

Production of Protopectinase by *Fusarium solani* the Incitant of Root Rot of Onion *

R. B. GAUR¹ and J. P. AGNIHOTRI²

Fusarium solani, causing root rot of onion in Rajasthan, was found to produce protopectinase. Out of the 14 carbon sources used, best activity of protopectinase was found with pectin followed by sucrose, inulin and dextrin. Ammonium purpurate and valine were found to be good sources of N for protopectinase production. Combination of Mn, Zn and Fe resulted in maximum enzyme production.

A severe root rot of onion incited by *F. solani* was observed in Rajasthan (Gaur and Agnihotri, 1978). Wood (1959) and Brown (1965) reviewed role of pectic enzymes in the breakdown of plant tissue and pathogenesis. Pectinolytic enzymes as pathogen factor in the physiology of disease caused by various species of *Fusarium* has been reported by various workers (Singh and Husain, 1968; Davis, 1970 and Perley and Page, 1971). Hence a detailed study was made on the production of protopectinase by this fungus and the results are presented in this paper.

MATERIAL AND METHODS

A monoconidial culture of the pathogen maintained on Richard's medium was used for the study. For investigation of the effect of carbon and nitrogen sources on enzyme production, different compounds were

substituted in the medium as the source of carbon or nitrogen. For studies with micronutrient, methodology of Steinberg (1935) was adopted. Manganese and zinc in the form of sulphates and iron as chloride were added to the purified medium to provide 1 ppm of the micronutrient individually and in combination. 20 ml of each of medium was distributed in 100 ml flasks and autoclaved. Thereafter the media were inoculated with a disc of 3 mm diameter cut from 9 days old fungal culture on PDA plates. The pH of the media and of the filtrate from all treatments were recorded. Each treatment was replicated thrice. The flasks were then incubated for six days at $30 \pm 1^\circ\text{C}$. The cultures were filtered and filtrates were centrifuged and used for testing the enzyme activity. The protopectinase enzyme was estimated by the potato disc method (Brown, 1915) by recording the reaction time (RT) in minutes.

* Part of the M. Sc. thesis submitted by the first author to Udaipur University, Campus-Jobner, 1. Regional Agricultural Research Station, Sriganganagar, and 2. Agricultural experiment Station, Jobner.

TABLE Production of protopectinase by *Fusarium solani* (Mart.) Sacc. on different sources of carbon, nitrogen and micronutrients.

Source	Final pH	RT in minutes	Dry weight (gm)
a—Carbon source			
D—Galactose	5.8	847—860	423.70
Sucrose	6.5	352—360	226.13
Inulin	6.3	496—530	283.47
Pectin	5.1	270—295	238.33
Dextrin	7.5	497—540	293.97
b—Inorganic nitrogen source			
Ammonium sulphate	2.8	200—1230	165.40
Ammonium chloride	2.9	375—380	147.90
Ammonium purpurate	3.0	300—320	257.50
Ammonium oxalate	3.1	380—400	334.47
c—Organic nitrogen source			
DL—alanine	4.7	315—325	372.77
DL—valine	4.3	197—205	296.80
L—leucine	4.2	305—325	283.33
L(—) serine	5.1	315—320	288.80
DL—tryptophan	4.1	319—340	165.37
L(+) cystein	3.4	310—330	42.43
Asparagine	4.3	305—325	268.40
Urea	4.1	200—230	218.30
L(—)histidin	3.3	310—330	180.83
L(—)prolin	4.0	210—220	311.87
d—Trace element			
Mn	5.9	848—870	167.13
Fe	6.6	587—600	141.17
Mn + Fe	6.2	587—600	172.50
Mn + Zn + Fe	6.6	250—270	217.88

RESULTS AND DISCUSSION

i) Effect of different sources of carbon on the production of protopectinase.

The results (Table) indicated that out of 14 sources used, maximum activity of protopectinase was found with pectin, though it did not yield maximum weight of the mycelium. It was followed by sucrose, inulin and dextrin.

ii) Effect of different sources of inorganic nitrogen on the production of protopectinase.

Ammonium purpurate accelerated the enzyme production followed by ammonium chloride and ammonium oxalate.

iii) Effect of different amino acids on the production of protopectinase.

Valine caused the maximum enzyme production followed by urea and prolin. Leucine and asparagin, cystein and histidin gave similar RT. Tryptophan, serine and alanine were found unsuitable for enzyme secretion.

iv) Effect of micronutrients on the production of protopectinase.

Combination of Mn, Zn and Fe resulted in maximum enzyme production though it did not support the best growth. The RT of Fe and Mn + Fe remained the same. Mn only was not found suitable for enzyme production.

Pectinolytic enzymes is known to help the pathogen in invasion and breakdown of plant tissues. Under present study *Fusarium solani* has also been found to produce pectinolytic enzymes. Production of enzymes is greatly influenced by nutritional and several other factors. As in this present study the activity of protopectinase was enhanced by pectin but it did not support the profuse growth of the fungus. Pectin was also found suitable for enzyme production in case of *Rhizopus* spp. (Gupta and Pandey, 1959). On the contrary, Davis (1970) found glucose accelerating the enzyme secretion in case of *F. oxysporum*. Agnihotri and Prasad (1971) in their studies on enzyme production by *Colletotrichum capsici* f. *Cyamopsicola* concluded that sucrose was quite effective. This fungus also gave quite a good response to sucrose. Out of the N sources tried, ammonium purpurate and valine were found most suitable for enzyme production but they also did not support the fungal growth. In case of micronutrients the combination of Mn, Zn and Fe coursed in maximum enzyme production but fungal growth was not heavy. The response of the agents used for enzyme activity may vary amongst species of the same genus. In conclusion, it can be stated that all those agents, irrespective of their chemical nature, that responded highly to enzyme production did not help the growth of the fungus in the medium. It can, therefore, be said that heavy mycelial growth is not essential for high enzyme production

(Gupta, 1962, Agnihotri and Prasad, 1971 and Egorov et al, 1871).

Thanks are due to Dr. V.N. Pathak, Head, Department of Plant Pathology, College of Agriculture, Jobner for providing necessary facilities and to Mr. S. R. Ahmed, Assistant Plant Pathologist, Sriganaganagar, for his valuable suggestions.

REFERENCES

- AGNIHOTRI, J. P. and N. PRASAD. 1971. Production of protopectinase by *Colletotrichum capsici* *Cyamopsiscola*. *Indian J. Mycol. Pl. Path.* 1: 51—55.
- BROWN, W. 1915. Studies in the physiology of parasitism. I The action of *Botrytis cinerea*. *Ann. Bot.* 29: 313—43.
- BROWN, W. 1965. Toxins and cell-wall dissolving enzymes in relation to plant disease. *Ann. Rev. Phytopathol.* 3: 1—18.
- DAVIS, D. 1970. Carbohydrate specificity for fusaric acid synthesis. *Phytopathology.* 60: 111—13.
- EGOROV, N. S., V. I. USHAKOVA and B. P. PRUDLOV. 1971. Study of the formation of proteolytic enzymes by fungi imperfecti of the genera *Ciadosporium*, *Fusarium* and *Alternaria* in connection with fibrinolytic activity. *Mikrobiologiya.* 40: 604—609.
- GAUR, R. B. and J. P. AGNIHOTRI. 1978. Growth of *Fusarium solani* in relation to carbon sources and C/N ratio. (ABST).
- Symposium on Plant Disease problems. Society of Mycology and Plant Pathology, Udaipur.
- GUPTA, M. N. 1962. Studies in the pectic enzyme by parasitic fungi. IV. Production of protopectinase enzyme by *Fusarium orthoceras* App. and Wr. var. *ciceri* Padwick on synthetic media. *Proc. Indian Acad. Sci.* 55: 120—27.
- GUPTA, S. C. and D. K. PANDER. 1950. Studies in the pectic enzymes by parasitic fungi II. Factors affecting the production of protopectinase enzymes by *Rhizopus stolonifer* (Ehrenb. ex Fr.) Lind. *Proc. Indian Acad. Sci.* 50 B: 75—81.
- PERLEY, A. E. and O. T. PAGE. 1971. Differential induction of pectolytic enzymes of *Fusarium roseum* (LK). emend. Synder and Hansen. *Can. J. Microbiol.* 17: 415—20.
- SINGH, G. P. and A. HUSAIN. 1962. Production of pectic and cellulolytic enzymes by arhar wilt fungus. *Curr. Sci.* 31: 110—12.
- SINGH, G. P. and A. HUSAIN. 1968. Role of enzymes in pathogenesis by *Fusarium lateritium* f. *cojani*. *Indian Phytopath.* 21: 361-73.
- STEINBERG, R. A. 1935. Nutrient solution purification for removal of heavy metals in deficiency investigation with *Aspergillus niger*. *J. agric. Res.* 51: 413—24.
- WOOD, R. K. S. 1959. Pathogen factors in the physiology of disease-pectic enzymes. In *Plant Pathology problems and progress 1908—1959*. pp. 100—199. The University of Wisconsin Press, USA.