*Indian J. mycol. Res.*, **17** (2) 71—76 (1979)

# IN VITRO PRODUCTION OF CELLULOLYTIC AND PROTEOLYTIC ENZYMES BY *RHIZOCTONIA* SP. AND *PYTHIUM BUTLERI*

By

K. C. BASUCHAUDHARY AND A. K. PAL

*Department of Plant Pathology, Banaras Hindu University, Varanasi 221005, India*

*Rhizoctohia* sp. and *Pythium butleri* produced cellulase, protease, polygalacturonase and pectinesterase enzymes in liquid culture medium with specific substrate. *Rhizoctonia* sp. produced more cellulase than the other enzymes whereas *P. butleri* produced negligible amount of cellulase. There was not much difference in the amount of protease, polygalacturonase and pectinesterase enzymes produced by two pathogens.

## INTRODUCTION

*Rhizoctonia* sp and *Pythium butleri* Subram. produce wilt, and wilt and damping-off symptoms respectively on sannhemp (*Crotolaria juncea* L.) (Pal, 1978). The present *Rhizoctonia* sp. differs from its closely related species, *R. solani* Kuhn. in having poor development of sclerotia, pigment, formation of mycelial strand and also in symptomatology. Various hydrolytic enzymes are known to be produced by other species of the genus *Rhizoctonia* (Matsumoto, 1921; Kernkamp *et al.*, 1952; Kohlmeyer, 1956; Elarosi, 1958; Barker & Walker, 1962;

Bateman, 1963; Sherwood, 1965; Ayers *et al.*, 1966. ) and *Pythium* (Dellingham, 1955; Gupta, 1956; Wood & Gupta, 1958; Winstead & McCombs, 1961; Moore & Couch, 1968.), but nothing is known about their production by the present *Rhizoctonia* sp. and *P. butleri* with only an exception of Janardhan and Husain (1974) who reported the production of pectolytic enzymes *in vitro* by latter species causing root-rot of belladona. Therefore, a study on the production of cellulolytic, pectolytic and proteclytic enzymes *in vitro* by these two pathogens were carried out.

## MATERIALS AND METHODS

An axenic culture, each of *Rhizoctonia* sp. and *P. butleri* from infected sannhemp were used in the present study. Isolates were cultivated on Potato-Dextrose-Agar and maintained at 10°C. The fungi were cultured in a liquid medium which had the following composition : $KH_2PO_4$-3.0 g, $K_2HPO_4$-2.0 g, $MgSO_4$ $7H_2O$-0.5 g. Asparagine 2.0 g Yeast extract-1,0 g and distilled water-1000 ml.To this medium 10 g/ 1 of different substrates,eg., gelatine, sodium polypectate,carboxymethyle cellulose, pectin,were added to improve the production of protease, polygalacturonase, cellulase and pectinesterase respectively. Isolates were grown in 50 ml of this medium in 250 ml Erlenmeyer flask at 21°C for eight days., The pH of culture media were adjusted to 4.5-5.3 depending on the enzyme in question (Table 1). Three replications for each enzyme were maintained. After the incubation period, the culture broths were filtered through 'Mill pore' filter and the filtrates were immediately tested for the enzyme by means of cup-plate method described by Dingle *et al.* (1953) and Khare and Bonpeix (1976).

20 ml of 2% agar and 1% substrate which contained 0.01% sodium merthiolate to prevent contamination, was poured in 90 mm diam. petriplates. Cups of 7 mm diam. were cut with the help of a sterilized cork-borer. Cups were filled with 0.1 ml culture filtrate. Plates were incubated at 30°C for 48 hours. The activity zone of enzyme after incubation were developed by flooding the pla-

tes with appropriate developing agents (Table 1). The enzyme activity was expressed as EA = D (Total diameter) — 7 (diameter of the cup). Four cups were cut in each plate for each enzyme.

Table 1. *The substrates, developing solution and pH used for detecting different enzymes secreted by* Rhizoctonia *sp. and* Pythium butleri

| Enzyme | Substrate | pH of the medium | Developing agent |
|---|---|---|---|
| Cellulase | Carboxyl methyl cellulose | 4.5 | 3% lead acetate |
| Protease | Gelatine | 4.5 | |
| Polygalacturonase | Sodium polypectate | 5.3 | 5N HCl |
| Pectinesterase | Pectin | 4.5 | 3% lead acetate |

## Result and Discussion

Cellulase— In cellulase, test plates when developed with 3% lead acetate, white band were formed around the cup against opalescent background. Enzyme in culture filtrate degraded carboxyl methyl cellulose first to cellubiose and then to glucose which reacted with lead acetate solution giving white band.

Protease—In protease test no developing agent is required. Clear zone developed around the cup of the opalescent background. This enzyme hydrolyzed proteins into free amino acids.

Polygalacturonase—White zones around the cup were formed when developed. Polygalacturonase hydrolysed sodium polypectate into glucose which gave white colouration.

Pectinesterase- Test-plates when developed with 3% lead acetate yielded clear zone on an opalescent background around the cup.

Table 2. *Cellulase, protease, polygalacturonase and pectinesterase secreted by* Rhizoctonia *sp. and* Pythium butleri *in liquid culture Diffusion zone (EA) in mm*

| Enzyme | Rhizoctonia sp. | Pythium butleri |
|---|---|---|
| Cellulase | 10.2 | 2.0 |
| Protease | 6.2 | 7.4 |
| Polygalacturonase | 8.0 | 7.6 |
| Pectinesterase | 6.5 | 5.0 |

It is evident from Table 2 that both *Rhizoctonia* sp. and *P. butleri* produced cellulase, protease, polygalacturonase and pectinesterase in liquid culture medium containing specific substrate. *Rhizoctonia* sp. pryduced cellulase much more than protease, polygalacturonase and pectinesterase. Cellulose enzyme produced by *P. butleri* was negligible in comparison to that produced by *Rhizoctonia* sp. There was not much difference in the amount of protease, polygalacturonase and pectinesterase produced by both the species.

Kohlmeyer (1956) observed that *R. solani* is capable of producing large quantities of cellulase *in vitro*. Barker and Walker (1962) reported that *Pellicularia filamentosa* produced pectolytic enzymes very readily *in vitro* on a pectin carbon source. In the cellulase production *Rhizoctonia* sp. seems alike to *R. solani*.

*P. butleri* produced very negligible cellulase compared to other three enzymes. Janardhan and Husain (1974) also reported *P. butleri* on belladonna to be very weakly cellulolytic while *P. aphanidermatum* (Dallingham,1955),*P. ultimum* (Winstead & McCombs, 1961) and *P. irregulare* (Moore & Couch, 1968) are strongly cellulolytic.

The histopathological investigation on the wilt and wilt/damping-off showed that *Rhizoctonia* sp. and *P. butleri* invaded the root tissues of sannhemp both intercellularly and intracellularly and the cells were disorganised (Pal and Basuchaudhary, unpublished) due to production of proteolytic enzymes which helped to dissolve the middle lamella of the cortical cells and pathogens made their way towards centre of the root. Christuo (1962) showed this type of

pectolytic activity by *Rhizoctonia solani* in beans. Thus, production of these enzymes *in vitro* by both the pathogens indicates their role in degrading cell walls of the susceptible sannhemp roots.

## REFERENCES


Ayers, W. A., Papavizas, G. O. and Dein, A. F. (1966). Polygalacturonase transeliminase and Polygalacturonase production by *Rhizoctonia solani*. *Phytopathology*, **56** : 1006-1011.

Barker, K. R. and Walker, J. C. (1962). Relationship of pectolytic and cellulolytic enzyme production by strains of *Pellicularia filamentosa* to their pathogenicity. *Ibid.* **52** : 1119-1125.

Bateman, D. F. (1963). Pectolytic activities of culture filtrates of *Rhizoctonia solani* and extracts of *Rhizoctonia* in infected tissues of bean. *Ibid*, **53** : 197-204.

Christou, T. (1962). Penetration and host-parasite relationships of *Rhizoctonia solani* in the been plant. *Ibid*, **52** : 381-389.

Dellingham, E. O. (1955). Cellulolytic activity and growth characteristic of *Pythium irregulare*. *Diss. Abst.*, **15** : 678-679.

Dingle. J., Reid, W. D., and Solomons, G. L. (1953). The enzymic degradation of Pectin and other Polysaccharides II-Application of the 'cup-plate' assay to the estimation of enzymes. *J. Sci. Food Agric.*, **3** : 149-L55.

Elarosi, H. (1958). Fungal associations. III. The role of pectic enzymes on the synergistic relation between *Rhizoctonia solani* Kuhn. and *Fusarium solani* Snyder and Hansen in the rotting of potato tubers. *Ann. Bot. (NS)*, **22** : 399-416.

Gupta, S. C. (1956). Studies in physiology of parasitism. XXII - The production of pectolytic enzymes of *Pythium debaryanum* Hesse. *Ibid.*, **20** : 179-190.

Janardhan, K. K. and Husain, A. (1974). Production of toxic metabolite and Pectolytic enzyme by *Pythium butleri*. *Mycopath, et. Mycol. appl.*, **52** : 325-330.

Kernkamp, M. F. de Zeeuw, D. J., Chen, S. M., Ortega, B. C., Tsianj, C. T. and Khan, A.M. (1952), Investigations on physiologic specialization and parasitism of *Rhizoctonia solani*. *Univ. Minnesota agric. Expt. Sta. Tech. Buil.*, **200** : 36.

Khare, K. B. and Bompeix, G. (1976). Activities proteolytiques des *Sclerotinia sclerotiorum* et *S. minor* : Role possible lors de la pathogenese. *Revue de Mycologie*, **40** : 65-84.

Kohlmeyer, J. (1966). Uber den cellulose-Abbau durch einige phytopathogene pilze. *Phytopath. Z.*, **27** : 147-182.

Matsumoto, T. (1921). Studies in the physiology of the fungi. XII. Physiological specialization in *Rhizoctonia solani* Kuhn. *Ann. Missouri Botan Garden*, **8**:1-62.

Moore, L. D. and Couch, H. B. (1968). Influence of calcium nutrition on pectolytic and cellulolytic enzymes activity of extracts of high land Bantage foliage blight by *Pythium ultimum*. *Phytopathology*, **58** : 833-838.

Pal, A. K. (1978). Studies on some root disease of sannhemp (*Crotalaria juncea* L.). Ph. D. Thesis, Banaras Hindu University, Varanasi, 221005, India.

Sherwood, R. T. (1966). Pectin lyase and polygalacturonase production by *Rhizoctonia solani* and other fungi. *Phytopatholog*, **56** : 279-286.

Winstead, N. N. and McCombs, C. L. (1961). Pectinolytic and cellulolytic enzyme production of *Pythium aphanidermatum*. *Phytopathology*, **51** : 270-273.

Wood, R. K. S. and Gupta, S. C. (1958). Some properties of pectic enzymes secreted by *Pythium*. *Ann. Bot. (NS)*, **22** : 309-319.