythium aphanidermatum* and *Rhizopus stolonifer*. *Phytopath. Z.* 59 : 49–54.

GUPTA, S. C. 1956. Studies in the physiology of a parasitism. xxii. The production of pectolytic enzymes by *Pythium debaryanum* Hesse. *Ann. Bot. Lond.* 77 : 176–90.

HANCOCK, J. G. and R. L. MILLAR. 1965. Relative importance of polygalacturonase and other pectolytic enzymes in southern anthracnose, spring black stem and *Stemphylium* leaf spot of alfalfa. *Phytopathology* 55 : 316–56.

HANCOCK, J. G., R. L. MILLAR and W. LORBEER 1964. Pectolytic and celluloytic enzymes produced by *Botrytis alli, B cinerea* and *B. squamosa in vitro* and *in vitro*. *Phytopathology*, 54 : 928 -31.

LIN REE. 1971. Studies on the factors influencing virulence and enzyme activity of *Pythium* spp. on potato tubers. *Indian Phytopoth* 24 : 74 – 88.

MELLANO, H. M., D. E. MUNNECKE and J. J. SIMS. 1970. Relationship of pectic enzyme activity and presence of sterols to pathogenicity of *Pythium ultimum* on roots of *Antirrhinum majus*. *Phytopathology* 60 : 943–50.

MUTHUSAMY, M. 1972. Studies on damping-off of tomoto incited by *Pythium aphanidermatum* (Edson) Fitz. M. Sc. (Ag.) Dissert, Tamil Nadu Agricultural University, 10 pp.

NORKRANS, B. 1950. Influence of cellulolytic enzymes from Hymenomycetes on cellulose preparations of different crystallinity. *Physiol. Plantarum*, 3 : 75–87.

URITANI, I. and M. A. STAHMANN 1961. Pectolytic enzymes of *Ceratocystis fimbriata*. *Phytopathology* 51 : 277–85.

VIDHYASEKARAN, P. and D. KANDASAMY. 1971. Effect of soil fertility on the physiology of corn plants in relation to helminthosporiose disease incidence. *Phytopath. Z.* 72 : 11–20.

VIDHYASEKARAN, P., C. PARAMBARAMANI and C. V. GOVINDASWAMY. 1971. Role of cellulolytic and proteolytic enzymes in pathogenesis of obligate and facultative parasites causing sorghum diseases. *Indian Phytopath.* 24 : 305–8.

WINSTEAD, N. N. and C. L. McCOMBS. 1961. Pectinolytic and cellulolytic enzyme production by *Pythium aphanidermatum*. *Phytopatopathogy* 51 : 270–3.