Proceedings of the Iowa Academy of Science

Volume 71 | Annual Issue Article 15

1964

Extracellular Pectolytic and Cellulolytic Enzymes of Pyrenochaeta terrestris

J. C. Horton
Iowa State University

consumo idrico delle piante. Consiglio Nazionale Delle Ricerche Comitato Per L'Agricoltura. Anonima Arti Grafiche S. A. Bologna.
3. Martin, E. V. and F. E. Clements. 1935. Plant Physiol. 10:613-636.
4. Martin, E. 1943. Carnegie Inst. Wash. Publ. No. 550.
5. Huber, B. 1930. Zeitschr. Bot. 23:839-891.
6. Ansari, A. Q. and W. E. Loomis. 1959. Amer. Jour. Bot. 46:713-717.
7. Brown, H. J. and F. Escombe. 1900. Phil. Trans. Roy. Soc. London 193B:223-291.
8. Ting, I. P. and W. E. Loomis. 1963. Amer. Jour. Bot. 50:866-872.
9. Ting, I. P. 1964. Unpublished Ph.D. Thesis. Iowa State University, Ames, Iowa.
10. Jeffreys, H. 1918. Phil. Mag. 35:270-280.
11. Woolley, J. T. 1961. Plant Physiol. 36:112-114.

Extracellular Pectolytic and Cellulolytic Enzymes of *Pyrenochaeta terrestris*[1]

J. C. Horton[2]

Abstract. Extracellular pectolytic and cellulolytic ensymes were produced *in vitro* and *in vivo* by *Pyrenochaeta terrestris*. Mycelial development was sparse on cellulose, very good on glucose and best on pectin. Cellulase synthesis was the same at culture temperatures from 15°C to 30°C while pectinase synthesis was optimum at 20°C.

Pink root disease of onions incited by *Pyrenochaeta terrestris* (Hans.) Gorenz *et al.* occurs annually in all onion growing areas. Onion roots are commonly attacked late in the season, and disease progress is slow. Under certain conditions of water stress, disease progress may be rapid and loss severe (1). The ubiquity of the pathogen and the constant inoculum potential, despite long rotations, suggest a well adapted saprophytic phase (2).

In culture, however, the fungus competes poorly with other common soil inhabitants. Despite an extremely wide host range, involvements with root of other plants are very casual. Asexual spore structures are produced only sporadically, suggesting that the probable overwintering structure is mycelia. These apparent contradictions in behavior pose the interesting question of how this fungus survives for years at a high inoculum potential in the absence of onion hosts? Disease symptoms of root maceration and softening suggest that cell disrupting enzymes, such as cellulase and pectinase, may be involved pathogenically and also provide a means for saprophytic survival.

Materials and Methods

Isolates of *P. terrestris* (Table 1) were grown in standing cul-

[1] Journal Paper No. J-4895 of the Iowa Agricultural and Home Economics Experiment Station, Ames, Iowa. Project No. 1524.
[3] The following in 1.0 liter of solutiin: $NaNO_3$ 4.25 g, KCl 0.5 g NaH_2PO_4 1.4 g,

Table 1. Sources of *P. terrestris* isolates

Isolate No.	Source	Contributor	Pathogenicity
Pt 8	Iowa	W. J. Hooker	severe
Pt 18	Louisiana	E. C. Timms	severe
Pt 30	Wisconsin	R. H. Larson	moderate
Pt 31	Iowa	W. J. Hooker	severe
Pt 33	Louisiana	E. C. Timms	———
Pt 40	Oregon	R. H. Larson	moderate

ture on 50 ml of a modified Czapeks medium[3] in 250 ml erlenmeyer flasks. Flasks containing 10 g/l of Alpha-cel[4], 10 g of citrus pectin[5], 156 g of glucose, or combinations of these carbon sources, were autoclaved for 20 min. at 15 psi and seeded with a 4 mm disk of a colony grown on PDA in petri plates. After seeding, flasks were swirled vigorously once daily for three days and then left undisturbed for 2-3 weeks at 25 ± 1°C. When required, flasks were incubated at 15 ± 1°, 20 ± 1°, 25 ± 1°, and 30 ± 1°C. Each experiment was replicated twice. Each replicate consisted of six flasks. Filtrates from individual flasks were filtered through sintered glass (C), diluted to 50 ml, and analyzed for cellulase (Cx) and pectinase (Px) enzymes.

The viscosity reduction of carboxymethylcellulose (CMC) has been established as an estimate of cellulase (Cx) activity (3). Viscosity changes were observed in Ostwald-Cannon-Fenske viscosimeters (size 300) at 40 ± .5°C. The reaction mixture was usually mixed in a small flask in the proportions: 4 ml of 1.0% w/v CMC[6], 5 ml of culture filtrate, and 1 ml of 10x McIlvaine's buffer at pH 5.0. The time of mixing enzyme and substrate was considered T_0 and efflux times were recorded every 5 minutes. For inactive enzyme comparisons, culture filtrates were autoclaved at 15 psi for 60 min and then handled as previously described.

Viscosity loss of sodium polypectate was considered indicative of the presence of pectin depolymerase enzymes. As in the cellulase assay, substrate-enzyme mixtures were made in small flasks in the proportions: 4 ml of 4% sodium polypectate (NaPP), 5 ml of culture filtrate, and 1 ml of 10x McIlvaine's buffer at pH 5.0. Again, time of mixing was T_0 and efflux times were recorded every five minutes. Inactive enzyme was prepared as previously described.

Diseased onion seedlings of variety Southport White Globe (SWG) and an unnamed hybrid (Hyb) were produced in pans of white quartz sand artificially infested with mycelial fragments of isolate Pt 8. Seedlings were harvested after four weeks when

[2] Associate Professor, Iowa State University, Ames.
$MgSO_4$ 0.23 g, $FeSO_4$ 0.01 g.
[4] Nutritional Biochemicals Corp., Cleveland, Ohio.
[5] Matheson Chemical Co., St. Louis, Missouri.
[6] Hercules Powder Co. Type 7HSXP Wilmington, Delaware.

disease involvement of SWG was severe and of Hyb was mild. Roots were detached, washed with distilled water, and 500 mg of each variety triturated in a mortar with 20 cc of buffered CMC or NaPP. The start of trituration was regarded as T_0. Macerates were filtered and placed in viscosimeters. One sample of each variety was autoclaved before trituration to provide an inactive enzyme check.

Results and Discussion

A comparison of enzyme production on different carbohydrate sources was made (Table 2).

Table 2. Enzyme production by isolate Pt 8 of *P. terrestris* on different carbohydrate sources *in vitro*

	Percent reduction in viscosity compared to inactive enzyme[a]		
	Substrate		
	Cellulose	Pectin	Glucose
Cellulase	93.4%[b]	2.6%	10.6%
Pectinase	8.6%	82.1%	0.7%
Growth	+[c]	++++	+++

[a] measured after 2 weeks incubation at 25°C.
[b] Each value is average of 2 replicates for each of 2 experiments.
[c] relative mycelial growth as observed visually.

A basal level of cellulase but not pectinase appears to be usual. The slight amount of pectinase synthesized on cellulase is probably due to residual amounts of pectin. Colony growth was visibly more luxurious on the pectin substrate than on either

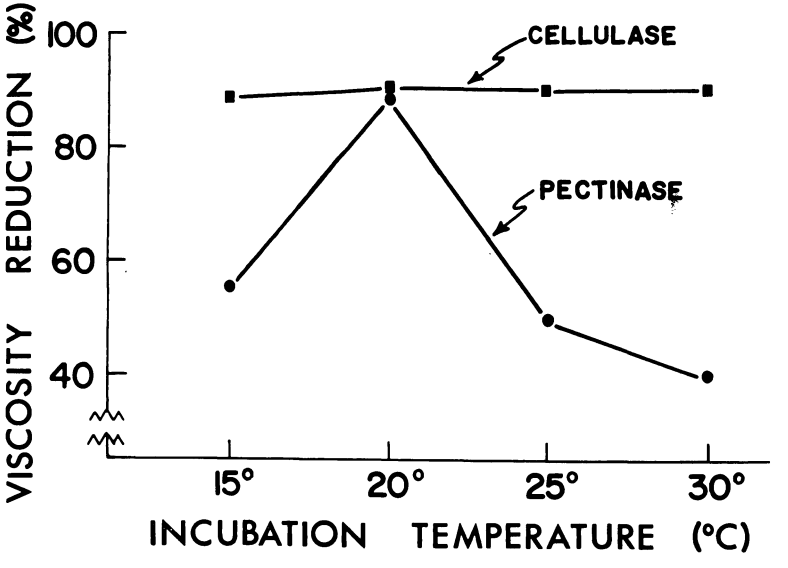

Figure 1. Cellulase and pectinase synthesis by *P. terrestris* afte r3 weeks incubation at various temperatures

cellulose or glucose. Growth on cellulose was always sparse. Production of enzymes by Isolate Pt 8 was determined at various temperatures of incubation (Figure 1). The lack of variation in Cx synthesis coincides with visual observations that approximately equal growth occurred at all temperatures on cellulose. Fungal growth was considerably less on cellulose than on glucose or pectin. Possible explanations include a slow rate of cellulosic hydrolysis or a low level of enzyme activity. Growth on cellulose does not explain rapid pathogenic development previously reported (1). Cellulase may be important in perpetuating the fungus in the absence of a host on soils having a high cellulose content, such as peats and mucks.

Mycelial development on pectin was excellent at all temperatures, although growth seemed to be best at 20°C. Growth occurred rapidly enough to explain the swift development of disease. The temperature optimum usually reported for this fungus is 24-28°C, and the generally assumed optimum for disease is 28°C. Soils on which onions are grown in Iowa rarely reach this temperature at a 4-6″ depth, but often reach 20°C, the optimum for pectinase production. While pectinase may be a factor in perpetuating the fungus in the absence of a host, the role of pectinase is probably most important in the initiation of disease, especially in cooler soils.

A comparison of enzyme synthesis by different isolates was made (Table 3).

Table 3. Comparative pectinase and cellulase production by various isolates of *P. terrestris*[a]

Isolate	Percent reduction in viscosity compared to inactive enzyme	
	Cx	Px
Pt 8	93.4[b]	88.5
Pt 18	89.6	93.2
Pt 30	95.7	96.2
Pt 31	92.5	96.6
Pt 33	84.4	88.8
Pt 40	69.4	95.2

[a] Analyzed after 2 weeks incubation and measured at T_{10}.
[b] Average of 2 replicates, each replicate consisted of 3 flasks.

Isolates exhibited only minor variation in cumulative synthesis of either enzyme, with the exception of Isolate Pt. 40. No correlation of synthetic ability and pathogenicity was apparent.

Although attempts to analyze enzymes quantitatively were not successful, cellulase and pectinase were consistently found in disease onion roots. Three different problems were encountered: (1) adsorption by macerated root cells of free enzyme, (2) variability in disease involvement per root and per plant, and (3) a highly variable decrease in viscosity of substrates by autoclaved filtrate. However, the ability of the fungus to grow

on cellulose and pectin and the presence of cellulase and pectinase in infected roots strongly suggests activity of these enzymes in pathogenesis.

Conclusion

Cellulase and pectinase enzyme systems are produced by *P. terrestris*. Mycelial growth on cellulose and cellulase synthesis did not vary greatly at temperatures from 15° to 30°C. Mycelial growth on pectin and pectinase production exhibited maximum response at 20°C. Growth on pectin equalled that on glucose and exceeded by many times that on cellulose. The data suggest pectinase may be of major importance in pathogenic behavior, while cellulase activity may play only a minor role. However, cellulase may be very important in saprophytic survival on plant debris in the soil. Attempts to correlate enzyme activity in infected roots with varietal response were not successful. A number of isolates of the fungus synthesized either enzyme equally well and other variations that were observed did not correspond to differences in pathogenicity.

Literature Cited

1. Borgman, R. P. and J. C. Horton. 1961. *Proc. Iowa. Acad. Sci.* 68: 103-105.
2. Garrett, S. D. 1956. *Biology of Root-infecting Fungi.* Cambridge Univ. Press. Cambridge.
3. Norkrans, B. 1963. *Ann. Rev. Phytopathology* 1:325-350.

Glucose Inhibition of Cellulase Synthesis by *Pyrenochaeta terrestris*[1]

J. C. Horton and N. T. Keen[2]

Abstract. The rate of synthesis of *Pyrenochaeta terrestris* cellulase was determined on the substrates of glucose, cellulose, and cellulose + glucose. Enzyme production was rapid on cellulose, almost negligible on glucose, and intermediate on cellulose + glucose. On the latter substrate, cellulase appeared after the near exhaustion of glucose. The authors suggest that extremely low concentrations of soluble carbohydrate regulate cellulase synthesis.

Cellulase is an extracellular inducible enzyme found in many fungi (1, 2). Cellulase synthesis typically occurs when fungi are grown on cellulose. Cellobiose, a β-1, 4 dimer of glucose, is also claimed to function as an inducer (3). Glucose, the end product of cellulose hydrolysis, does not induce cellulase syn-

[1] Journal Paper No. J-4892 of the Iowa Agricultural and Home Economics Experiment Station, Ames, Iowa Project No. 1524.
[2] Associate Professor of Botany and Graduate Assistant, respectively Iowa State University, Ames, Iowa.