Purification and Properties of an Endoxylanase from a Corn Stalk Rot Strain of *Erwinia chrysanthemi*

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ABSTRACT

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An extracellular xylanase (EC 3.2.1.8) was purified approximately 20-fold from liquid cultures of *Erwinia chrysanthemi* strain SR120A grown with glycerol as a carbon source. Ammonium sulphate fractionation, cation-exchange chromatography (CM-Sephadex), chromatofocusing, and gel filtration (Sephadex G-75) isolated the enzyme from culture filtrates. The xylanase had a molecular mass of approximately 42 kDa and had a pI of 8.8. The pH optimum was 5.5, and the temperature optimum in a 10-min assay was 55 C. About 40% of the enzyme activity was lost after incubating for 1 h at 40 C. The enzyme was shown to be an endoxylanase, cleaving xylan polymers internally. The xylanase and an endopectate lyase (EC 4.2.2.2) isolated from *E. chrysanthemi* were tested, both alone and together, for cell-killing and tissue-macerating ability on oat, corn, and selected dicotyledonous plants. The xylanase demonstrated both a cell-killing and a tissue-macerating capability on grasses.

Additional keywords: cell wall-degrading enzyme.

Soft rot pathogens produce a wide array of enzymes capable of breaking down polymers found in plant cell walls (10,21). Numerous genetic, biochemical, and physiological studies indicate that the pectolytic enzymes play a central role in soft rot pathogenesis (8). Virtually all these studies have involved dicotyledonous plants.

Cell-wall composition in grasses (Gramineae) differs a great deal from that found in dicotyledonous plants and most other monocotyledonous plants (10,11,20). Pectic polysaccharides typically account for 30–40% of the primary cell-wall material in dicotyledons and for 4–6% of the material in grasses. There are also important differences in the hemicelluloses. Whereas a typical dicotyledon wall might contain 20% xyloglucan and 5% xylan, a typical grass wall contains 30–40% arabinoxylan and only trace amounts of xyloglucan. There has been speculation that xylans play an important role in cross-linking the polysaccharides in grass cell walls (14,18). Grass cell walls also contain a unique type of mixed linkage β-D-glucan that is absent or extremely rare in dicotyledons as well as in other monocotyledons. Substantial differences in wall composition between grasses and dicotyledons raise the question of whether different cell wall-degrading enzymes are needed by pathogens of these two plant groups.

In an earlier study (6), several strains of *Erwinia chrysanthemi* from grass (corn) and nongrass hosts were screened for their ability to produce cell wall-degrading enzymes. All the strains produced an array of enzymes (endopectate lyase, exopolygalacturonase, pectin methylesterase, endoglucanase, and protease), and there usually were only minor differences in the amounts produced by different strains. Xylanase, however, was produced in substantially greater amounts by corn strains than by strains from nongrass hosts. The difference in xylanase production was correlated with the ability to macerate corn leaf tissues in vitro.

Our objective was to purify and characterize the *E. chrysanthemi* xylanase and to determine what effects the xylanase and endopectate lyase produced by this corn pathogen would have on the cells and tissues of selected grasses and dicotyledonous plants. Such information might suggest whether either or both of these

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enzymes contributes to the development of soft rot symptoms observed in infected corn plants.

MATERIALS AND METHODS

Culture conditions. *E. chrysanthemi* strain SR120A initially was isolated from *Zea mays* L. in Hawaii by A. C. Hayward. Stock cultures were stored in 10% skim milk in liquid nitrogen or as suspensions in sterile distilled water at room temperature. To produce enzymes, bacterial cells from storage were first streaked onto crystal violet-pectate medium (29), and cells from colonies with typical morphology were suspended in sterile distilled water and were used as inoculum for liquid cultures. Bacteria were grown in either MOPS minimal medium (23) or in the mineral-salts medium of Zucker and Hankin (37) supplemented with 0.8% glycerol and 0.015% Triton X-100 to produce xylanase or 0.25% pectin and 0.25% sodium polypectate (Sigma Chemical Co., St. Louis, MO) to produce pectate lyase (PL). Cultures were incubated on a rotary shaker (80 rpm) at 22 C for 72 h.

Enzyme and protein assays. Xylan from larchwood (Sigma) was repurified before use (32). For xylanase assays, 0.5 ml of 1% xylan in 0.05 M sodium acetate buffer, pH 5.5, was mixed with 0.1 ml of enzyme solution and was incubated for 30 min at 30 C. Xylanase activity was determined by measuring the increase in reducing sugars in the reaction mixtures (22). One unit of enzyme activity was defined as the amount of enzyme releasing 1 μmol of product per minute under the conditions just described. PL activity was determined by measuring the increase in absorbance at 235 nm of a reaction mixture containing 1.9 ml of 0.05 M Tris-HCl buffer, pH 8.5, 0.25% (w/v) sodium polypectate (Sigma), 0.1 mM calcium chloride, and 0.1 ml of enzyme solution (31). This mixture was incubated for 30 min at 30 C, and the reaction was stopped by adding 0.1 ml of 40 mM EDTA. The absorbance blank consisted of a reaction mixture with 0.1 ml of boiled enzyme added. Protein was determined using the Bio-Rad protein assay (Bio-Rad Laboratories, Richmond, CA) with bovine gamma globulin as a standard.

Xylanase purification. Bacteria were removed from culture fluids by centrifugation at 12,000 *g* for 20 min. All subsequent steps were carried out at 5–8 C. Culture filtrate was concentrated to about one-third of the original volume by dialysis against 20% polyethylene glycol (PEG 20,000; Fisher Scientific, Pittsburgh, PA). Ammonium sulfate fractionation was performed, and the 55–90% fraction was collected by centrifugation at 10,000 *g* for 30 min. The precipitate was taken up in a small volume of 0.02 M phosphate buffer, pH 7.0, and was desalted by passage through a Sephadex G-25 column (Pharmacia LKB Biotechnology, Piscataway, NJ) into 0.02 M MES buffer, pH 5.5.

The desalted enzyme solution was applied to a CM-Sephadex column (1.5 × 19 cm) equilibrated with 0.02 M MES buffer, pH 5.5. After washing with two column volumes of buffer, the proteins were eluted with a 0–0.2 M NaCl gradient in the same buffer. Active fractions were pooled, concentrated by dialysis against solid PEG, and dialyzed into 0.025 M ethanolamine-HCl buffer, pH 9.4. The enzyme solution was loaded onto a 1- × 29-cm chromatofocusing column (PBE 94; Pharmacia) and was eluted with Polybuffer 96 (Pharmacia), forming a gradient from pH 9 to 7. Active fractions were collected, concentrated by dialysis against solid PEG, and loaded onto a 1.5- × 48-cm column of Sephadex G-75 equilibrated with 0.05 M phosphate buffer, pH 6.0, containing 0.05 M NaCl. The enzyme was eluted with the same buffer, and active fractions were stored at −20 C until use.

PL purification. The overall procedure was similar to the xylanase purification procedure described above. The culture filtrate underwent ammonium sulfate fractionation, without prior concentration. The precipitate from the 55–90% salt fraction was collected by centrifugation, was taken up in a small amount of 0.02 M phosphate buffer, pH 7.0, and was desalted into 0.02 M acetate buffer, pH 5.5, by passage through a Sephadex G-25 column. The desalted solution was loaded onto a CM-Sephadex column (1.5 × 20 cm) equilibrated with 0.02 M acetate buffer, pH 5.5, was washed with two column volumes of acetate buffer, and the PL was eluted from the column with a 0.0–0.5 M NaCl gradient. Active fractions were concentrated by dialysis against solid PEG and were dialyzed into 0.025 M triethylamine-HCl buffer, pH 11.0. The enzyme solution was loaded onto a PBE 118 chromatofocusing column (1 × 28 cm; Pharmacia) and was eluted with Pharmalyte-HCl (Pharmacia), pH 8.0, forming a pH gradient from pH 11 to 8. PL fractions were collected from pH 9.2 to 9.4, were concentrated, and were passed through a Sephadex G-75 column (1.5 × 48 cm) equilibrated with 0.05 M Tris-HCl buffer, pH 8.0, containing 0.05 M NaCl. Enzyme solutions were stored at −20 C . The purified PL was assayed for the following enzymatic activities: pectin lyase, polygalacturonase, pectin methylesterase, xylanase, and cellulase. Only PL activity was detected.

Electrophoresis. Xylanase purity and molecular weight were assessed via sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using the procedure of Laemmli (presented in [28]). Molecular mass standards (Sigma) included bovine albumin (66 kDa), egg albumin (45 kDa), glyceraldehyde-3-phosphate dehydrogenase (36 kDa), carbonic anhydrase (29 kDa), trypsinogen (24 kDa), soybean trypsin inhibitor (20 kDa), and alpha-lactalbumin (14 kDa). Proteins were stained with Coomassie blue R 250.

Effect of pH and temperature. Influence of pH on xylanase activity was determined using a range of buffers in the standard xylanase assay. The buffers included citrate-phosphate (pH 2.5–6.5), phosphate (pH 7), Tris-HCl (pH 7.5–9), and carbonate-bicarbonate (pH 9.5–10). Enzyme stability at various pH values was determined by dialyzing enzyme solutions against 250 volumes of the appropriate buffer solutions for 5 h at 8 C. Dialysis bags were transferred to phosphate buffer, pH 6, and were dialyzed for an additional 3 h at 8 C. Residual xylanase activity was determined using the standard assay. Buffers used in the pH stability studies included those already listed, except triethylamine-HCl, which was used for pH 10–11. Temperature stability was examined by incubating enzyme solutions for 1 h in water baths held at appropriate temperatures, after which enzyme solutions were returned to an ice bath, and residual enzyme activity was measured using the standard assay.

Mode of xylanase action. Enzyme action was determined using both viscometric and chromatographic methods. For the viscometric assay, soluble hydroxyethylxylan was prepared as described by Baker et al (1). One milliliter of enzyme solution was added to 5 ml of 1.7% hydroxyethylxylan in 0.05 M phosphate buffer, pH 6.0. At various time intervals, the viscosity of the solution was estimated by determining the percentage of loss in efflux time using a 100 Cannon-Fenske viscometer held at 25 C. At each time interval, the percentage of hydrolysis of the hydroxyethylxylan substrate also was determined. Reducing sugars were measured using the method of McFeeters (22), and total carbohydrate was measured with the phenol-sulfuric acid procedure (28).

Reaction products were separated via paper chromatography (28). Reaction mixtures consisted of 0.4 ml of substrate (1% xylan in 0.05 M acetate buffer, pH 5.5) and 0.1 ml of enzyme solution incubated at 30 C. At 0, 15, 30, 60, and 120 min of incubation, 50 μl of the reaction mixture was spotted on Whatman No. 1 filter paper, and spots were dried with a stream of warm air. A mixture of xylose, xylobiose, and xylotriose (a gift from P. Reilly, Iowa State University, Ames) was spotted as standard, and the chromatograph was developed by three ascending runs in butanol/acetic acid/water (3:1:1). The paper was developed with methanolic $NaOH/AgNO_3$ (28).

Cell death assay. Root cap cells were collected from 2- and 3-day-old seedlings of cucumber (*Cucumis sativus* L. 'Spacemaster'), corn (*Zea mays* hybrid A619 × A632 Ht), cotton (*Gossypium hirsutum* L.), oat (*Avena sativa* L. 'IA 576'), and bean (*Phaseolus vulgaris* L. 'Pinto') (17). Treatments included PL alone, xylanase alone, and both enzymes together. Control trials used boiled enzyme. All treatments were carried out in 0.02 M phosphate buffer, pH 7.0, and enzyme preparations were dialyzed into this buffer before use. The size of the drops con-

TABLE 1. Purification of xylanase from culture filtrates of *Erwinia chrysanthemi* strain SR120A

Step	Volume (ml)	Protein (mg)	Total activity (IU)	Specific activity (IU/mg)	Purification	Yield (%)
Culture filtrate	1,928	48.20	35.24	0.73	1.0	100
$(NH_4)_2SO_4$ fractionation	18	5.40	7.1	1.31	1.8	20.15
Ion-exchange (CM-Sephadex)	56	0.448	3.21	7.17	9.8	9.11
Chromatofocusing (PBE 94)	16	0.16	2.01	12.60	17.2	5.70
Gel filtration (Sephadex G-75)	5.8	0.075	1.10	14.59	19.9	3.12

taining root cap cells was adjusted so the enzyme concentration would be constant, and the total volume would equal 20 μl for all repetitions. Each drop contained at least 80 root cap cells; they were placed on microscope slides and were kept at room temperature in petri plates lined with moistened paper. Cell death was determined after 1, 2, and 3 h by adding a 20-μl drop of Evan's blue stain (1% aqueous) and then incubating an additional 10 min. Evan's blue stains dead cells and is excluded from living cells (15). Each treatment was repeated 10 times, and data were analyzed using *t* tests ($P = 0.05$).

Tissue maceration tests. Tissue maceration was determined using the method developed by Ishii (18). The fully expanded first leaves of corn (A619 × A632 Ht), oat (IA 576), and cowpea (*Vigna unguiculata* (L.) Walp. 'California Blackeye') were used, along with expanded cotyledon tissue of cucumber (Spacemaster) and leaves of 7- to 9-wk-old tobacco plants (*Nicotiana tabacum* L.). The lower epidermis of the leaves was peeled to expose the mesophyll cells. Disks 0.5 cm in diameter were cut with a borer and floated on the surface of enzyme solutions. All treatments were carried out at pH 7.0 in 0.02 M phoshpate buffer, and enzyme preparations were dialyzed into this buffer before use. Treatments included PL alone, xylanase alone, and both enzymes together. A buffer control was used for each treatment. For cucumber, polygalacturonase (PG) from *Rhizopus* (pectinase; Sigma) was used in place of PL. The amount of buffer added to the enzyme solution was adjusted so the enzyme concentration would remain constant, and the volume would be 0.5 ml for all repetitions. A 10 μl portion was removed hourly for 4 h and was examined with a hemacytometer to determine the number of cells released. Each treatment was repeated three times, and data were analyzed using *t* tests ($P = 0.05$). To estimate the total number of mesophyll cells that could be released enzymatically in a tissue disk, leaf-tissue disks were treated with a mixture of 2 U/ml of commercial pectinase, 2 U/ml of Driselase (Sigma), and 10% (v/v) Celluclast (NOVO Biochemical Industries, Inc., Franklinton, NC). The proportion of cells released by each treatment was determined by subtracting the number of cells released in the control from the cells released by the enzyme treatment and dividing this number by the total number of cells present that could be released enzymatically.

RESULTS

Xylanase purification. Table 1 summarizes the enzyme purification procedure. The procedure was repeated several times with purifications ranging from 19- to 27-fold. Cation-exchange chromatography resulted in significant purification of the xylanase (Fig. 1). Most of the proteins passed through the column, and the adsorbed xylanase was eluted when the NaCl concentration reached about 0.13 M. The chromatofocusing step also resulted in substantial purification, with the peak of xylanase activity eluting at a pH of about 8.8 (Fig. 2). After gel filtration, the purified xylanase preparation was concentrated by ultrafiltration and was analyzed by SDS-PAGE. A single protein band was present after staining the gel with Coomassie blue R 250 (Fig. 3). The purified xylanase was stable for up to 1 yr when stored at −20 C in 0.05 M phosphate buffer, pH 6.0, containing 0.05 M NaCl.

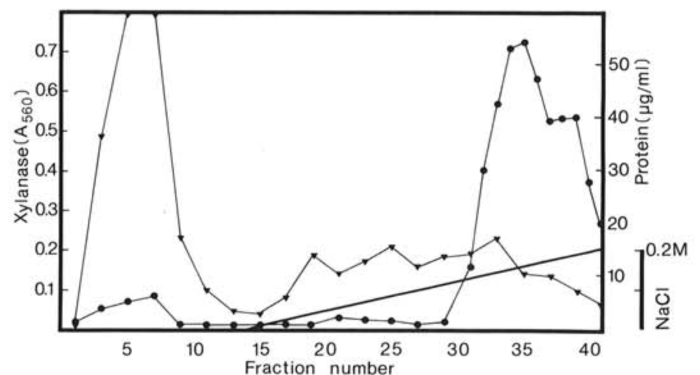

Fig. 1. Elution of *Erwinia chrysanthemi* xylanase from a CM-Sephadex column. The enzyme was precipitated from concentrated culture filtrates with ammonium sulphate, desalted into 0.02 M MES buffer, pH 5.5, and loaded onto the column. After a wash in buffer, the enzyme was eluted with a 0–0.2 M NaCl gradient in 0.02 M MES buffer (—). Fractions were assayed for xylanase activity (●-●-●) and protein (▲-▲-▲).

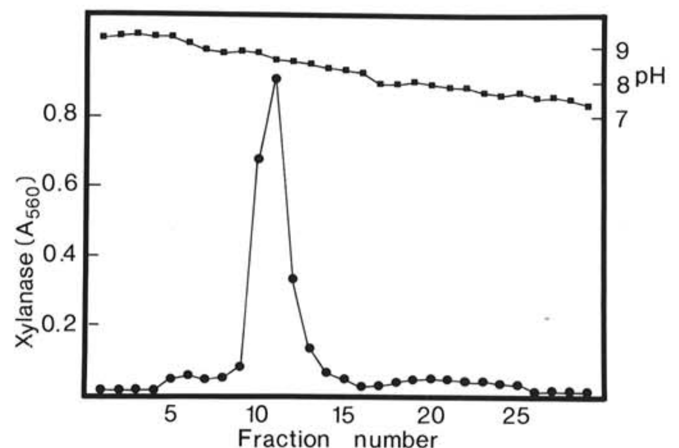

Fig. 2. Elution of *Erwinia chrysanthemi* xylanase from chromatofocusing column. Xylanase fractions from a CM-Sephadex column were concentrated, dialyzed into 0.025 M ethanolamine-HCl buffer, pH 9.4, and loaded onto column of PBE 94. Enzyme was eluted with Polybuffer 96, forming a gradient from pH 9 to 7. Fractions were tested for xylanase activity (●-●-●) and pH (■-■-■).

Substrate specificity of the purified xylanase was examined using the following polysaccharides: dextran, starch, carboxymethylcellulose, pectin, sodium polypectate, locust bean gum, gum arabic, arabinogalactan, barley glucan, and xylan. Barley glucan was obtained from U. S. Biochemical Corp., Cleveland, OH; all other polysaccharides were obtained from Sigma. Substrates were prepared in 0.1 M phosphate buffer, pH 6.0, at a concentration of 0.5% (w/v). Reactions were carried out at 30 C for 1 h, and increases in reducing sugars were measured as in the standard xylanase assay. The purified xylanase preparation was not active on any of the substrates, except xylan.

Enzyme characterization. The molecular mass of the xylanase was estimated, by SDS-PAGE, to be approximately 42 kDa (Fig. 3). The isoelectric point of the enzyme was determined, by chromatofocusing, to be 8.8 (Fig. 2). The purified xylanase had a pH optimum of 5.5 and was fairly stable over a wide pH range (Fig. 4), but residual xylanase activity fell rapidly at levels above pH 10. The optimum temperature in a 10-min assay was 55 C, and about 40% of the enzyme activity was lost after incubation for 1 h at 40 C (Fig. 5).

The effect of metal ions on enzyme activity was examined by conducting the standard xylanase assay in the presence of 1- or 10-mM concentrations of various metal salts (Table 2). Xylanase activity was completely inhibited by 1 mM Hg^{+2} and 1 mM Ag^{+} and was substantially reduced by 1 mM Cu^{+2} and 1 mM Zn^{+2}.

The xylanase was an endoxylanase, as shown by both viscometric techniques and chromatographic analysis of reaction products. Simultaneous measurement of viscosity reduction and production of reducing sugars in reaction mixtures containing 1.7% hydroxyethylxylan revealed that viscosity was reduced by 50% when only 1.3% of the glycosidic bonds in the substrate had been cleaved. Paper chromatographic analysis indicated that reaction products released from xylan in the standard xylanase assay included xylobiose, xylotriose, and higher oligomers. No xylose was detected.

Biological activity of enzymes on corn, oat, and selected dicotyledonous plants. Treatment with PL resulted in abundant death of pinto bean, cotton, and cucumber cells but had considerably less effect on corn and oat cells (Fig. 6). Xylanase caused moderate

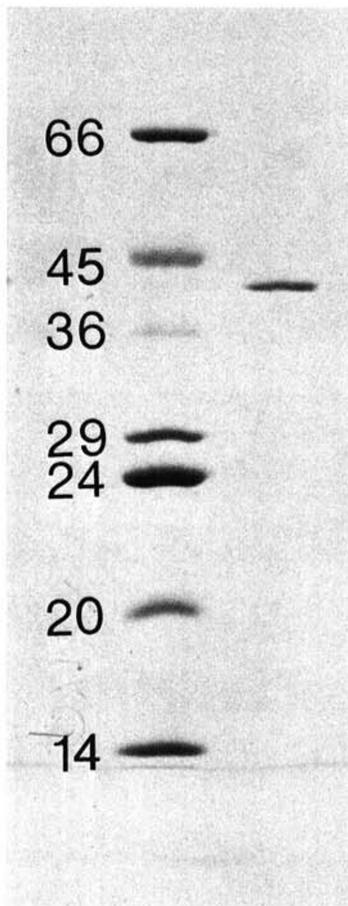

Fig. 3. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of purified *Erwinia chrysanthemi* xylanase (right lane) and molecular mass standards (left lane). Standards include: bovine albumin (66 kDa), egg albumin (45 kDa), glyceraldehyde-3-phosphate dehydrogenase (36 kDa), carbonic anhydrase (29 kDa), trypsinogen (24 kDa), soybean trypsin inhibitor (20 kDa), and alpha-lactalbumin (14 kDa). Purified xylanase (10.5 μg) and protein standards (26 μg) were loaded onto the gel. Proteins were stained with Coomassie blue R 250.

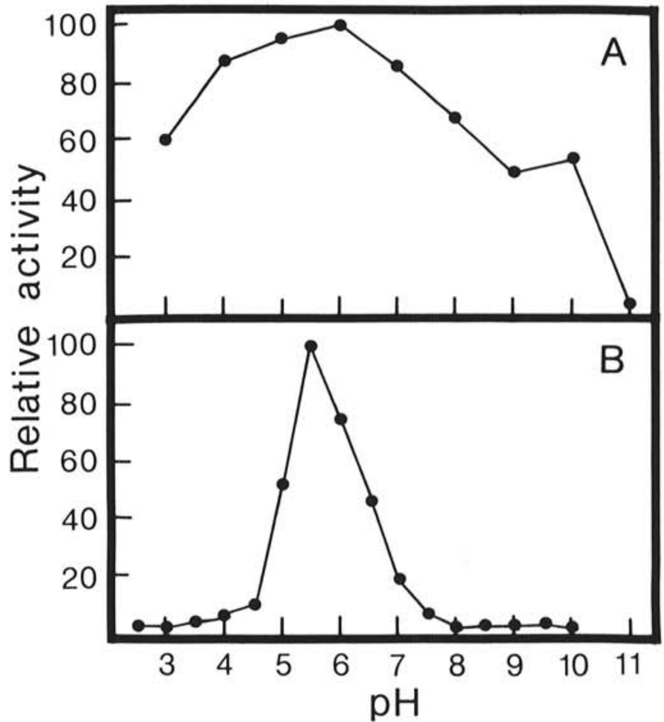

Fig. 4. Effect of pH on **A,** stability and **B,** activity of *Erwinia chrysanthemi* xylanase. Stability was determined by dialyzing enzyme solutions against appropriate buffers for 5 h at 8 C. Dialysis bags were transferred to phosphate buffer, pH 6, for 3 h at 8 C, and residual activity was determined. The pH optimum was determined by using a range of buffers in the standard xylanase assay.

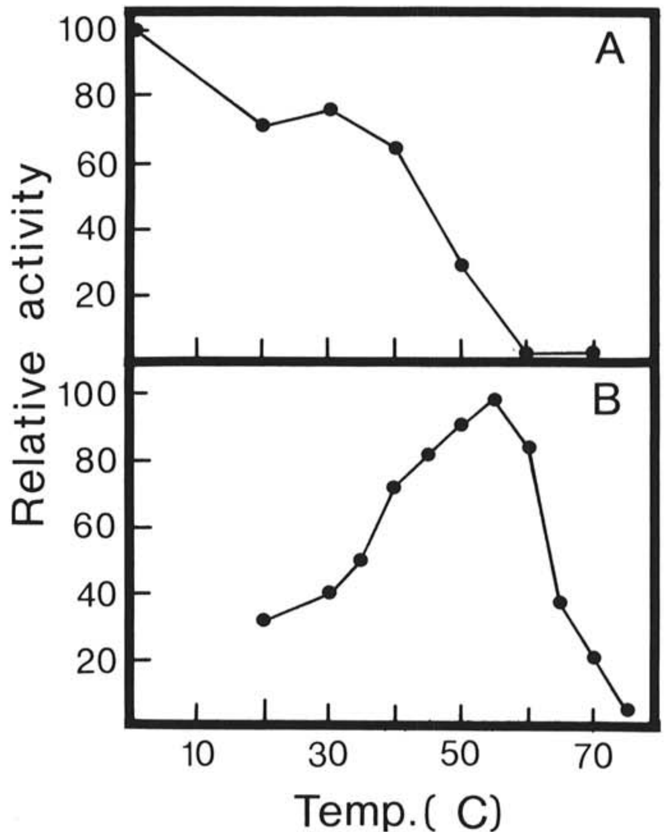

Fig. 5. Effect of temperature on **A,** stability and **B,** activity of *Erwinia chrysanthemi* xylanase. Residual activity was determined after incubation of enzyme solutions in water baths at appropriate temperatures for 1 h. Optimum temperature was determined in a 10-min assay.

mortality of corn, oat, and cucumber cells but had little effect on pinto bean and cotton cells. When treated with both enzymes, mortality of cotton and pinto bean cells differed only slightly from treatment with PL alone. Xylanase and PL acted synergistically in causing death of corn, oat, and cucumber cells.

PL teatment caused extensive maceration of leaf tissues of tobacco and cowpea, and a commercial PG preparation (having no xylanase and little or no cellulase activity) caused abundant maceration of cucumber cotyledon tissue (Fig. 7). PL treatment released very few cells from corn and oat leaf tissue. Xylanase caused negligable maceration of tobacco, cowpea, or cucumber tissue but caused significant maceration of corn and oat tissues. When treated with both PL and xylanase, tobacco and cowpea maceration differed little from that seen with PL alone. Likewise, the amount of cucumber maceration in the presence of PG and xylanase was similar to that observed with PG alone. Xylanase and PL acted synergistically, however, in causing maceration of corn and oat tissues.

DISCUSSION

Xylanases have been reported in a wide range of terrestrial, aquatic, and rumen-inhabiting bacteria (2,12,16,24,26,). However, surprisingly little attention has been paid to xylanases produced by plant-pathogenic bacteria or to the possible role these enzymes play in disease development. Within the soft rot erwinias, xylanase production has been reported for some strains of *E. carotovora* subsp. *carotovora* (21) and *E. chrysanthemi* (6).

We previously have shown that *E. chrysanthemi* xylanase is produced constitutively and is apparently subject to catabolite repression (6). This is similar to the way in which endoglucanase production is regulated in this organism (4,21) and contrasts with the inductive regulation of the pectolytic enzymes (3,9,21).

The properties of the xylanase we purified from *E. chrysanthemi* SR120A are, for the most part, typical of those reported for many xylanases of bacterial or fungal origin (12,27). The molecular mass of the *E. chrysanthemi* xylanase was estimated, by SDS-PAGE, to be 42 kDa. It should be noted that SDS-PAGE provides an estimate of the molecular weight of protein subunits but not necessarily the weight of the holoenzyme. However, based on the size and characteristics of other cell wall-degrading enzymes of *Erwinia*, we feel that our estimate reflects the size of the holoenzyme. Most microbial xylanases with alkaline isoelectric points are somewhat smaller, with molecular masses ranging from 15 to 30 kDa (35). The *E. chrysanthemi* xylanase, however, falls well within the size range of the other cell wall-degrading enzymes this pathogen produces (9,21).

We previously reported the molecular mass of *E. chrysanthemi* xylanase to be only 24 kDa, based on gel filtration on Sephadex G-75 (5). We believe that this estimate was erronious and possibly the result of an interaction between the enzyme and the gel material. Tan et al (33), found that a xylanase from *Trichoderma*, which had a molecular mass of 20 kDa, based on SDS-PAGE, was severly retarded on gel filtration, resulting in molecular mass estimates of 11.5 kda with Sephadex G-75 and 8 kDa with Bio-Gel P-100. Others also have reported erratic behavior of xylanases on gel filtration (1,25,35).

It is quite common for microorganisms to produce multiple xylanases (35). Biochemical and genetic studies have indicated that, in some instances, the multiple-xylanase isozymes are formed as a result of postranslational modifications of a single gene product (2), while in other cases, the isozymes are products of distinct xylanase genes (16,36). In the present study, we detected only a single xylanase produced by *E. chrysanthemi* SR120A.

TABLE 2. Effects of metal ions on activity of *Erwinia chrysanthemi* xylanase

Chemical	Concentration (M)	Relative Activity
None	. . .	100
$HgCl_2$	10^{-3}	0
$FeCl_3$	10^{-3}	77
	10^{-2}	0
$ZnSO_4$	10^{-3}	8
	10^{-2}	0
$MnCl_2$	10^{-3}	107
	10^{-2}	23
$CaCl_2$	10^{-3}	96
	10^{-2}	51
$AgNO_3$	10^{-3}	0
$MgCl_2$	10^{-3}	110
	10^{-2}	101
$CuSO_4$	10^{-3}	29

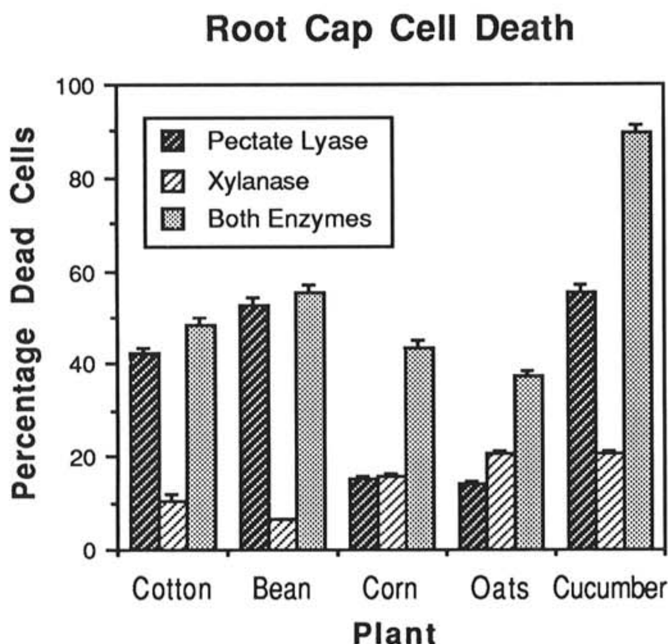

Fig. 6. Effect of pectate lyase, xylanase, and pectate lyase-xylanase mixtures on viability of isolated root cap cells. Cell death was determined by staining with 1% aqueous Evan's blue after incubation of cells in enzyme solutions for 3 h. Bars indicate standard errors.

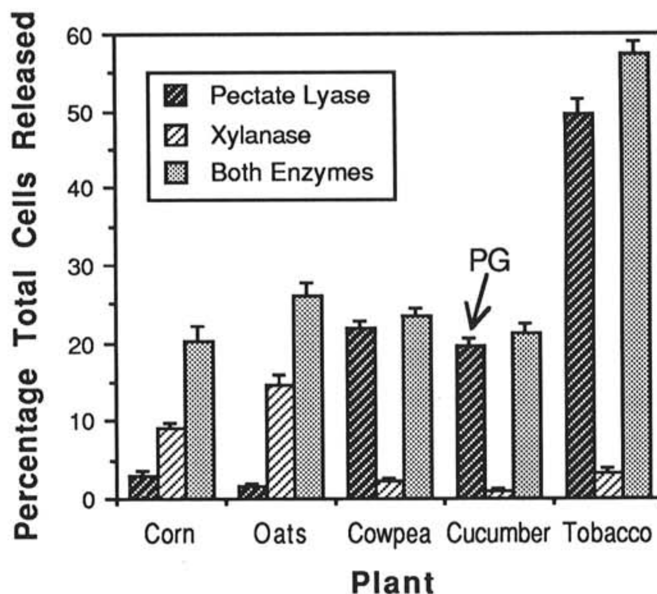

Fig. 7. Maceration of corn, oat, cowpea, and tobacco leaf tissue by pectate lyase (PL), xylanase, and PL-xylanase mixtures. Cucumber cotyledon tissues were treated with commercial polygalacturonase (PG), xylanase, or PG-xylanase mixtures. Percentage of cells released from the tissues was determined after 4 h of incubation in enzyme solutions. Bars indicate standard errors.

This result should be viewed with some caution, however. Xylans are very heterogeneous polymers and xylanases often are quite specific concerning the region of the xylan polymer they are able to cleave (27,35). We used only a single type of xylan (from larchwood) in our studies. It is possible that *E. chrysanthemi* SR120A produces other xylanases that, while unable to attack our larchwood xylan substrate, might be capable of degrading xylan from another source with a different composition.

Pectolytic enzymes have been shown to play important roles in a wide range of dicotyledon diseases caused by necrotrophic pathogens (8,10). The importance of these enzymes in diseases of grasses, however, is less clear. Ishii (18) found that a PG that caused extensive maceration of radish, potato, and tobacco had little ability to macerate oat tissues. In the same paper, he reported that a pectin lyase from *Aspergillus japonicus* had some ability to release single cells from oat leaves, and he later showed that this PL could reduce the viability of suspension-cultured rice cells (19). Bucheli et al (7) treated suspension-cultured corn cells with a range of purified pectolytic enzymes (pectin lyase, PL, pectinmethylesterase, and PG) and found that none of the enzymes caused a significant reduction in ^{14}C-leucine incorporation (an assay for cell viability). Our observations indicate that, by itself, PL from *E. chrysanthemi* caused minimal maceration and only a small reduction in viability of corn and oat cells relative to the effects seen with dicotyledonous plants. In conjunction with xylanase, however, PL contributed significantly to both cell killing and maceration in corn and oat tissues. Scott-Craig et al (30) recently found that a mutant strain of the corn pathogen *Cochliobolus carbonum*, which no longer produced endopolygalacturonase, retained pathogenicity equivalent to the wild-type parent strain, but caution should be used in extrapolating their results to other host-parasite systems. Any role of endopolygalacturonase as a cell-killing factor probably would have been masked in their system because of the production of a host-specific toxin by the pathogen and the use of a toxin-sensitive inbred line in pathogenicity tests.

Only recently has the effect of xylanase on plant cells and tissues been investigated. Ishii (18) found that an endoxylanase from *Trichoderma viride* could cause some maceration of oat leaf tissue by itself, and it acted synergistically with PL in macerating oat, corn, barley, and wheat tissues. He also found that the *Trichoderma* xylanase caused a reduction in viability of suspension-cultured rice cells and suggested that the toxicity was the result of the production of activated oxygen as the xylanase acted on the walls of the cultured rice cells (19). Bucheli et al (7) postulated that xylanase was one of two or more factors secreted by the rice pathogen *Magnaporthe grisea*, which has the ability to kill suspension-cultured corn cells. They determined that the xylanase (as well as other factors) released cell-wall fragments that were toxic to the cultured cells. Regardless of the mechanism involved, our results also indicate that xylanase can have a toxic effect on grass cells and can contribute to the maceration of grass tissues, and that the enzyme acts synergistically with PL in causing these effects.

Cucumber responded differently to *E. chrysanthemi* xylanase than did the other dicotyledonous plants. Treatment with xylanase alone killed a substantial number of cucumber root cap cells, and xylanase and PL acted synergistically in reducing cell viability. Others have suggested that xylanase might be a factor in diseases of cucurbits. Muskmelon stem cell walls have been shown to possess considerable amounts of xylose-containing polymers, including a xylan with a β-1,4-linked backbone (13). In addition, xylanase has been implicated as a virulence factor in the infection of melon with the pathogen *Colletotrichum lagenarium* (34). In spite of its ability to induce mortality, however, xylanase seemed to have no ability to macerate cucumber tissues.

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