Effect of Pectin Source and Sugars on Polygalacturonase Production by Ceratocystis ulmi

W. L. Biehn and A. E. Dimond

Assistant Plant Pathologist and Chief, respectively, Department of Plant Pathology and Botany, The Connecticut Agricultural Experiment Station, New Haven 06504.

ABSTRACT

Polygalacturonase (PG) production by *Ceratocystis ulmi* was more than 50 times greater on an ethanol-insoluble fraction from green elm shoots than on citrus pectin. Glucose (0.1 M) repressed PG production by *C. ulmi* 80-99% on an ethanol-insoluble fraction from green elm shoots and on citrus pectin; while galacturonic acid (0.05 M) reduced PG production 70-85%. Phytopathology 61: 745-746.

Pectic enzymes have been suggested to be important in pathogenesis by vascular wilt fungi (2). Beckman (1) reported that *Ceratocystis ulmi* (Buis.) produces a pectin depolymerase; while Husain & Dimond (3) reported that *C. ulmi* produces a polygalacturonase (PG). The characteristics of the *C. ulmi* pectic enzymes reported by Beckman (1) and Husain & Dimond (3) indicate that they were probably working with an endopolygalacturonase. Our studies also indicate that *C. ulmi* produces mostly an endopolygalacturonase in the culture media we used (*unpublished data*). For example, supernatants of *C. ulmi* cultures cause a rapid decrease in viscosity of sodium polypectate solutions and a slow liberation of reducing groups. Thus, the hydrolysis of sodium polypectate is of a random nature. In addition, we detected galacturonic acid as a reaction product. We have not detected any polygalacturonate *trans*-eliminase in culture supernatants of *C. ulmi*. Husain & Dimond (3) reported that *C. ulmi* produced more PG on autoclaved elm twigs than on citrus pectin, but in both cases the PG produced was considerably less than was reported for *Fusarium oxysporum* f. sp. *lycopersici*. Recent studies suggest that most of the vessel blockage causing the wilting of elm leaves may occur in green elm shoots (4, 6, 7). Therefore, we studied PG production by *C. ulmi* on a medium containing an ethanol-insoluble fraction from green elm shoots (elm preparation) compared to that on a citrus pectin medium. This paper also reports on the effect of glucose (0.1 M) and galacturonic acid (0.05 M) on PG production by *C. ulmi*.

PG was obtained by growing *C. ulmi* in shake culture on a pectin-asparagine-salts medium, adjusted to a pH of 3.5 with lactic acid after autoclaving. The medium contained 0.15% KH_2PO_4, 0.001% $FeCl_3$, 0.1% yeast extract, 0.2% L-asparagine and 1% of either citrus pectin or an ethanol insoluble fraction from green elm shoots. The elm preparation contained about 0.15% pectin on a dry wt basis. Galacturonic acid, previously adjusted to a pH of 3.5 with NaOH, and glucose were passed through a sterile Millipore filter (0.22 $\mu \pm 0.02\ \mu$) (Millipore Corp., Bedford, Mass.) prior to addition to the autoclaved medium. Citrus pectin was purchased from Nutritional Biochemicals Corp., Cleveland, Ohio.

The elm shoots (stripped of leaves), harvested in mid-May from American elm (*Ulmus americana* L.) trees 15 ft in height, were immediately frozen in polyethylene bags with dry ice.

The ethanol-insoluble fraction from green elm shoots

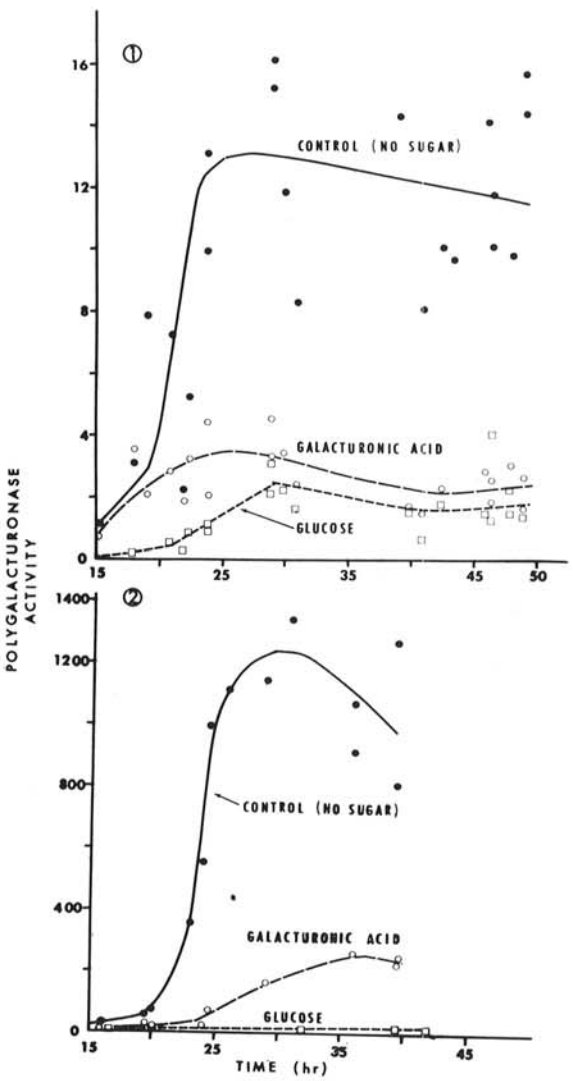

Fig. 1-2. **1)** Polygalacturonase (PG) production by *Ceratocytis ulmi* in a citrus pectin (1%)-asparagine salts medium without sugar (control), with glucose (0.1 M), and with galacturonic acid (0.05 M); as a function of time. **2)** PG production by *C. ulmi* in an elm preparation (1%)-asparagine-salts medium without sugar (control), with glucose (0.1 M), and with galacturonic acid (0.05 M); as a function of time. PG activity is expressed as $\frac{1{,}000}{t}$, where t equals min required for the relative viscosity of the sodium polypectate reaction mixture to be reduced by 50%/mg dry wt of mycelium.

was prepared as follows: the frozen tissue was chopped into smaller sections with a knife, dropped into boiling 95% ethanol (3.7 ml/g fresh wt of tissue) and left to stand in ethanol for 3 days at 22-24 C, filtered, resuspended in fresh 95% ethanol, and allowed to stand 1 day. After filtering, the shoots were dried in an oven at 60 C and ground to pass a 60-mesh screen in a Wiley mill.

Ceratocystis ulmi was maintained on potato-dextrose agar. A 1-ml aliquot containing mostly conidia was transferred to a glucose-casamino acids medium (5). Inoculum was prepared after 3 days of shake culture on a Gyrotary (New Brunswick) shaker (212 cycles/min) at 21-23 C by centrifuging and washing the resulting cells with sterile deionized water. A 1-ml aliquot of a spore suspension (1×10^8 cells/ml) was added to 42 ml of the pectin-asparagine-salts medium in a 250-ml flask. After incubation on a shaker, the contents were centrifuged. The supernatants were dialyzed overnight and assayed for PG activity according to a standard viscometric assay with 1% sodium polypectate as the substrate (5), except that a pH 5.5 citrate buffer was used.

We have previously observed that *C. ulmi* produces only 1-4 units of PG/mg dry wt of mycelium in a casamino acids medium (5), with or without glucose, containing 1% sodium polypectate (*unpublished data*). PG production by *C. ulmi* on citrus pectin reached a maximum of about 14 units compared to 1,000-1,300 units on the elm preparation (Fig. 1, 2). PG production was similar for two different isolates of *C. ulmi*. In addition, PG production was similar on elm shoot media prepared from shoots collected in either 1969 or 1970.

Glucose (0.1 M) repressed PG synthesis more than 80% on citrus pectin and more than 98% on the elm preparation (Fig. 1, 2). In the presence of galacturonic acid (0.05 M), PG production by *C. ulmi* was reduced at least 70% after 25-50 hr of shake culture (Fig. 1, 2). Glucose (0.1 M) and galacturonic acid (0.05 M) incubated with the PG of *C. ulmi* for 2 hr had no effect on its activity.

It appears that host pectin is a good inducer of PG production by *C. ulmi*. Recent work suggests that most of the vessel blockage causing the wilting of leaves may occur in green shoots (4, 6, 7). The large amount of PG produced by *C. ulmi* on the ethanol-insoluble fraction from green elm shoots suggests that PG production by *C. ulmi* might play a role in this vessel blockage.

Since glucose represses PG production by *C. ulmi*, the evaluation of nonmetabolized compounds related to glucose as chemotherapeutants against Dutch elm disease would seem worthwhile. Such studies might provide information on the role of hydrolytic enzymes in pathogenesis as well as lead to further means of controlling plant diseases.

LITERATURE CITED

1. Beckman, C. H. 1956. Production of pectinase, cellulases, and growth-promoting substances by Ceratostomella ulmi. Phytopathology 46:605-609.
2. Dimond, A. E. 1970. Biophysics and biochemistry of the vascular wilt syndrome. Annu. Rev. Phytopathol. 8:301-322.
3. Husain, A., & A. E. Dimond. 1958. The function of extracellular enzymes of Dutch elm disease pathogen. Nat. Acad. Sci. Proc. 44:594-601.
4. MacHardy, W. E. 1970. Sites of water transport interference contributing to elm wilt caused by Ceratocystis ulmi. Phytopathology 60:576 (Abstr.).
5. Patil, S. S., & A. E. Dimond. 1968. Repression of polygalacturonase synthesis in Fusarium oxysporum f. sp. lycopersici by sugars and its effect on symptom reduction in infected tomato plants. Phytopathology 58:676-682.
6. Pomerleau, R. 1968. Progression et localisation de l'infection par le Ceratocystis ulmi dans l'orme d'Amerique. Phytopathol. Z. 63:301-327.
7. Pomerleau, R. 1970. Pathological anatomy of the Dutch elm disease. Distribution and development of Ceratocystis ulmi in elm tissues. Can. J. Bot. 48: 2043-2058.